Sunil Kumar Talapatra · Bani Talapatra

Chemistry of Plant Natural Products

Stereochemistry, Conformation, Synthesis, Biology, and Medicine

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With a Foreword by Professor K.C. Nicolaou

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Dedicated
To the loving memories of our mothers
Chapala-Basanti Talapatra and Sushama Rani Chaudhuri

And

A gift with love
To our students
Who inspired us through years
And
To our children
Anupam and Sharmishtha
Who ungrudgingly cooperated during our early professional years and
Helped in all possible ways during the preparation of this manuscript

Foreword

Striving to understand nature provided the impetus for much of our philosophical ideas, scientific discoveries, and technological advances whose beneficial results we came to enjoy as a species throughout history. Understanding the molecules of life—nucleic acids, proteins, and secondary metabolites, commonly referred to by organic chemists as natural products—is arguably one of the most important human endeavors, one in which the benefits reaped by science and society are both immense and undisputed. Indeed, the emergence of the structure of the molecule and the art of its synthesis in the nineteenth century set the stage for the development of all of organic chemistry and biochemistry and much of modern biology and medicine. Natural products played a protagonist role in these developments. Their isolation, structural elucidation, and synthesis challenged and stimulated analytical and purification techniques and instrumentation, method development, and synthetic strategy design. Today, because of the enormous advances made over the last two centuries in the chemistry, biology, and medicine of natural products, people around the world enjoy untold benefits with regard to healthcare, nutrition, cosmetics, and fashion, just to name a few areas.

I was honored by the invitation of Professors Sunil and Bani Talapatra to provide a Foreword to their book and enjoyed reading various versions of their prepublication manuscripts. Much to my delight, I found them to be stunningly illuminating in terms of breadth and depth of coverage of essentially the entire field of chemistry, biology, and medicine of plant-derived natural products. Their accomplishment in putting together this tome is beyond the normal boundaries of most books written on the subject, and they deserve our admiration and respect for bringing together the various aspects of this important field.

In the chapters that follow, the authors focus on the molecules of life, particularly those natural products derived from plants, dwelling on such wide ranging aspects as enzymatic transformations, biosynthetic pathways, isolation, and structural elucidation (including techniques and instrumentation), conformation, total synthesis, biological activities and functions, symbiotic relationships, medical applications, biochemistry, and stereochemistry. The latter topic is discussed in considerable detail and its impact on the chemical and biological properties of the

molecule and its asymmetric synthesis are articulated and explained. The special emphasis on conformation is particularly informative and educational and provides useful insights and understanding of the nature of the molecule to students of organic chemistry. The various aspects of the science of natural products are elaborated upon by using selected natural product classes and individual compounds, such as terpenoids and alkaloids, to underscore the influential impact of these endeavors in advancing the discipline of organic chemistry, including theory, experimental methods and techniques, chemical synthesis, biology, medicine, and nutrition.

Three appendices at the end of the book provide interesting and useful biographical information on selected pioneers, major discoveries and inventions, and extra educational material for learning. Indeed, this opus is intended as both a reference book and a teaching text where one can find important facts, inspiration, and pedagogy. The structure of the book allows for easy selection of separate topics for reading and teaching. Congratulations and many thanks to Professors Sunil and Bani Talapatra for an outstanding treatise which will remain a classic in the field and hopefully serve to maintain its momentum and proliferation with new admirers, recruits, and supporters.

Houston, TX K.C. Nicolaou

Preface

Linus Pauling [NL 1954 (Chemistry), 1962 (Peace)] once said "*A good book or a speech should be like a Lady's dress. It should be long enough to cover the essentials and short enough to reveal the vital statistics*."

With this caveat in mind, it becomes a challenging task to author an adequately balanced textbook which shall capture the essentials and address the leading frontiers, while staying focused within the broader scope of the subject area. During our long postgraduate-level teaching career spanning more than three decades, we felt the unmet need of a textbook on Plant Natural Products, the Secondary Metabolites, that covered the fundamental aspects of the relevant chemistry, stereochemistry, biosynthesis, and bioactivity in sufficient depth, elaborated using well-known representative members.

Here we have made an attempt to write a book intended to serve as a textbook for advanced undergraduate and graduate/postgraduate students as well as a reference guide for researchers and practicing organic and pharmaceutical chemists who may wish to gain a better understanding of various aspects of the chemistry of natural products. It is almost an impossible task to write a comprehensive textbook dealing with all facets of natural products chemistry of even some of the selected classes of molecules due to the rapidly expanding literature in this area. Hence, we have deliberately not roamed too far to make this a comprehensive textbook, but rather tried to create one that will provide sufficient background in the general area of natural products chemistry and serve as an adequate launching pad for an in-depth and specialized investigation.

This book grew primarily out of our 68 years of combined postgraduate teaching experience in natural products chemistry and stereochemistry at the Calcutta University and at some other universities. We have limited our discussions to some well-studied natural products of plant origin, which have contributed significantly to the advancement of organic chemistry. The choice from the endless variations of natural products is entirely personal, driven by our perception of model natural products for our target readers. Our coverage of the material is illustrative of the vastness, diversification, and continued growth of this particular area of chemistry.

The distinguishing feature of this book is the discussion on the stereochemical aspects, the hallmark of chiral natural products, and the inclusion of a chapter exclusively dedicated to stereochemistry (Chap. 2). This chapter will be helpful in understanding various updated nomenclatures, stereostructures, asymmetric synthesis, biosynthesis, and bioactivities (chiral recognition in vivo) of natural products that appear in subsequent chapters. Furthermore, wherever possible, we have given plausible mechanistic rationalizations of product formations which are less obvious, during the portrayal of reactions and synthesis of natural products. Original literature has been referenced, with titles of the articles (with the exception of titles of some very old references, which could not be procured), to give interested readers an idea of the subject matter contained in such articles.

Chapter 1 offers a preliminary introduction to enzymes, coenzymes, and primary and secondary metabolites (natural products), illustrates various biological functions of the metabolites, and summarizes the identified metabolic pathways leading to the different types of natural products.

Chapter 3 deals with few important biochemical events related to the discussions in the subsequent chapters on the biogenesis and biosynthesis of major skeletal patterns formed sometime during three billion years of evolution.

Various synthetic methodologies, separation techniques, and instrumental analysis have been invented and developed in connection with the natural products chemistry research. In Chap. 4, we have discussed some widely used isolation procedures and separation techniques (Sect. 4.1). The chapter also touches upon traditional and state-of-the-art methods for structural elucidation of naturally occurring molecules (Sect. 4.2).

During the discussions on the "Biosynthesis of Terpenoids: the oldest Natural Products" (Chap. 5) or on the biosynthesis of other natural products (other Chapters), no extensive description of the enzymes and their processes has been provided. The biosynthetic conversions are explained in terms of the currently accepted mechanisms of organic reactions, with special attention to the stereochemical features of the processes.

Mother Nature uses organic molecules as the building blocks for the natural product framework. Barton (NL 1969) said, "*if we assume that enzymatically-induced reactions follow the same mechanistic principles as ordinary organic reactions we can at least make an approach to the subject*" [1]. Likewise, R. Kluger wrote in an article [2] "*It is useful if the reactions can be systematically divided into mechanistic types (such as Ingold formulation [Ingold, 1953]. The most common examples of this type of classifications are the two general nucleophilic substitution mechanisms, S_N1 and S_N2.*" The synthetic methodologies used by Nature are amazingly simple, as exemplified by aldol, acyloin, and Claisen condensations, olefin-cation addition/cyclization, Markonikov additions, simple S_N1, S_N2, S_N2', E1, and E2 reactions, and 1,2-*trans* migrations for creating carbon–carbon bonds—the life string of organic molecules. Nature's substrates for the biosynthesis of complex natural products are also simple (e.g., small carbonyl compounds, L-amino acids, olefins, etc.).

Chapters 6–29, including a number of sections (sub-chapters) of Chaps. 6–8, 10, 13, and 14, deal with the chemistry of individual compounds classified according to the major biogenetic pathways. Many basic concepts and ideas have been discussed. Though the tone of discussions on natural products has changed during the last few decades, we have given adequate attention to the work of past great chemists in dealing with the individual compounds, because we believe that their work will never go out of date; rather it will exist to impart the spirit of enquiry and forms the foundation to our current understanding. In this context we quote from the abstract of Barton's talk entitled "Oxygen and I" in the 10th Johnson Symposium 1995 held at Stanford University: "*In Chemical Sciences, the distant past, the near past and the present join together in continuous harmony.... The lecture will illustrate how past chemistry should not be forgotten.*"

Chapter 15 is a general introduction to alkaloids. Chapters 16–29 include detailed discussions on some well-known alkaloids derived from various biogenetic precursors. Chapter 30 contains structures with relevant references of a number of alkaloids of diverse skeletal patterns (not discussed due to space constraint).

Important concepts such as conformational analysis, stereochemistry, biomimetic synthesis, retrosynthetic approach, pericyclic reactions, Fischer and Newman projection formulas, and many organic reactions owe their genesis and/or refinement to the natural products chemistry. With his understanding of squalene-2,3*S*-oxide (a natural product), the phenomenon of catalysis, and his passion for the transition metals of the Periodic Table, "the most elegant organizational chart ever devised" [3], Sharpless (NL 2001) invented Sharpless epoxidation—a near chemical substitute for an enzymatic reaction. The trisubstituted olefinic alcohol, geraniol, a natural product with an attractive smell that was a great favorite of Sharpless [4], was used by him to successfully implement the most challenging asymmetric epoxidation and dihydroxylation. Chapter 31 attempts to bring together such important outcomes of natural products chemistry research.

The contribution of natural products in asymmetric synthesis is immense. Natural chiral auxiliaries take the place of enzymes in some chemical reactions, and the chemical literature is flooded with reports on such chiral auxiliaries. Chapter 32 has been dedicated to chiral recognition in biological systems and the use of natural products and their derivatives as chiral auxiliaries. This chapter illustrates the differences in biological properties exhibited by each component in a pair of stereochemically nonequivalent enantiomeric twins, i.e., enantiomeric stereoselectivity.

The medicinal values of many natural products are now evaluated scientifically to give them the drug status. Further, many of them served as the lead molecules for the synthesis of cost-effective therapeutic molecules with promising medicinal values. This is one of the important value-added dimensions of natural products chemistry. Chapter 33 deals with *The Natural Products in the Parlor of the Pharmaceuticals*. Chapter 34, entitled *Organic Phytonutrients, Vitamins and Antioxidants*, presents the multitude of useful biological functions of nutraceuticals, as well as natural products that are consumed daily through diet in the form of spices,

vegetables, fruits, and drinks. This chapter may also stimulate interest of the uninitiated readers.

Carbohydrates form a very important class of bioorganic molecules whose chemical and biochemical properties revolve around their stereochemical and conformational features. The chemistry of carbohydrates has grown enormously during the last few decades and involves their use as templates in the synthesis of complex chiral natural products. However, since their biosynthesis and biodegradation are routed through primary metabolic/catabolic pathways, they are referred to as primary metabolites according to conservative definition and do not fall within the scope of secondary metabolites and hence have not been included.

Brief biographical sketches of 31 pioneering chemists, who we believe immensely enriched the realm of natural products chemistry, appear in Appendix A. We hope that these great personalities will inspire the young readers and motivate them in their professional pursuits. In the absence of space constraint, several more such life sketches could have been included, since concepts and ideas often develop through the collaborative and collective intellectual engagement and may not be attributed to a single discoverer. We sincerely regret our inability to be all-inclusive in this regard.

Appendix B chronologically lists some landmark inventions/discoveries in the field of natural products which may be interesting and inspiring to readers.

Appendix C contains miscellaneous information geared towards students and some often-overlooked conventions. For convenience, a list of Abbreviations has been provided before the Table of Contents**.**

Both in the Preface and in the body of the text, we took the liberty of quoting from books, articles, lectures, and letters (original references provided wherever appropriate), in order to impart deeper insights and broader perspectives related to the topics discussed.

We hope that the present version of the text will be well adapted to the teaching of natural products chemistry at the senior undergraduate and graduate/postgraduate levels. Students should get a flavor of the subject and be able to comfortably move forward with more advanced topics both within the domain of natural products chemistry and beyond. The teachers with limited teaching time and resources should be able to pick and choose topics of interest to them, aligned with the level of the course they intend to offer.

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We are solely responsible for errors, inconsistencies, and infelicities that may persist in the text and Figures, despite our most sincere efforts to eliminate them. Our endeavors will be meaningful if the book benefits the target readers. We welcome any corrective suggestions and recommendations for the future edition of this book.

Kolkata, India
July, 2013

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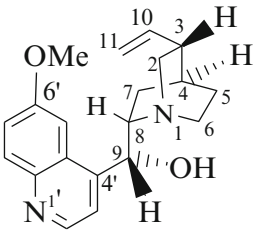
About the Images



(—)-Quinine
3(R), 4(S), 8(S), 9(R)

Cinchona calisaya

Cinchona officinalis

Quinine has been one of the most celebrated natural products since its first isolation in 1820 from the *Cinchona* bark by J. Pelletier and J. Caventou. It remained as the only effective drug against malaria for more than a century. The pictures of *C. calisaya* and *C. officinalis* are shown. Malaria is thus one of the first few diseases to be treated by a pure organic compound. The French Government issued a postal stamp (shown) in 1970 in honor of the discoverers of quinine to mark the 150th year of its discovery and also to introduce this molecule of immense health importance to the general public.

Quinine also served as the lead compound for a number of effective synthetic antimalarial drugs. Its chiral and basic character allowed its use for the first chiral

resolution of a racemate acid, and a new horizon called stereochemistry appeared in the sky of chemical sciences. *Cinchona* alkaloids with some chemical manipulations have been extensively used as chiral auxiliaries and chiral catalysts in asymmetric synthesis and also as phase-transfer catalysts. For the multifarious importance of quinine along with its inspiring structure with four chiral centers, it has served as a target molecule for its first elegant synthesis in 1944. Its stereoselective synthesis appeared thrice even in the early twenty-first century. In fact, quinine serves as an interesting illustrative example of the (*R*,*S*)-designation of its four chiral centers. Thus, quinine deserves the front matter recognition of a book on the chemistry of bioactive plant natural products.

List of Abbreviations

α	Observed optical rotation in degrees
$[\alpha]$	Specific rotation [expressed without units; the actual units, deg mL/g dm) are understood].
Ac	Acetyl
ACP	Acyl carrier protein
acac	Acetylacetonate, acetylacetonyl
AD	Asymmetric dihydroxylation
ADP	Adenosine 5′-diphosphate
AIBN	2,2′-Azobisisobutyronitrile
aka	Also known as
AMP	Adenosine-5′-monophosphate
AO	Atomic orbital
anhyd	Anhydrous
Ar	Aryl
atm	Atmosphere(s)
ATP	Adenosine 5′-triphosphate
ATPase	Adenosine triphosphatase
aq.	Aqueous
	9-Borabicyclo[3.3.1]nonyl
BBEDA	*N*,*N*′-bis(benzylidene)ethylenediamine
9-BBN	9-Borabicyclo[3.3.1]nonane
BINAL-H	2,2′-Dihydroxy-1,1′-binaphthylaluminum hydride
BINAP	(2*R*,3*S*),2,2′-bis(diphenylphos- phino)1,1′-binaphthyl
Bn	Benzyl
BOC, *t*-BOC, Boc	*tert*-Butoxycarbonyl
BOM	Benzyloxymethyl
bp	Boiling point
bpy, bipy	2,2′-Bipyridyl

BTMSA	Bis(trimethylsilyl)acetylene
Bu	*n*-Butyl
s-Bu	*sec*-Butyl
t-Bu	*tert*-Butyl
Bz	Benzoyl
18-C-6	18-Crown-6
c	Cyclo-
^{o}C	Degrees Celsius
calcd	Calculated
CAM	Carboxamidomethyl
CAN	Ceric ammonium nitrate
cat	Catalyst
Cbz, CBZ	Benzyloxycarbonyl
CD	Circular dichroism
CHD	Coronary heart disease
CI	Chemical ionization (in MS)
CIP	Cahn-Ingold-Prelog
cm	Centimeter (s)
CoA	Coenzyme A
COD/cod	1,5-Cyclooctadiene (legand)
concd	Concentrated
COSY	Correlation spectroscopy (NMR)
cot, (COT)	Cyclooctatetraene (ligand)
Cp	Cyclopentadienyl
CSA	10-Camphorsulfonic acid
CTP	Cytidine triphosphate
Cy Cy-Hex	Cyclohexyl
δ	Chemical shift in parts per million downfield from TMS (NMR)
d	Day(s); doublet (spectral)
DABCO	1,4-Diazabicyclo[2.2.2]octane
DAIB	3-*exo*-(dimethylamino)isoborneol
DAST	Diethylaminosulfur trifluoride
dba	*trans*,*trans*-Dibenzylideneacetone
DBE	1,2-Dibromoethane
DBN	1,5-Diazabicyclo[4.3.0]non-5-ene
DBS	5-Dibenzosuberyl
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCBI	*N*,*N*′-dicyclohexyl-*O*-benzylisourea
DCC	*N*,*N*-dicyclohexylcarbodiimide
DCE	1,2-Dichloroethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
de	Diastereomer(ic) excess
DEA	Diethylamine
DEAD	Diethyl azodicarboxylate

DEIPS	Diethylisopropylsilyl
DEPT	Distortionless enhancement by polarization transfer (NMR)
DET	Diethyl tartrate
DHP	3,4-Dihydro-2*H*-pyran
DHQ	Dihydroquinine
DHQD	Dihydroquinidine
DIAD	Diisopropyl azodicarboxylate
Dibal-H, DIBALH	Diisobutylaluminum hydride
DIOP	2,3-*O*-isopropylidene-2,3-dihydroxy-1,4-bis (diphenylphosphino)butane
DiPAMP	1,2-Bis(*o*-anisylphenylphosphino)ethane
DIPT	Diisopropyl tartrate
DMA	*N*,*N*-dimethylacetamide
4-DMAP, DMAP	4-(dimethylamino)pyridine
DMB	3,4-Dimethoxybenzyl
Dmca	(*S*) and (*R*)-6,7-dimethoxy-4-coumaryl) alanines
DMDO	Dimethyldioxane
dppe	Bis(diphenylphosphino)ethane $Ph_2PCH_2CH_2PPh_2$
dppf	Bis(diphenylphosphino)ferrocene
dppm	Bis(diphenylphosphino)methane $(Ph_2P)_2CH_2$
dppp	1,3-Bis(diphenylphosphino)propane
dr	Diastereomer ratio
dvd	Divenylbenzene
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMS	Dimethyl sulfide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
L-DOPA	3-(3,4-dihydroxyphenyl)-L-alanine
DPC	Dipyridine chromium(VI) oxide
DTBMS	Di(*tert*-butyl)methylsilyl
EDC (EDCI)	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
E_1	Unimolecular elimination
E_2	Bimolecular elimination
ED_{50}	Dose that is effective in 50 % of test subjects
EDTA	Ethylenediaminetetraacetic acid
e	Electron
ee	Enantiomeric excess
EE	1-Ethoxyethyl
EI	Electron impact (in MS)
Et-DuPHOS	1,2-Bis(2′,5′-diethylphospholano)ethane

ESR	Electron spin resonance
Et	Ethyl
equiv	Equivalent(s)
FAB	Fast atom bombardment (in MS)
FAD	Flavin adenine dinucleotide (oxidized form)
$FADH_2$	Flavin adenine dinucleotide (reduced form)
FD	Field desorption (in MS)
FID	Flame ionization detection
Fmoc	9-Fluorenylmethoxycarbonyl
FPP	Fernesyl pyrophosphate
FT	Fourier transform
g	Gram (s)
GC	Gas chromatography
GFPP	Geranylfarnesyl pyrophosphate
GPP	Geranyl pyrophosphate
GGPP	Geranylgeranyl pyrophosphate
GSSG	Oxidized glutathione
h	Hour (s)
HETE	Hydroxyeicosatetraenoic
hfc	3-(heptafluoropropylhydroxymethylene)-D-camphorato
HDL	High-density lipoprotein
His	Histidine
HMPA	Hexamethylphosphoramide
HMG	Hydroxymethylglutaryl
HPLC	High-pressure liquid chromatography
HWE	Horner-Wadsworth-Emmons
Im (imid.)	Imidazole
IND	Indoline
Ipc	Isopinocampheyl
KHMDS	Potassium hexamethyldisilazide, potassium bis(trimethylsilyl) amide
L	Liter(s)
LASER	Light Amplification by Stimulated Emission of Radiation
LDA	Lithium diisopropylamide
LDL	Low-density lipoprotein
LHMDS	Lithium hexamethyldisilazide, lithium bis(trimethylsilyl)amide
LTMP	Lithium 2,2,6,6-tetramethylpiperidide
*m*CPBA	3-Chloroperoxybenzoic acid
Me	Methyl
MEM	(2-Methoxyethoxy)methyl
Mes	Mesityl,2,4,6-trimethylphenyl (not methanesulfonyl)
MHz	Megahertz (NMR)
min	Minute(s)
mM	Millimoles per liter

MO	Molecular orbital
MOM	Methoxymethyl
MS	Mass spectrometry
Ms	Methanesulfonyl(mesyl)
mol	Mole(s)
(+)-MTPA	(R)-(+)-α−Methoxy-α−trifluoromethyl- phenyl acetate / acetyl chloride
m/z	Mass to charge ratio (in MS)
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced NAD
NaHMDS	Sodium bis(trimethylsilyl)amide
NB	2-Nitrobenzyl
nbd	Norbornadiene
*p*NB	*p*-Nitrobenzyl
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NIS	*N*-iodosuccinimide
NMM	4-Methylmorpholine
NMO	4-Methylmorpholine *N*-oxide
NMP	1-Methyl-2-pyrrolidinone
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
NOESY	NOE spectroscopy
Nu	Nucleophile
OD	Optical density
ORD	Optical rotatory dispersion
op	Optical purity (discouraged, see *ee*)
ORTEP	Oak Ridge thermal ellipsoid plot
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
PDF	Portable document transfer format
PG	Prostaglandin
Ph	Phenyl
PHAL	Phthalazine
Phth	Phthalimido (phthalate)
Piv	Pivaloyl
PMB	4-Methoxybenzyl
PNB	4-Nitrobenzyl
PMP	4-Methoxyphenyl
PPA	Poly(phosphoric acid)
ppm	Parts per million (in NMR)
PNNP	*N*,*N*′-bis(1-phenylethyl)-*N*,*N*′-bis-(diphe-nylphosphino) ethylenediamine
PPTS	Pyridinium 4-toluenesulfonate

Pr	Propyl
i-Pr	Isopropyl
psi	Pounds per square inch
py (pyr,Py)	Pyridine
PYR	Diphenylpyrimidine
q	Quartet (spectral)
Ra-Ni	Raney nickel
Red-Al	Sodium bis(2-methoxyethoxy)aluminum hydride
Rf	Retention factor (in chromatography)
rt	Room temperature
rf	Radio frequency
s	Second(s)
SEM	2′-(trimethylsilyl)ethoxymethyl
SET	Single electron transfer
S_N1	Unimolecular nucleophilic substitution
S_N2	Bimolecular nucleophilic substitution
S_N2'	Bimolecular nucleophilic substitution with rearrangement
Sia	Siamyl
TADDOL	α,α,α',α'-Tetraaryl-4,5-dimethoxy-1,3-dioxolane
TASF	*Tris*-(diethylamino)sulfonium difluorotrimethyl silicate
TBAF	Tetra-*n*-butylammonium fluoride
TBAI	Tetra-*n*-butylammonium iodide
TBDMS	*tert*-Butyldimethylsilyl
TBDPS	*tert*-Butyldiphenylsilyl
TBHP	*tert*-Butylhydroperoxide, (*t*-BuOOH), l
TBS	*tert*-Butyldimethylsilyl
TCNE	Tetracyanoethylene
TEBA	Triethylbenzylammonium $Bu(Et)_3N^+$
TEMPO	Tetramethylpieridinyloxy free radical
TEOC	2-(trimethylsilyl)ethoxycarbonyl
TES	Triethylsilyl
Tf	Trifluoromethanesulfonyl (triflyl)
Tf(OTf)	Triflate $–SO_2CF_3(–OSO_2CF_3)$
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
thexyl	1,1,2-Trimethylpropyl
THF	Tetrahydrofuran
THP	Tetrahydropyran-2-yl
TIPDS	1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl
TIPS	Triisopropylsilyl
TMEDA	*N*,*N*,*N*′,*N*′-tetramethyl-1,2-ethylenediamine
TMG	1,1,3,3-Tetramethylguanidine
TMS	Trimethylsilyl (tetramethylsilane)
TMS N_3	Trimethylsilylazide

Tol	4-Methylphenyl
Torr	1 mg Hg, 1/760 atm
TPAP	Tetra-*n*-propylammonium perruthenate
TPS	*tert*-Butyldiphenylsilyl
Tr	Triphenylmethyl (trityl)
Triton B	Benzyltrimethylammonium hydroxide
Ts (Tos)	Tosyl, 4-toluenesulfonyl
TS	Transition state
T_R	Retention time in chromatography
UV	Ultraviolet
X_c	Chiral auxiliary

Common Latin Abbreviations

De novo (L)	A new, a fresh, again from the beginning
et al. (L)	And others
Ibid (Ibidim L)	In the same book, Chapter, pages etc.
Idem (L)	The same as previously given
infra	Below
in situ	Existing place or position
in vitro (L)	Controlled experimental environment rather than within the living system
in vivo (L)	Made to occur within a living system
modus operandi (L)	Mode of operation of working
supra	Above
tincture	Medicinal solution of drug in alcohol

For more terms see Appendix C, item (**9**)

LIST 2

Compound Names

At the beginning of sentences or in headings, the **first** letter of the chemical name after the prefix is capitalized.

Plant Names in Italics

See Sect. 4.1.2, p. 245 for details

Element Symbol Locants in Italics

N,*N*′-dimethylurea
N-ethylaniline
O,*O*,*S*-triethyl-3*H*-fluorene

Positional and Structural Prefixes in Italics

o, *m*, *p*, *n*, *1°*, *2°*, *3°*, *sec*, *t*, *tert*, *peri*

Configurational Prefixes in Italics

(R), *(S)*, *(Z)*, *(E)*, *cis*, *trans*, *cisoid*, *transoid*, *rel*, ***d***, ***l***, *meso*, *sn*, *endo*, *exo*, *sym*, *syn*, *anti*, *amphi*, *erythro*, *threo*, *altro*, *ribo*, *xylo*, *vic*, *gem*, *Pref*, *Parf*

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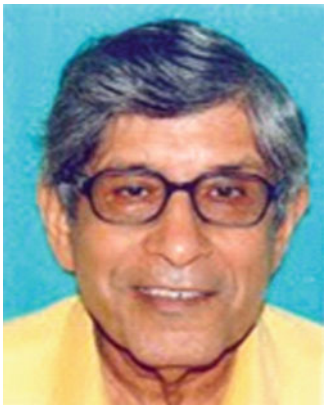
About the Authors



Sunil Kumar Talapatra's scholastic association with his mentor, a renowned natural products chemist Professor (Mrs.) Asima Chatterjee at the Calcutta University (CU) for over 6 years, and with an outstanding organic chemist Professor Michael P. Cava at the Ohio State University (OSU), USA, for over 4 years and his close association with Professor M. S. Newman at OSU served as inspiration for him to pursue the career of teaching and fundamental research. During his active career as Lecturer (1965–1971), Reader (1971–1975), and UGC Professor of Chemistry (1975–1998), CU, he taught stereochemistry, conformational analysis, reaction mechanism, and alkaloids in the postgraduate classes of CU and Presidency College (now University), Kolkata, for more than three decades and has been teaching stereochemistry for the last three decades in some other universities of W. Bengal and Tripura as a Guest teacher. He has been a pioneer in teaching reaction mechanism in CU and stereochemistry and conformational analysis in the country. His research contributions include the discovery and structure elucidation of more than a hundred new bioactive secondary metabolites, mostly alkaloids, terpenoids, polyphenolics, etc., of different classes and cardiac glycosides from medicinal plants and one new C_{26} tetracyclic diamine from a marine sponge (with Professor P. Crews at UCSC). In 1968 he proclaimed for the first time from chemical and model studies a double skew-boat conformation of rings D/E of multiflorenol and friedelin (confirmation appeared in the literature much later in early 1980s by X-ray studies) and a skew-boat conformation of ring E in bauerenol. His more than two dozens of PhD students and several postdoctoral fellows have served in universities, IITs, research institutes, and colleges and many of them have carried out high standard research works. He published more than 175 papers, is a recipient of Premchand Roychand Scholarship

(CU) and Fulbright Travel Fellowship, and was selected for an 1851 Exhibition Scholarship of UK (but did not avail it since he was already offered a postdoctoral fellowship of OSU by Professor Cava). He extensively visited many major laboratories, universities, and institutions of USA, UK, Europe, Japan, Singapore, Bangkok, Hong Kong, Seoul, Kuala Lumpur, Bandung, Hanoi, Karachi, Kathmandu, Colombo, etc., on Exchange Programs, as a British Council Guest Visitor, as the FACS General Assembly Member (1981–1987), and as Executive Committee Member (1981–1983 and 1987–1988) from India, for International Conferences, participated in more than 30 International Conferences, and lectured on his research findings. As a Visiting Scientist he was associated with Professors N. R. Farnsworth and G. A. Cordell (Illinois U, Chicago) (19740, P. Crews (UCSC) (1995) and T. C. McMorris (UCSD) (2000) for 3 months at each place and lectured. As a UNESCO Senior Visiting Scientist in 1982, he was associated with Professors Koji Nakanishi (Columbia U), J. Meinwald (Cornell U), E. J. Corey (Harvard U), H Rapoport and C. H. Heathcock (UC Berkeley), N. Takahashi and K. Mori (Tokyo Agri. U), and Y. Kanaoka (Hokkaido U) for 2 weeks at each place. He has been elected C.Chem., FRIC (London) in 1974, President of the Indian Chemical Society (2002 and 2003), Vice President, Indian Science News Association (2008-), and an Ex officio Vice President, Indian Chemical Society since 2004, and was a recipient of Mouat Gold Medal of the CU. He is interested in photography, visiting museums and art galleries, watching cricket, tennis, and football, and reading books, and he loves traveling and making friends.

Bani Talapatra Professor of Chemistry (1983–2002), Calcutta University (CU), taught natural products chemistry covering their all aspects in the postgraduate classes for more than three decades and in some other universities of W. Bengal and Tripura as a Guest teacher for more than a decade. She also taught general chemistry in a local undergraduate college for several years during her early professional career. She obtained the D.Sc. degree of the CU working in the laboratory of Professor (Mrs) Asima Chatterjee. She was offered the Smith-Mund Scholarship by USEFI and also a Postdoctoral Fellowship at the Vanderbilt University (VU) by a renowned microbiologist, Professor V. Najjar, and she accepted the latter offer. At VU very fortunately she came in personal contact in 1964 with Sir Christopher K. Ingold, who was completing there the second edition of his famous book "Structure and Mechanism in Organic Chemistry." She learnt from Professor Ingold much about the fundamental concepts on organic reaction mechanism. As a UNESCO Senior

Visiting Scientist (in 1976 and 1983) she extensively traveled and visited many major universities and research institutes of USA, Europe, Japan, and South East Asia and was briefly associated with Professors W. Parker (Stirling U), W. Kraus. (Hohenheim U, Stuttgart), F.Bohlmann (Tech. Univ. Berlin), H. Wagner (Munich U), S. Masamune (MIT), W. Herz (Florida State U), H. Rapoport (UC Berkeley), and N. Takahashi (Agri. U, Tokyo). She participated in many international conferences and presented her work. Her research interest includes the various aspects of natural products chemistry with special reference to terpenoids, alkaloids, polyphenolics, and reaction mechanism. She published more than 130 research papers; 15 students obtained PhD degree of CU under her guidance. She received Premchand Roychand Scholarship of CU. She is fond of books, friends, music, paintings, museums, art gallery visits, and traveling. She authored two books, one of poems in her mother tongue Bengali (1998) and the other one mostly a collection of articles and poems (2013).

Chapter 1
Introduction: Enzymes. Cofactors/ Coenzymes. Primary and Secondary Metabolites. Natural Products and their Functions. Plant Chemical Ecology. Biosynthesis. Metabolic Pathways

The evolution of natural products (secondary metabolites) in plants, their functions, environmental interactions with living organisms, and some participating components involved in these dynamic biological processes are briefly presented in this chapter.

1.1 Nature. Life. Cells. Molecules. Self-Replication

Nature is the mysteriously integrated network of incredibly simple to immensely complex phenomena that are vastly unknown and very little known and will remain forever in the thoughts and quests of scientists and philosophers as the childhood rhyme, "How I wonder what you are!" [1].

In absence of any definitive evidence, it is believed that ours is the only planet that is blessed with the supreme gift of Mother *Nature*, the life often referred to as carbon–liquid water life [2] enjoying the environmental conditions of this planet. Anything living should grow, respond to external stimuli, be capable of self-replication, and eventually die; and for that, all living organisms need to do an enormous variety of actions. These biological actions include reactions within organisms by way of assimilation, synthesis, transformations, interconversions, etc. involving the participation of simple to complex organic molecules and thus within the cell "... these diverse materials can take part in the amazing dance that we call life" [3]. In biochemical as well as energy sense, a continuous process of bond breaking and bond formation is going on during the biological processes, as long as life is there. And as a consequence, an enormously large number of simultaneous, parallel and recurring chemical events are going on too. Thus, the *fundamental biological processes of life involve* (1) the *biosynthesis* of small molecules and their further conversion to complex molecules (*anabolism*), (2) *self-replication* by which life is sustained through generations, and (3) *catabolism*, i.e., the breaking down of molecules into smaller ones [4]. These reactions proceed with profound stereospecificity and at enormously rapid rates in the cells.

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"The cell has been compared to many things, from 'a complex chemical refinery' (by the physicist James Trefil) to 'a vast teeming metropolis' (the biochemist Guy Brown). A cell is both of those things and neither. It is like a refinery in that it is devoted to chemical activity on a grand scale, and like a metropolis in that it is crowded and busy and filled with interactions that seemed confused and random but clearly have some system to them" [5].

1.2 Enzymes [6–16]

1.2.1 Nature of Enzymes

Enzymes are highly evolved biological catalysts that virtually catalyze almost all metabolic reactions with high specificities at ambient temperature at very rapid rates (with few exceptions, e.g., *rubisco,* see Chap. 3). Jack bean urease was the first enzyme to be recognized as a protein, which was crystallized in 1926 by James B. Sumner (NL 1946). It was shown to catalyze the hydrolysis of urea to carbon dioxide and ammonia [9]. Sometimes, they also serve as targets for useful therapeutics (Chap. 5, footnote 1). Enzymes are natural proteins, made up of 20 amino acids {of L-configuration at the α-carbon atom (save achiral glycine) carrying both NH_2 and COOH groups, see Appendix C item (7)}, and *obviously, exist in only one enantiomeric form*; thus, their optical antipodes do not occur in nature. They elaborate their primary structures by way of sequencing the amino acids. Ribonuclease A (having 124 amino acids, molecular mass 13,680 Da) is the first enzyme to be sequenced by Christian B. Anfinsen, Stanford Moore, and William. H. Stein in 1960. They shared the Nobel Prize in 1972 for the methodology they developed for this purpose and for their contribution to the chemistry of enzymes. The other aspects of enzyme structures [6, 7, 16] include the tetrahedral configuration of sp^3 carbons, planar amide (–CO–NH–) bonds, appropriate stereochemically comfortable hydrogen bondings, hydrophobic character, and various elements of protein folding, playing chemical origami. The ability of polypeptide chains to fold into a great variety of topologies, combined with the enormous variety of sequences that can be derived from 19 different L-amino acids and achiral glycine (Appendix C), confers on proteins their prowess of recognition and selectivity of catalysis, and of specificity as structure forming elements. With all the armory currently available in experimental and theoretical methods, we still cannot predict reliably or even comprehend rationally how the sequence of amino acids in a given polypeptide chain would determine and control the ultimate spatial arrangement of the protein.

1.2.2 Functions of Enzymes

Catalysis and specificity are the central tenets of enzymatic reactions, i.e., "stereospecificity is inherent in the catalytic actions of enzymes, and that enzymes would not be equally efficient as catalysts of nonstereospecific reactions" [7]. Enzymes can have molecular masses of several thousands to several million daltons; still they can catalyze conversions on molecules as small as carbon dioxide and nitrogen. At least 21 different hypotheses for the mechanisms of enzyme-catalyzed reactions have been proposed [9, 10]. It is an accepted proposition that an enzyme recognizes the substrate and catalyzes the reaction by formation of an *enzyme–substrate* (or *E–S*) *complex*. In general, an enzyme accelerates the reaction rate by lowering the transition state energy and thus decreasing the free energy of activation. They provide the reaction surface and suitable environment for bringing the reactants together in a favorable configuration to attain the transition state (TS) and weaken the bonds of the reactants. The loss of entropy on binding the enzyme is compensated by the favorable enthalpic interactions; the substrates come in the productive alignment from the randomly moving individuals; thus, though a considerable part of the entropy of reaction is removed, a favorable situation of intramolecular reaction results [10]. The enzymes may also raise the ground state energy (*cf.* steric acceleration). The structure of the TS of an enzyme catalyzed reaction cannot be detected by any spectroscopic method. The reasons for the specificities of enzymes as well as their profound catalytic power are not fully understood. Enzymes, being composed of only 20 amino acids, pose a limited repository of functional groups. Thus, enzymes by themselves, with their limited built-in resources, are quite often incapable of performing complex biochemical reactions entrusted upon them by the need of the complexity of living organisms.

1.2.3 Enzyme Classification and Nomenclature [12–14]

The name of an enzyme reveals the type of reaction it catalyzes. The names of enzymes usually carry the suffix "ase," while proteolytic enzymes, e.g., pepsin, chymotrypsin, trypsin, etc. are exceptions. Thus, an oxidase enzyme catalyzes an oxidation reaction. It should be noted that enzymes can catalyze the forward and backward reactions of an equilibrium reaction. An oxidase enzyme can catalyze oxidations as well as reductions, depending on the nature of the substrate, i.e., whether it is in the reduced or oxidized state. So, this type of enzymes may be classified as oxidoreductases (Class 1, stated below).

Enzymes are classified according to the general class of the reaction they catalyze and are coded with an EC number [12]. EC stands for Enzyme Commission, a body set up by the International Union of Biochemistry. In the system of nomenclature recommended and accepted, each enzyme is designated by a *four*

digit number separated by dots. The *first digit* indicates the main class to which the enzyme belongs, and, *spells out the function the enzyme performs, viz*.

1. *Oxidoreductase* (oxidase/oxygenase/dehydrogenase/reductase) (causing oxidation or reduction)
2. *Transferase* (causing transfer of functional groups like acyl, glycosyl, etc. or one-carbon compound)
3. *Hydrolase* (causing hydrolysis)
4. *Lyase*
5. *Isomerase* (causing isomerization and intramolecular group transfer)
6. *Ligases* (*Synthetases*)

Thus, the names of the classes excepting *lyase* and *ligase* indicate the functions they perform. A *lyase* effects removal of a group (not by hydrolysis) from the substrate leaving a double bond. Conversely, an enzyme which adds a group to a double bond is also a lyase. As stated above, enzymatic reactions are reversed depending upon the substrate, although the same name is given to the enzyme, which sometimes is confusing. A *ligase* catalyzes the joining of two substrates, where energy is provided by the hydrolysis of ATP, etc. (see Chap. 3). *Ligase* is also classified as *synthetase* or *synthase*, causing synthesis.

The second digit constitutes a subclass, since it elaborates the class by specifying the substrate or reaction. The third digit or subsubclass gives further details of the reaction. The fourth digit is a specific number allocated to only one enzyme. Thus, there may be several enzymes with the same first three figures but a different fourth figure. However, these codes for enzymes will be understood only from a Table of the codes of classes, subclasses, and subsubclasses, and hence the codes are not handy. The interested reader may consult reference [13] for the complete rules of enzymatic nomenclature. Generally, the simplified way to classify an enzyme is to *spell out the function indicated by the first figure*. Some other specific functions of enzymes, which may be included in one of the six main classes (first digit) mentioned, are usually expressed by the specific names, e.g., a *cyclase* effecting cyclization, a *kinase* effecting phosphorylation, a *carboxylase* affecting carboxylation, a *transaminase* effecting transfer of amino group, etc. Further, these functional words, which fall in class 1, are tagged with the substrate/product to confer specificity. Some examples of nomenclature of enzymes in vogue are *phenylalanine transaminase*, *tyrosine aminotransferase, acetylcoenzyme synthetase, hydroxyphenyl-pyruvate reductase, squalene:hopene cyclase*, *taxadiene synthase, strictosidine synthase, geraniol 10-hydroxylase, secologanin synthase, abietadiene synthase, chorismate lyase, 4-amino-4-deoxychorismate lyase, anthranilate-CoA ligase, etc*. Sometimes, a single enzyme can perform more than one function, e.g., *rubisco* the most abundant enzyme on this planet, functions both as a *carboxylase* and as an *oxygenase* (see Chap. 3).

1.3 Cofactors/Coenzymes

Enzymes are Nature's catalysts. The catalytic activity of many enzymes depends on the presence of small organic molecules, or metals or, metal ions. They are termed **cofactors** (Chap. 3). They are Nature's version of common organic reagents. Their precise role varies with the enzyme. In general, these cofactors are able to perform complex biochemical reactions by activating the enzymes. An enzyme without its cofactor is called *apoenzyme*. The apoenzyme along with the cofactor becomes catalytically active and is called *holoenzyme*. Some coenzymes possess innate catalytic activity.

Cofactors can be divided into two groups: (1) elemental cofactors, e.g., polar metal ions like K^+, Mg^{++}, Ca^{++}, etc., transitional metal ions like Fe^{++}/Fe^{+++}, Ni^{++}, Cu^{+}/Cu^{++}, Zn^{++}, and metals like Mn, Co, Se, Mo, etc. and (2) small organic molecules called **coenzymes** (mostly derived from vitamins), e.g., *coenzyme* A (CoA), *nicotinamide adenine dinucleotide* (NAD^+) and *its hydride* (NADH), *nicotinamide adenine dinucleotide phosphate* ($NADP^+$) and *its hydride* (NADPH), *flavin adenine dinucleotide* (FAD+), *thiamine pyrophosphate(diphosphate)* (TPP/TDP), *pyridoxal phosphate* (PLP), *adenosine triphosphate* (ATP), *biotin, tetrahydrofolate*, etc. A tightly bound (covalently bonded) coenzyme is called a *prosthetic* group, while a loosely associated (noncovalently bonded) coenzyme behaves like a *cosubstrate*; like substrates and products, they bind to the enzyme to come to a compatible conformation and are finally released from it. Enzymes that use the same coenzyme usually execute catalysis by similar mechanism.

Arthur Harden (1865–1940, an English biochemist, shared 1929 Nobel Prize with Hans August Simon von Euler-Chelpin of Germany) first recognized the existence of coenzymes and their assistance to coenzyme-dependent enzymes [16, 17]. Statistics suggests that the coenzyme-dependent enzymes are more in number than the enzymes capable of doing work by themselves. The involvement of some coenzymes in various enzymatic reactions has been discussed in Chap. 3 and other chapters in appropriate cases.

In summary, we quote Birch [15], "Enzymes selectively assemble molecules (often chemically activated by combination with coenzymes), arrange them structurally, and promote, sterically and chirally, bond-forming processes within an asymmetric molecularly engineered cavity." In essence, enzymes catalyze reactions that cells need to live and replicate. *In the Nature's laboratory and in a chemical laboratory, the same chemistry exists. The difference is that Nature is superb and unparallel at chemistry (e.g., biosynthesis), and chemists are only learning little bit and trying to emulate to some extent even at this 21st century. The name enzyme was given by Kuhne in 1878/1881 to some active components present in yeast which caused fermentation. The two words* ***in yeast*** *are expressed in* ***Greek as en(in) and zyme (yeast)*** *and hence the name.*

1.4 Metabolism. The Vital Biological Processes. Primary Metabolites

Each organism is carrying out within itself an integrated network of enzyme-catalyzed cellular reactions, sum total of which is regarded as *metabolism*. It includes anabolism, reactions concerned with an increase in molecular complexity, and catabolism, a reverse process, i.e., reactions concerned with molecular degradation. However, the extent of such network varies from one organism to another because of the differences in the diversifications and individual characteristics. The paths of these reactions constitute *metabolic pathways*. But, whatever may be the diversification—the mode of formation of the most important and basic chemical ingredients of life, *viz.,* proteins, fats, nucleic acids, carbohydrates, etc. follows more or less the paths where a fundamental commonality is observed. They are formed from a small number of fundamental units (*building blocks*), which, in turn, are formed by *photosynthesis*, directly or indirectly.

Of all living organisms, plants are most efficient in elaborating organic compounds with superb molecular diversity, initiated by the pivotal natural synthetic tool *photosynthesis* (Sect. 3.1), by which light energy of the sun is utilized by the green plants to produce organic compounds from carbon dioxide in presence of water. The initial products of photosynthesis are carbohydrates which upon subsequent metabolic alterations give rise to a series of simple and universally distributed organic compounds of low molecular weights, like common sugars and sugar derivatives, the 20 or so amino acids that make the majority of the proteins, and the low molecular weight aliphatic carboxylic acids which form common fats and lipids. These small molecules are needed for sustaining life and are present in almost all living systems, and hence they are known as *primary metabolites*. In plants they all have the same metabolic functions. The pathways involved in their formations and transformations during anabolism and catabolism are described as *primary metabolic pathways* and constitute the *primary metabolism* of plants. Unlike the plants, the animals and the microorganisms derive these raw materials from their food and diet, and hence animals develop mechanisms for locomotion and perception of external objects.

1.5 Metabolism. Secondary Metabolites (Natural Products)

There is also another area of metabolism that falls outside the domain of *primary metabolism* where simple *primary metabolites* enter into further reactions to form more complex molecules. *These reactions are genetically controlled and enzymatically catalyzed.* The resulting products are of *limited or restricted distribution, i.e., unlike primary metabolites* they are found to occur in certain groups of organisms or plant genera and species only. These compounds are classified as

secondary metabolites and the pathways involved in their formation fall in the domain of *secondary metabolism*.

> "The term 'secondary metabolites' was coined by the biochemist Albrecht Kossel in 1891 to characterize cell components that contrast with 'primary metabolites', and are not found in any developing cell. The former term was adopted by Friedrich Czapek in his book *Biochemistry of Plants* and has been used ever since." [18]

Incidentally, it may be mentioned that Christophersen [19] commented, "An exact definition of primary and secondary metabolites is not very useful since it is deemed to be artificial. . . .From a purely formal point of view this distinction is meaningless and best abandoned."

In view of the above comment, the present authors opine that such definition as well as classification helps in harmonizing the chemical chaos of Nature. It serves as the useful working proposition for understanding the purpose of primary and secondary metabolisms and evokes serious interest to unveil the cryptic functions of secondary metabolites, which are distinctly different from those of primary metabolites.

1.5.1 Natural Products

The secondary metabolites are termed by the chemists as *"natural products"* to distinguish them from those compounds which traditionally have been the province of biochemists. They are unique in the sense of being of restricted occurrence and are more characteristic of specific botanical sources (i.e., a family or a genus and sometimes a single species of plants). Most of the natural products, usually of relatively complex structures, possess biological activities. Many of them find their way to the parlor of pharmaceuticals (Chap. 33) and also are used as insecticides, pesticides, plant growth hormones, etc. Their interesting structural variations, stereochemical configurations, conformations, molecular shapes, etc. claim the academic attention of chemists all over the globe.

1.5.2 Biomacromolecules

It should be pointed out that *plants elaborate the vital life-essential biopolymers like proteins, nucleic acids, polymeric carbohydrates, lipids,* etc. Though *like primary metabolites* they occur in all living organisms, but unlike the same they possess complex structures—a characteristic of many *secondary metabolites*. But *unlike secondary metabolites* they are not of restricted occurrence, and the purpose of their formation by the organisms is well understood. Hence, they fall outside the conservative definitions of both primary and secondary metabolism. They have their own domain—a defused overlapping area of metabolism that cannot be

associated with either primary or secondary metabolites, as long as the conservative definition of the latter is in vogue.

However, all metabolic products may be looked upon as biological entities whose origin, function, transformation, and fate constitute a vast unlimited area of research toward our understanding of their purpose in the living systems.

1.6 Functions of the Natural Products: Chemical Ecology—Plant Chemical Ecology

The functions of the secondary metabolites in the organisms producing them are yet to be deciphered in most cases. However, in some cases, the functions have been revealed through observations and experiments.

1.6.1 Chemical Ecology

Chemical ecology means chemically mediated interactions between organisms and their living (biotic) and nonliving (abiotic) environments [18]. It includes a broad range of *chemical interactions/communications and signaling processes as follows:*

(a) Chemical defenses of organisms, plant defenses against herbivores
(b) Chemical communication with insects and plant–insect interactions (*pheromones, ecdysones, and pollination*)
(c) Mutualistic interactions of plants and fungi (*endophytes*)
(d) Plant–plant interactions (*allelopathy*)
(e) Plant–microorganism interactions (*phytoalexins)*
(f) Natural products and human being

Ecological factors or geographical parameters (e.g., altitude, season, climate, etc.) influence the nature, yields, and the chemical ecology of the secondary metabolites (Sect. 4.1.2). The above interactions involving essential ecological role of natural products may be regarded as *chemical ecology of natural products or plant chemical ecology*. Such interactions will be briefly discussed with a few illustrations. *However, this classification is not mutually exclusive; there is some overlapping*. The pioneering work of A. Kerner (1831–1898), L. Errera (1858–1906), and E. Stahl (1848–1919) on the ecological role of plant secondary metabolism in interactions between plants and a mostly hostile environment finally seems to be broadly accepted [18]. These interactions reveal enormously amazing facts. The chemical ecological research is now regarded as an important area of natural products research, since it is possible at present to isolate and do the structures of the responsible secondary metabolites even in microgram scales using the available

sophisticated instruments. The intensive works of Schildknecht, Meinwald, Rembold, Towers, and others have significantly enriched the chemical ecology area.

1.6.2 Chemical Defenses of Organisms. Plant Defenses Against Herbivores

Plants elaborate natural products for their own benefit. Kerner emphasized the importance of chemical defenses for plants' survival [18].

1.6.2.1 Antifeedants. Repelling Insects

The bitter principles produced in some Meliaceae plants (e.g., *Melia azadirachta, neem*/*nim*) might repel the insects and protect the plants. Some toxic materials produced in certain organisms prevent the attack on them by *predators*. Many secondary metabolites act as *antifeedants to insects*, i.e., they inhibit feeding but do not kill the insects directly and the insects perhaps die of starvation [20]. Azadirachtin, a complex terpenoid constituent of *Azadirachta indica* (syn. *Melia azadirachta*) (neem/*nim* tree) (Fam. Meliaceae), and polygodial (*Polygonum hydropiper*) (Fam. Polygonaceae) (Fig. 1.1) are perhaps *two most promising antifeedants of* plant origin, known till date [20]. In case of polygodial, the enedial functionality probably blocks the insect chemoreceptor by reacting with an amino group to form a pyrrole derivative. The homodrimanes (**I**) and (**II**), synthesized from the diterpene (−)-sclareol, showed significantly more antifeedant activity, perhaps since they may interact with an amino group of the insect chemoreceptor more strongly yielding pyridine derivatives [21]. Interestingly, it has been mentioned that if the predator eats the opium plant/exudate, it will soon feel drowsy and will not be able to do any further harm to the plant.

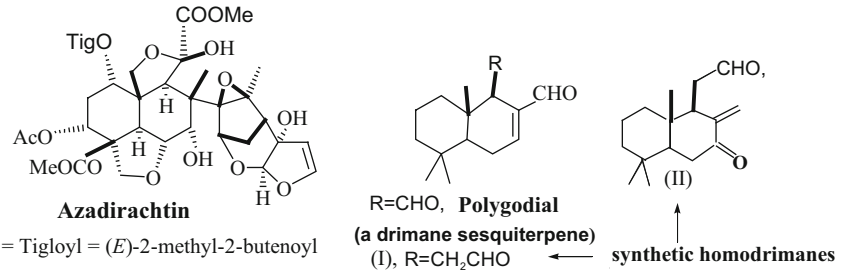

Fig. 1.1 Antifeedants azadirachtin and polygodial, and two synthetic homodrimanes

1.6.2.2 Wounded Plants Emitting Prussic Acid

To protect themselves from herbivores many plants, when wounded by animals emit defense chemicals. Almost 10 % of all plants use *prussic acid* (HCN), a very potent poison, as defense against being eaten by animals. It is an inhibitor of cytochrome *c* oxidase affecting the final step in the respiratory electron transport chain and is present widely in plants in the bound form as *cyanogenic glycosides* [22, 23]. *When wounded, the compartmentalization of the glycoside and the enzyme glycosidase gets disturbed and their contact results in the hydrolysis of the glycoside liberating prussic acid* [22, 23] (Fig. 1.2). Many cyanogenic glycosides have been isolated from several flora, most of them are O–β-D-glucosides. The old name of hydrocyanic acid is prussic acid since it was prepared by Scheele in 1782 by heating Prussian blue(ferric ferrocyanide) with sulfuric acid.

1.6.2.3 Wounded Plants Emitting Volatile Mustard Oils

Likewise, an isothiocyanate, R–N=C=S, poisonous at high concentration, is generated when radish, cabbage, and mustard plants, containing a thioglycoside, *glycosinolate,* also named *mustard oil glycoside*, are wounded; then the thioglycoside comes in contact with the enzyme *thioglycosidase,* located in separate compartments of the plant tissues and gets hydrolyzed and rearranged to form the isothiocyanate [22, 23] (Fig. 1.3). When cells of these plants get damaged, the pungent smell of mustard oil is detected indicating the formation of the isothiocyanate. Several dozens of glycosinolates are known to occur in Capparaceae and Cruciferae families. Most of them are S–β-D-glucosides.

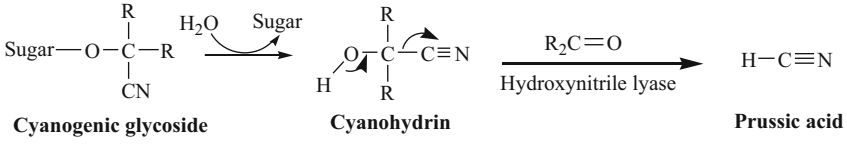

Fig. 1.2 Enzymatic hydrolysis of cyanogenic glycoside to prussic acid (R=Me); two substituents may be different, e.g., Me, Et; Ph, H (both enantiomers); *p*-hydroxyphenyl, H (both enantiomers). Most of them are O–β-D-glucosides

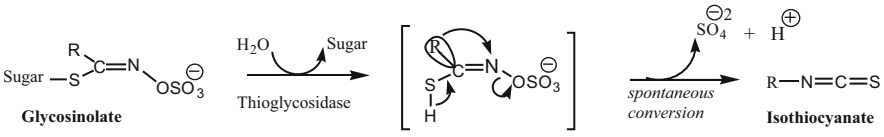

Fig. 1.3 Enzymatic hydrolysis of glycosinolate to isothiocyanate (R=Me, 4-oxo-n-heptyl, indolyl-3-methyl, CH_2=CH–CHOH–, EtC(Me)(OH)CH_2−). Most of them are *S*–β-D-glucosides

1.6.2.4 Plants Toxic to Animals

A number of secondary metabolites also provide plants protective means from herbivores. Ruminants (e.g., cows, buffaloes, and other animals living on plants) generally do not browse plants containing alkaloids. For example, pyrrolizidine alkaloids present in some Leguminosae (*Crotalaria* species) and Boraginaceae (*Heliotropium* species) plants cause severe liver damage. They are hepatotoxic. They have attracted a lot of attention because of the heavy loss of livestock in many countries. Even human food (cereal grains) contaminated with the seeds of these plants, when consumed, may cause food poisoning and liver damage.

Molded (fermented) sweet clover (*Melilotus officinalis*, Leguminosae) contains the toxic principle dicoumarol (**1**) (a bis-4-hydroxycoumarin derivative) (Fig. 1.4) with pronounced blood anticoagulant properties which can cause the death of livestock (e.g., cows) by severe internal bleeding (hemorrhage). Dicoumarol interferes with the effects of vitamin K in blood coagulation. This observation led to the synthesis of some medicinal anticoagulants, having 3-substituted-4-hydroxycoumarin structures, e.g., warfarin (Sect. 13.2, Chap. 33) used in thrombosis. Kerner realized that plants toxic for some animals may not be toxic for others, e.g., *Belladonna* berries are toxic to ruminant animals but harmless to many birds [18].

1.6.2.5 Plants Deceiving Herbivores with False Amino Acids

Many plants elaborate unusual amino acids, e.g., (*S*)-L-canavanine (**2**) (Fig. 1.4) (occurring in *Canavalia ensiformis, jack bean)* which is a structural analogue of (*S*)-L-arginine (**3**), a protein building amino acid. Herbivores consume canavanine from their food. This false amino acid cannot be distinguished by the arginine-transfer RNAs of those herbivores during protein biosynthesis. The wrong incorporation changes the desired three-dimensional structure of proteins

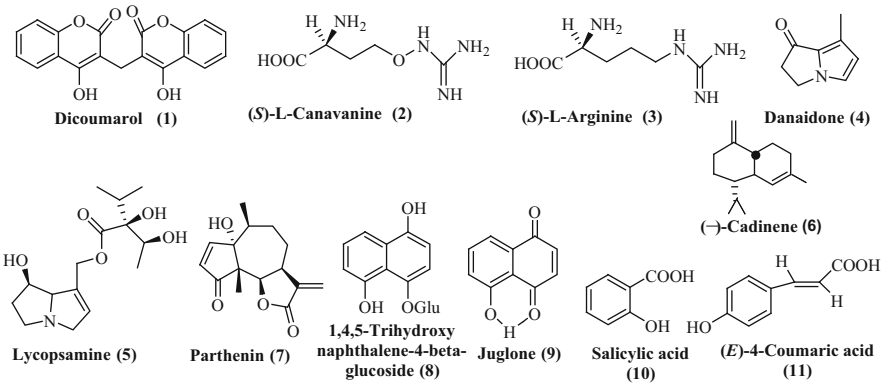

Fig. 1.4 Some natural products involved in plant chemical ecology

which lose their biological function partially or completely, and hence become toxic to those herbivores. The arginine-transfer RNAs do not react with canavanine in plants which synthesize it, so it is nontoxic for such plants. Plants contain many false amino acids, which are toxic for herbivores in an analogous manner.

1.6.3 Chemical Communication with Insects and Plant–Insect interactions

1.6.3.1 Formation of Courtship Pheromones

Chemical interactions between plants and insects constitute a type of signal exchange. Animals or insects of the same species quite often release some secondary metabolites or chemicals, broadly known as *pheromones*, which serve to influence the physiology and behavior of other members of the same species so as to attract them and effect courtship. While feeding on plants some insects receive the precursor chemicals of their courtship pheromones. For an example, pyrrolizidine alkaloids occurring in the *Crotalaria* (Leguminosae) and *Heliotropium* (Boraginaceae) plants, though poisonous to livestock as stated above (see Sect. 1.6.1.4), are useful as precursor pheromones to some insects such as danaid butterflies and arctiid moths. The dual role of these plant alkaloids as defensive chemicals and as the precursors of male courtship pheromones in danaid butterflies and arctiid moths have been thoroughly studied by Meinwald [24–27] and coworkers during several decades. They isolated a heterocyclic ketone danaidone (**4**) (Fig. 1.3) from the hairpencils' secretion of Trinidad butterfly, *Lycorea ceres*. The ketone is the courtship pheromone in the Florida queen butterfly (*Danaus berenice*). It was found to be *biosynthesized* in African monarch, *Danaus chrysippus* from the pyrrolizidine alkaloid lycopsamine (**5**), delivered to their system from the plant food *Heliotropium steudneri*. This observation establishes a deep relationship between arctiid moth and its *Crotalaria* plant food elaborating the pyrrolizidine alkaloid that the moth thus utilizes on becoming an adult butterfly in making the courtship pheromone.

1.6.3.2 Formation of Ecdysones (Molting Hormones)

Insects require ***ecdysones*** *having molting hormone properties for their larval development and metamorphosis*. Biosynthetically, ecdysones are derived from steroids (Sects. 11.2 and 11.3). However, insects are unable to synthesize steroids. They use the plant food as the source of ecdysones. Since ecdysones are not widely elaborated by plants, they use more abundantly occurring plant steroids (e.g., sitosterol, stigmasterol, etc.) as well as less abundant cholesterol from plant food as the precursors of ecdysones biosynthesis. The structural features 14*a*-OH,

a,β-unsaturated ketone system and *cis*-A/B rings are responsible for their bioactivity. The involvement of secondary metabolites in plant–insect interactions, their utilization as signaling agents, the biosynthetic conversion of a plant alkaloid to an insect sex pheromone, and of plant steroids to molting hormones and various other interesting findings are significant extensions of natural products chemistry research.

1.6.3.3 Pollination

The beautiful colors of the flowers, caused by the presence of flavonoids, anthocyanins, etc. (Chap. 14) and other pigments *and their smells* (due to monoterpenes like geraniol, linalool, β-ionone, etc.; aromatic compounds like vanillin, eugenol, etc.; aliphatic compounds like isooctanol; various amines; etc.) and nutritious exudates (e.g., honey*)* might attract the insects like bees, butterflies, flies, etc. to help in the *pollination*—the first step in the fertilization in flowering plants. This step is performed unknowingly by all these visitors (including birds and bats) collecting male gametes from the flowers while in search for food in nectar and pollens. Different pollinators are attracted to different colors, e.g., butterflies like bright colors, while flies prefer green and brown colors. These colors are due to various plant pigments belonging to the classes of secondary metabolites, which include quinones, chalcones, flavonoids, anthocyanins, carotenoids, etc., to be discussed in appropriate chapters. Pollinators visit the same plant species as they are capable of distinguishing the species.

Interestingly, it may be mentioned that the fragrance of *Ophrys* flowers (orchids) resembles the odor coming from the odor glands of *Andrena* female bees. Further, one of the components of the flower fragrance, identified as (−)-cadinene (**11**) (Fig. 1.4), has been the sex pheromone of the female bees. The deceived *Andrena* male bees are attracted, and this attraction results in the pollination.

1.6.3.4 Plant–Insect Interactions: Another Example

Insects are sometimes attracted by the nutritious exudates of some plants. Many ants use the nutritious exudates of some *Acacia* species as their food and in turn protect the host plants from herbivores by forming ant colonies on them. If the ant colonies are removed from the plants, the herbivores damage the plants. This proves the mutualistic beneficial relationship between insects and plants. Some *Acacia* species, where ants do not form colonies to protect the plants from herbivores, produce cyanogenic glycosides, toxic to herbivores (see Sect. 1.6.1.2).

Both pollination (Sect. 1.6.2.3) and plant–insect interaction (Sect. 1.6.2.4) stated above may be grouped under mutualistic interactions of plants and insects, since they both are benefitted. In case of the former, propagation of plants is caused while the insects, etc., are getting their food.

1.6.4 Mutualistic Interactions of Plants and Fungi: Endophytes [28]

1.6.4.1 Endophyte Fungus and Host Plant

Endophyte refers to a situation when one organism lives inside another organism. Endophyte fungus is a beneficial fungus and holds a symbiotic relation with the host plant in the cells of which the former grows. When it feeds on the host plant, it produces toxic chemicals (e.g., alkaloids) that protect the host plants from environmental influences (e.g., pests, diseases, etc.). Thus, in this phenomenon, the endophyte fungus can grow and at the same time the host plant gets a natural protection. Hence, a mutualistic beneficial relationship exists.

An example: the turf grass has a built-in protection provided by some specific endophytes, present in high percentage in the seeds. It protects the turf grass from the pests eating the surface of the leaves, and sometimes enhances drought tolerance; chemical pesticides are not needed to be added. Endophytes are transferred from plant to plant through seeds. Many perennial rye grass and tall fescue varieties (*Festuca* genus) contain endophytes. There are specific endophytes that can be used for forage grasses, which produce alkaloids toxic to pests and not to livestock.

An important as well as interesting study revealed that the endophytic novel fungus *Taxomyces andreanae* isolated from the inner bark of a yew tree (*Taxus brevifolia*) growing in northwestern Montana elaborates, though in small amount, the same compound taxol as its host plant [29]. Taxol is an extremely important drug for cancer. Hopefully, microbial synthesis of taxol [30] will help in procuring the drug (Sect. 8.4). Likewise, many endophytic fungi have been shown to produce a number of interesting biologically active natural products like their host plants. Endophytic fungus can thus potentially serve as an alternative source for the bioactive natural product/s.

1.6.5 Plant–Plant Interactions: Allelopathy [31]

The chemical communicative phenomenon between some plants where their chemical constituent/s (natural product/s) disallow or inhibit the growth of other plants in their vicinities is termed as allelopathy. The phenomenon takes place through the soil. *Parthenium hysteroporus* (Asteraceae), a troublesome weed of India and notorious for its virulent growth, contains a sesquiterpene lactone, parthenin (**7**) (Fig. 1.4), identified as a growth inhibitor for other plants. The leaves of the walnut tree, *Juglans nigra* (Juglandaceae), elaborate juglone (**9**) (=5-hydroxynaphthaquinone) and 1,4,5-trihydroxynaphthalene-4-glucoside (**8**). The latter being a glucoside is washed down by rainwater and dew. On coming in contact with the microorganisms in the wet soil, it gets hydrolyzed to the aglycone which is readily oxidized to juglone (**9**). Juglone inhibits the growth of other plants

under this walnut tree. However, some species like Kentucky blue grass, *Poa pratensis* can grow under a walnut tree, showing that such plants somehow neutralize the adverse effect of juglone and can compete for soil nutrients. Some allelopathic agents (allomones) are produced by some plants, e.g., salicylic acid (**10**) by the oak tree, *Quercus falcate*; *E*-4-coumaric acid (**11**) (*E*-4-hydroxycinnamic acid) and some quinones by some plants including the shrubs *Adenostoma fasciculatum and Arctostaphylos glandulosa,* native to southwest California where they inhibit the growth of other shrubs in the neighborhood.

Incidentally, it may be mentioned that parthenins/parthenolides possess fairly good insecticidal properties. They affect the nervous system of the insect. Flowers containing parthenins as the chemical constituents [*Chrysanthemum cinerariaefolium* (Asteraceae)] are processed as insecticidal spray for domestic and agricultural purposes. Parthenins are biodegradable and nontoxic to mammals but toxic to fish and amphibians. Various synthetic parthenins/parthenolides with improved insecticidal properties and decreased toxicity are in use.

The undergrowth in groves of different trees generating allomones varies considerably. The bare belt area around such plants thus differs. *Salvia leucophylla* (mint) and *Artemisia californica* thickets do not allow other herbs to grow in their vicinity. The allomones from these trees have been identified as volatile monoterpenes like camphor and cineol. When these thickets are removed by way of burning, grass and annual herbs start growing on the bare patches. Allelopathic effect is caused by E-cinnamic acid or its derivatives. Allelopathic effect has been studied in vitro with some compounds, which inhibit the growth of some seeds.

1.6.6 Plant–Microorganism Interactions: Phytoalexins [32, 33]

The loss of crops by microbial attack is a global problem and thus a matter of great concern. Much needed intense research in this area revealed that the microorganisms—the pathogens—are host specific; they multiply in the plant tissues and produce toxic metabolism by generating compounds termed as *Elicitors*. *The latter* activate the genes in the plant so as to produce chemicals which are capable of detoxifying the microbial-generated toxins by way of oxidation, glycosylation, or counteractingly by the host–plant-generated defense chemicals. The defense chemicals becomes of maximum concentration immediately after the microbial attack on the host plants. In course of studies on plants exposed to microorganisms and various infected tubers, hundreds of defense chemicals of diverse skeletal patterns have been discovered. These defense chemicals are termed as phytoalexins, e.g., Orchidaceae plants elaborate phenanthrene derivatives, while Compositae plants elaborate acetylenes as phytoalexins.

Plants and Fungi/Bacteria/Pathogens Secondary metabolites often protect plants from pathogenic microorganisms. The latter are provided by fungi and or

bacteria while they are using the plant sources for their nutritional purposes. Varied classes of secondary metabolites comprising *isoprenoids* (Chaps. 6–12), *phenylpropanoids* (Chap. 13), *polyketides* (Chap. 14), and *alkaloids* (Chaps. 15–30) include natural pesticides that protect plants against herbivores and pathogenic microorganisms.

For more information on different aspects of chemical ecology, as discussed in Sect. 1.6, as well as sequestration of natural products by insects, etc. two excellent reviews by J. J. Harborne [32, 33] may be consulted.

1.6.7 Natural Products and Human Being

Many natural products, being remarkably bioactive, serve as the cradle for pharmaceuticals. A number of molecules not only saved many lives in the past but also continue to do so even after the advent of modern day pharmaceutical sciences consisting of various scientific disciplines. Their activities have largely been studied in relation to human benefit or compatibility resulting into the discovery of many important drugs, namely morphine, quinine, reserpine, vincristine, vinblastine, taxol, artemisinin, dicoumarol, camptothecin, etc. (Chap. 33)—to name only a few well-known ones, and various crude drugs as well. However, the bioactivity of most natural products still remains unknown. The occurrence of many natural products in fruits, vegetables, and spices are responsible for our liking the odors and tastes and finally for our health benefit (Chap. 34).

Some poisonous natural products like curare alkaloids (quaternary bisbenzylisoquinoline alkaloids) of calabash-curare [22, 23] are strong dart poisons, causing the voluntary muscle relaxation (paralysis) to a great extent. It was used in hunting in South America, particularly in Amazon and Orinoco basins. Taking advantage of the muscle relaxation property of the curare alkaloids, by the judicial use of curare preparations along with light anesthesia, some surgical operations could be performed without recourse to deep anesthesia.

Some plants (leaves), exudates, fruits, etc. elaborate natural products that cause addiction when taken and immensely affect the human behavior. Cannabinoids (*charas*), morphine, acetyl morphine, cocaine, and nicotine (tobacco leaves) are a few prominent examples of such types of natural products.

The beautiful *colors* of the flowers, due to the presence of flavonoids, anthocyanins, etc. (Chap. 14) and other pigments are visual delight to human being from time immemorial. The *fragrance* of flowers and natural perfumes (prepared from plant sources) satisfy our olfactory nerves to a great extent. However, pollen volatiles of sweet-scented flowers often act as vital aeroallergens (generally allergens are mostly proteins) and cause seasonal fever to susceptible people, and bring to them a lot of physical miseries.

These are few of the many relations of natural products with human being, and the relationship or interactions are directly understood.

However, in defining the role of natural products in the internal economy of the organisms producing them, William et al. [34] suggested that biosynthesis of natural products is programmed by many kilobytes of DNA, and the energy expenditure involved is allowed, since they serve to improve the fitness of the natural product forming organisms by acting at specific receptors in the competing organisms. This is an important aspect of natural product formation under the pressure of the survival; otherwise, Darwin's natural selection would have precluded this energy expenditure, if necessary.

In this context, we may quote [35] Prelog (NL 1975), "Every natural product, which has appeared sometime during the three billion years of evolution and survived, carries in its structure a message and many of them have a yet unrevealed function. To decipher the message and to find out their function will remain for a long time one of the most challenging tasks of chemistry."

1.7 Biosynthesis: Studies with Isotopically Labeled Precursors[1] [36–38]

With the advent of isotope chemistry and advanced technology, it has been possible to synthesize molecules by putting isotopic (mostly ^{14}C, ^{13}C, ^{15}N, ^{2}H, or ^{3}H) labels into the assumed precursors. In the feeding experiments, the labeled precursors are administered into the plants. After a suitable period of growth, the expected incorporation of the precursors with radiolabels could be detected by trapping the intermediate/s or by degradation of the product/s, which appear with isotopic signature. In cases of ^{2}H-labeled precursors mass shifts in the products are observed in mass spectroscopy.

In the last few decades of the twentieth century, ^{13}C NMR spectroscopy has become an important tool for biosynthetic studies of natural products, as evident from the accumulation of a large body of data in the literature. ^{13}C Rich precursor is given in an adequate culture media during a biosynthetic study. The isolation of the specifically ^{13}C-labeled product/s showing larger intensity for some carbons in its ^{13}C NMR spectra will allow the identification of the site of incorporation, and hence help in elucidating the biosynthetic pathway of the compound under study. Generally, enrichment of 0.5 % ^{13}C above the natural abundance (1.1 %) is sufficient to locate the labeled site/s [39]. The biosynthesis of camptothecin [40] demonstrated

[1] Since the discovery of carbon14 by Martin Kamen and his fellow chemist Sam Ruben in 1940 while working at E.O. Lawrence's famous radiation laboratory at the UC, Berkeley, this radioactive isotope has been used as a very important and useful tracer in the study of various biological pathways including photosynthesis. It has profoundly influenced the studies of biochemistry and biology. Using $^{14}CO_2$ Melvin Calvin deciphered the photosynthetic pathway and received the Nobel Prize in 1961. William Libby also used ^{14}C decay in radioactive dating technique and received the Nobel Prize in 1960.

for the first time the suitability of ^{13}C NMR spectroscopy for biosynthetic studies in higher plants.

Thus, labeled compounds used as probable precursors help to map the various biological events with stereochemical implications involved in the biosynthesis of natural products. This provides an insight regarding the synthetic strategies of *Nature*. However, one has to be very careful about the biosynthetic experiments because sometimes the labeled precursor gets degraded prior to its incorporation, and also measurements of the various parameters are to be done with much repetitive care. The results are to be carefully interpreted to avoid inadvertent misleading conclusions. The negative results should also be rationalized with caution, keeping in mind that the experiment might not have been done at the opportune time for the natural biosynthesis of the concerned compound.

Despite the unbelievable diversity occurring in natural products, the plants utilize only a few amazingly simple molecules [e.g., acetate, C_5-units (isoprenoids), and amino acids (phenylalanine, tyrosine, ornithine, etc.)] as the building blocks. More amazingly, plants use very simple reactions at ambient temperature revolving around carbonyl chemistry, cation-olefin addition/cyclization, 1,2-alkyl or H migration, Michael addition, Diels–Alder reaction, Mannich condensation and a few other reactions to create carbon–carbon bonds during the construction of organic molecules of all conceivable structural patterns.

1.8 Metabolic Pathways: Mevalonic Acid Pathway, 1-Deoxy-D-xylulose Phosphate Pathway, Shikimic Acid Pathway, and Polyketide Pathway

Metabolic pathways provide definite sequence of biological reactions and elaborate discrete and controlled steps. Some of the identified well-known metabolic pathways are mevalonic acid (MVA), 1-deoxy-D-xylulose phosphate (DXP), shikimic acid, and polyketide pathways. It is helpful to classify the compounds based on their precursors from which the natural products are formed, as well as based on the metabolic pathways. Identification of the metabolic pathways brings a harmony in the chaotic chemical richness of *Nature*.

Mevalonic acid pathway and deoxy-D-xylulose phosphate pathway are responsible for the biosynthesis of terpenoids, steroids, and related natural molecules in the plant cells, while shikimic acid pathway forks out at the point of formation of chorismic acid, and the latter then participates in different pathways leading to the formations of natural products with structural carbon contents C_6–C_3, C_6–C_3–C_6, C_6–C_3–C_3–C_6, etc., and various aromatic amino acids and related products. The polyketide pathway allows the formation of simple to complex phenolic compounds (Table 1.1). There are many compounds which are of mixed biogenetic origin, i.e., certain fragment of the molecule originates following a route, while the

Table 1.1 Metabolic pathways and natural products[a]

Metabolic pathways	Class of compounds
Mevalonic acid (MVA)/deoxy-D-xylulose P (DXP)	Terpenoids, steroids, carotenoids, etc. and also the prenyl (C_5, C_{10}, C_{15}, C_{20}, etc.) units as such or in their modified forms as part/s of other compounds of different biogenetic origin, e.g., monoterpene indole alkaloids and other strictosidine-derived alkaloids, etc.
Shikimic acid (via chorismic acid)	Coumarins, lignans, etc., aromatic amino acids, aromatic phenolic acids, etc. ↓ Different classes of alkaloids
Polyketide	Aromatic phenolic compounds, polyphenolic compounds, flourenones, phenanthraquinones, antibiotics (mostly in microorganisms), etc.
Shikimic acid + polyketide	C_6–C_3–C_6 Compounds which include flavonoids, isoflavonoids, anthocyanins, kava pyrones, etc.
Shikimic acid + MVA/DXP	Coumarins with prenyl (C_5, C_{10}, C_{15}, C_{20}, etc.) units or, their modified forms
Shikimic acid + polyketide + MVA/DXP	Flavonoids, isoflavonoids, and anthocyanins carrying prenyl (C_5, C_{10}, C_{15}, C_{20}, etc.) units or, their modified forms

[a]Many other natural products fall outside these classifications and will not be discussed

other part is formed from another route, and then they get united to form the natural products, e.g., flavonoids having carbon content C_6–C_3–C_6 are formed via polyketide and shikimic acid pathways: C_6 (from polyketide) and C_3–C_6 (cinnamic acid equivalent) (from shikimic acid) (Table 1.1). *The above pathways will be dealt in fairly detail with the stereochemical implications while discussing the chemistry of the compounds derived from these pathways.*

The L-amino acids serve as the source of the nitrogen atom in the molecules of a major class of natural products called *alkaloids*. In fact, in most cases the amino acids as such get incorporated in the alkaloidal framework (decarboxylation takes place at some stage of biosynthesis). Rarely nitrogen is delivered to the alkaloidal skeleton by way of direct amination as in case of coniine (Chap. 17). The associated pathways and the derived natural products are summarized in Table 1.1 in a very general fashion.

Biosynthesis of the simple molecules like 3-phosphoglyceraldehyde, 3-phosphoglycerate, erythrose phosphate, ribose 1,5-diphosphate, fructose 1,6-diphosphate, pyruvic acid, phosphoenol pyruvate, acetone dicarboxylic acid, dihydroxyacetone diphosphate, etc. (Calvin cycle) (fixation products of C_3-plants), and of the C_4-acids like aspartate and malate (the primary fixation products in cases of C_4-plants) will be discussed briefly in Chap. 3. These are the *key primary metabolites,* which enter into the formation of natural products.

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Chapter 2
Fundamental Stereochemical Concepts and Nomenclatures

2.1 Introduction

In this chapter some essential stereochemical concepts associated with organic molecules (natural or synthetic), as reflected in course of their many reactions, their asymmetric synthesis, biosynthesis, and biological activities, have been discussed. This treatment is expected to be quite handy, advantageous, and helpful to the readers to understand the chiral/achiral designations (nomenclatures), the stereochemical features, and related properties of the natural products dealt in the chapters that follow. Without having adequate stereochemical concepts, it may not be possible to understand and appreciate properly the stereochemistry of the natural or synthetic products. Thus, some essential static and dynamic aspects of stereochemistry will be dealt with sufficient illustrative examples in figures along with discussions in the text to reasonable extents. Further, this treatment will give an idea of some basic stereochemical concepts as applied to organic molecules in general.

The chirality of the natural products imparts specific remarkable medicinal properties (in vivo interactions) (Chap. 32) and opened up in vitro two new chemically important avenues:

1. Synthesis of chiral molecules using chiral templates
2. Asymmetric synthesis of chiral molecules, based on stereoselectivity, in profitable enantiomeric or diastereomeric excess (involving development of chirality from a prochiral system).

Optically active natural products are synthesized in the plant cells in genetically controlled and enzymatically catalyzed processes with well-defined stereochemistry and with definite orientations (*Z*/*E*) of double bond/s, if present.

S.K. Talapatra and B. Talapatra, *Chemistry of Plant Natural Products*,
DOI 10.1007/978-3-642-45410-3_2, © Springer-Verlag Berlin Heidelberg 2015

2.2 Chirality. Symmetry Elements. Optical Rotation

In a lecture in 1893 Lord Kelvin introduced the term *chirality*. The word *chiral* is from the Greek name *cheir* (for hand or pertaining to hand). Chirality means handedness (topological). It is a purely geometrical property. An object, for that matter, a molecule is chiral if it is not superposable on its mirror image. Organic molecules may be conformationally mobile. *A chiral molecule must be nonsuperposable on its mirror image even after operation of rotation* around single bond/s or translation, *.i.e.*, it must be chiral in all conformations. Thus, in order to ascertain whether a flexible molecule is chiral or achiral (not chiral), all possible conformers must be analyzed.

Chirality of a particular molecular conformation of known stereostructure may be decided by

(i) Intuition (not useful for beginners),
(ii) Constructing the molecular models of the stereostructure of the compound and its mirror image and examining their probable superposability (not always possible)
(iii) Looking for *symmetry elements* of that structure by *symmetry operation(s)*.

Molecules (a particular conformation in case of a flexible molecule) which have a plane of symmetry (σ or S_1), a center of symmetry (*i* or S_2), or other alternating axis of symmetry (S_n, *n* even) are said to have reflection symmetry. Such molecules are superposable on their mirror images and are termed *achiral*. Molecules lacking any reflection symmetry are nonsuperposable on their mirror images and are termed *chiral*. *Thus, nonsuperposability on the mirror image of a molecule is the necessary and sufficient condition for chirality and for displaying optical activity*. The principal symmetry elements and symmetry operations are summarized in Table 2.1 for their convenient application and for subsequent finding out their point groups, which will be illustrated in the sequel.

A preliminary introduction to the different symmetry elements and their operations follow.

Table 2.1 Symmetry elements (symbols expressed in italics) and symmetry operations

Symbol	Symmetry elements	Symmetry operations
(i) C_n	Simple or proper axis of symmetry	Rotation about an axis through 360°/n
(ii) σ (S_1)	Plane of symmetry	Reflection in a plane
(iii) *i* (S_2)	Center of symmetry	Inversion through a center
(iv) S_n	Alternating or improper, or rotation-reflection or reflection-rotation axis of symmetry	*Rotation-Reflection*: Rotation about an axis by 360°/*n*, followed by reflection in a plane orthogonal (⊥r) to the axis. *Reflection-Rotation*: The order of the above two operations may be reversed to give the same result.

2.2.1 Simple or Proper Axis of Symmetry

A simple or proper axis of symmetry of order (multiplicity) *n* is such that rotation of the model or structure of the molecule around an axis by 360°/n leads to a structure indistinguishable from the original. Such an axis is denoted by *Cn*, expressed in *italics*. Some examples are shown in Fig. 2.1.

Principal Axis If a molecule possesses several simple axes of symmetry, the axis with maximum multiplicity (order) is designated as the principal axis of symmetry. If the simple axes of symmetry are of the same order, the simple axis passing through the atoms is regarded as the principal or main axis. For an example see allene (Fig. 2.3).

C_1 Axes. *Any object* or *molecule* contains an infinite number of C_1 axes since its rotation by 360° around any axis passing through the object or molecule results in the original three-dimensional orientation.

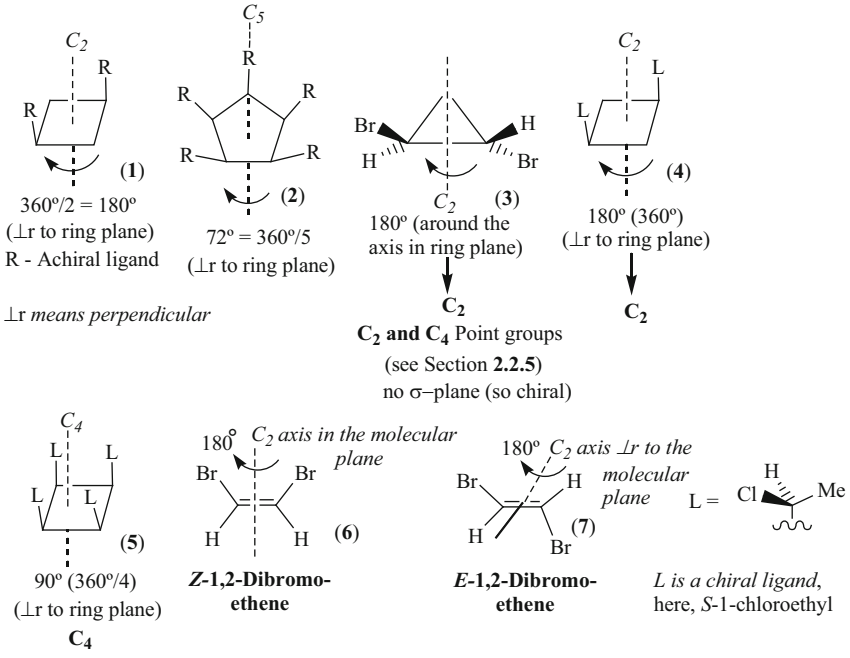


Fig. 2.1 Examples of molecules with C_2, C_4, and point groups $\mathbf{C_2}$ and $\mathbf{C_4}$

2.2.2 *Plane of Symmetry*

A plane of symmetry or a sigma plane (**σ plane**) is defined as a *mirror plane* which bisects a rigid object or a molecule so that one-half of it coincides with the reflection of the other half in the mirror, i.e., one-half of it reflects its enantiomeric or identical half.

Examples of some common objects with only one plane of symmetry are chairs, cups, file cabinets, spoons, and tooth brushes. Some examples of molecules with one σ-plane are shown in Fig. 2.2.

σ_v and σ_h C_n axes and σ planes often occur together in molecules. The principal proper axis of symmetry is conventionally taken as vertical. The symbol σ_ν is used to designate a vertical plane of symmetry containing the principal axis; σ_h is used to designate a horizontal plane of symmetry perpendicular to the principal axis of symmetry; and σ_d is used to represent a diagonal plane bisecting the angle between two C_2 axes. The three types of planes are illustrated with examples in Fig. 2.3.

A plane of symmetry is equivalent to onefold alternate axis of symmetry S_1 (*vide infra*).

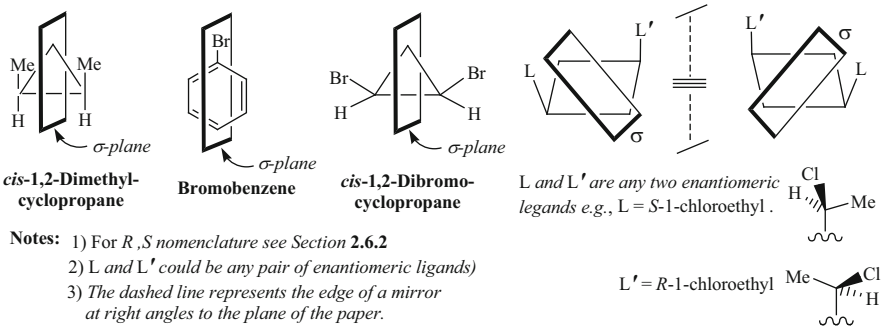


Fig. 2.2 Achiral molecules with only one symmetry plane

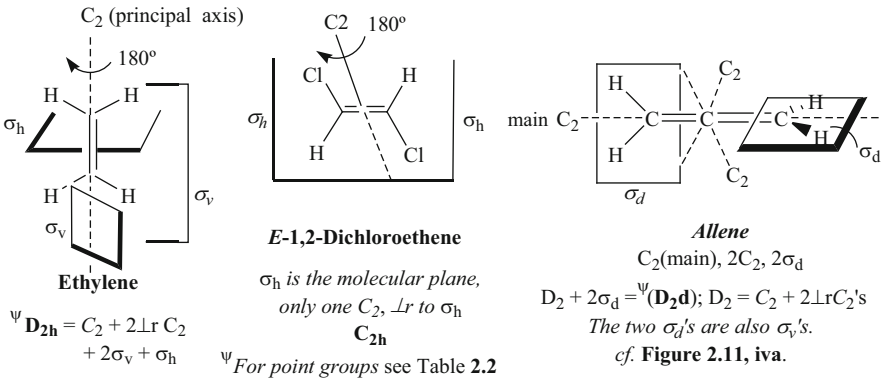


Fig. 2.3 Examples of σ_ν, σ_h, and σ_d planes

2.2.3 Center of Symmetry or Inversion Center

The center of symmetry designated *i* is a point such that all imaginary straight lines that can be drawn through it meet identical atoms at equal distance from the point. The objects including molecules with a center of symmetry (point symmetry) are termed *centroasymmetric*.

A center of symmetry is equivalent to twofold alternating axis of symmetry S_2, as illustrated in Fig. 2.4 (see Sect. 2.2.4).

2.2.4 Alternating or Improper or Rotation-Reflection Axis (S_n)

An alternating or an improper or a rotation-reflection axis of order n (S_n) present in a molecule is such that it can be rotated about the axis by an angle of 360°/n and then reflected across a plane perpendicular to the axis to provide a structure indistinguishable from the original. The order of the two operations may be reversed (reflection-rotation) to give the same result. It has been exemplified for *meso*-2,3-butanediol and *meso*-tartaric acid, each having an S_2 axis (Fig. 2.4), and also for substituted cyclobutane with two mirror image (enantiomeric) ligands at 1,3 positions having an S_4 axes (Fig. 2.5). Another example with an S_4 axis is illustrated in Fig. 2.5.

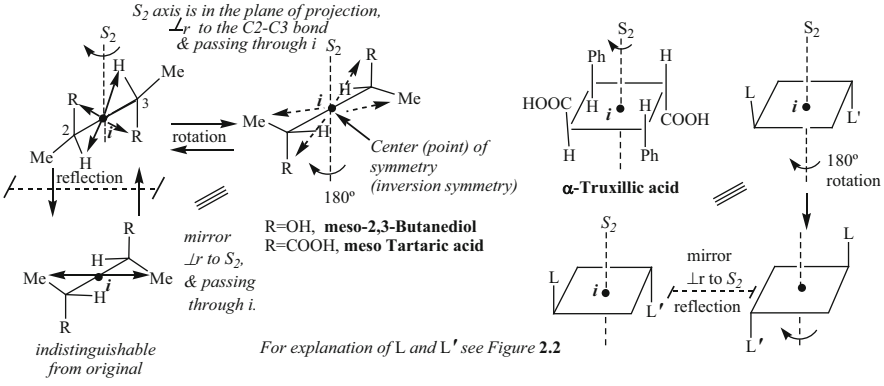


Fig. 2.4 Achiral molecules with center of symmetry (S_2) (inversion symmetry, *i*)

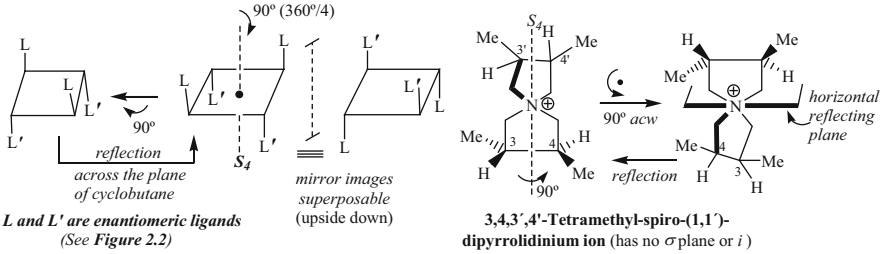


Fig. 2.5 Achiral molecules with S_4 axis explained

2.2.5 *Dissymetric and Asymmetric Molecules. Chiral and Achiral Point Groups. Central Chirality*

All chiral molecules are *dissymmetric* since they lack the symmetry elements S_n, *i*, and σ. A dissymmetric molecule may have one axis or more axes of symmetry (C_n). On the other hand, the chiral or dissymmetric molecules which lack even C_n axis (save C_1 axis) is termed *asymmetric*. Thus, a molecule can be classified as shown in the following Fig. 2.6.

A chiral or dissymmetric compound having at least one or more (a) chiral centers (central chirality), (b) chiral axis (axial chirality), (c) chiral plane (planar chirality), or (d) helicity (helical chirality) is nonsuperposable on its minor image (enantiomer) and hence is optically active. Axial chirality, planar chirality, and helicity—lacking any chiral center—will be discussed in Sects. 2.16, 2.17, and 2.18, respectively.

The ***point group*** defines the symmetry class to which a molecule belongs. The symmetry operations (or symmetry elements) are combined to form a point group (notation by Schönfliess), since each operation *leaves a point*, the center of gravity of the molecule, *unchanged*. The C_n and S_n operations organize a point group which is by convention expressed in **bold letters**. The chiral point groups (Fig. 2.6) are illustrated by examples in Figs. 2.7, 2.8, 2.9, and 2.10).

Some Examples of Chiral Point Groups

(i) **Point group C_1**

All organic molecules with only one stereogenic center or axis or plane belong to $\mathbf{C_1}$ point group.

C_n axis and C_n operation

The compounds (**3**) and (**4**) of Fig. 2.1 are chiral and belong to the point groups $\mathbf{C_2}$ and $\mathbf{C_4}$. Compounds (**1**), (**2**), (**6**), and (**7**) possess σ-planes and hence achiral (Fig. 2.1). A few more compounds belonging to $\mathbf{C_2}$ point group are illustrated in Figs. 2.8 and 2.9.

(ii) **Point group C_2** (*possesses the only symmetry element*, $\mathbf{C_2}$)

(iii) **Point group C_3**

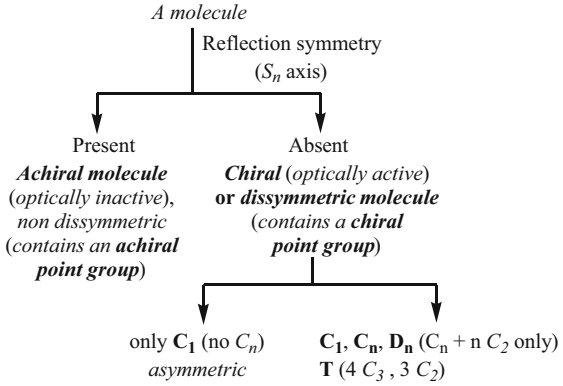


Fig. 2.6 Chiral and achiral molecules based on reflection symmetry. Chiral point groups

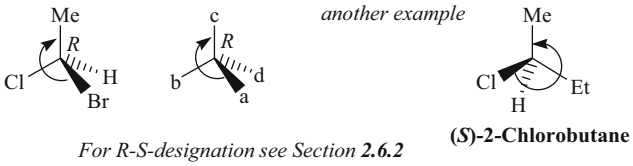
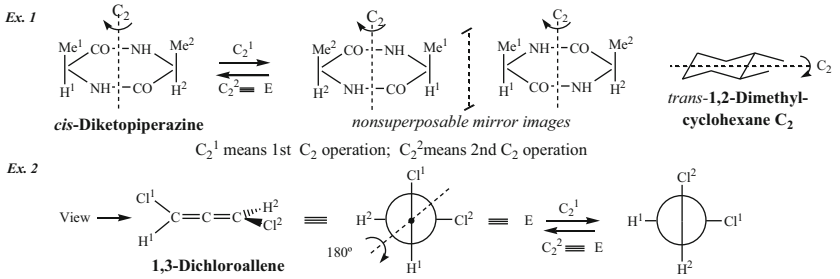


Fig. 2.7 Asymmetric compounds with $\mathbf{C_1}$ point group

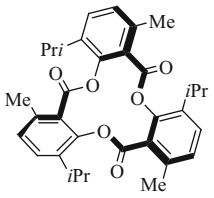


- *The C_2 axis passes through the central carbon and bisecting the dihedral angle between the two Cl's and two H's (taking the three allene carbons as a C-C bond in the Newman projection formulas.*
- *Any operation like C_2^2 in the above examples, producing a shape identical with the original one is called identity or 'E' or 'I' operation.*
- *The C_2 symmetry of 1,3-dichloroallene is best seen in its Newman projection, as shown.*

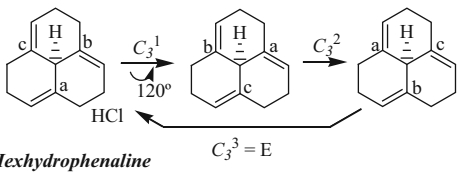
Fig. 2.8 Asymmetric compounds belonging to $\mathbf{C_2}$ point group

(iv) **Point groups $\mathbf{D_n}$ and T.** The chiral point group $\mathbf{D_n}$ consists of a principal C_n axis of maximum multiplicity (or passing through some atoms of the molecule) and n C_2 axes orthogonal to the principal axis (Fig. 2.10).

T point group. Tetrahedral (**T**) point group is also chiral, possessing four C_3 and three C_2 axes, but no σ-plane. The four chiral ligands are necessarily having same structure and absolute configuration (Fig. 2.10).



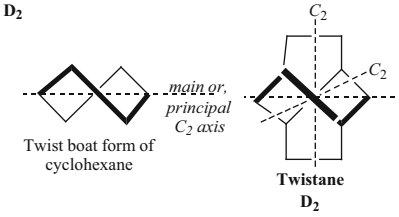
Tri-*o*-thymotide
(*no σ-plane, a known example of* **C_3** *point group having the shape of a 3-bladed propellar*)

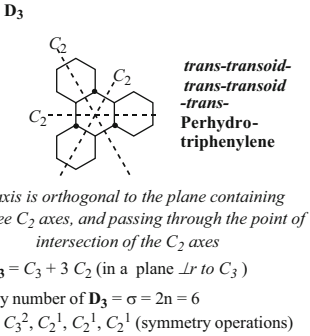


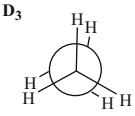
Hexhydrophenaline
(*no σ-plane*)

Imaginary hydrocarbon (*propellar type*) (*C_3 axis ⊥r to the paper plane, passing through central H-C bond*)

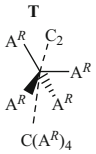
Fig. 2.9 Asymmetric compounds belonging to **C_3** point group







Ethane
(*neither staggered nor eclipsed*)



T = 4 C_3 + 3 C_2
Symmetry number σ = E, 4 C_3^1, 4 C_3^2, 3 C_2^1 = 12
Symmetry order also 12
} see Section **2.2.6**

For distribution of all simple axes of symmetry *vide infra* (point group T_d)

Fig. 2.10 Few molecules belonging to **D_2**, **D_3**, and **T** chiral point groups

2.2.6 *Symmetry Number, Order of Point Groups, Achiral Point Groups*

Point group notations of *achiral* molecules are enumerated in Table 2.2.

Symmetry number σ of a molecule is the number of indistinguishable but nonidentical positions into which the molecule can be turned by rigid rotation (around all simple axes of symmetry), taking the parent position as 1.

Order of the point group is defined as the total number of symmetry operations that can be performed in that point group. Table 2.3 lists the order and symmetry number (σ) of various chiral and achiral point groups.

Achiral Point Groups: Several examples of achiral molecules belonging to different point groups (Table 2.2) are displayed in Fig. 2.11. The point groups could be allocated to each molecule by finding out the C_n, S_1 (σ-plane), and S_2 (center of symmetry) possessed by the molecule.

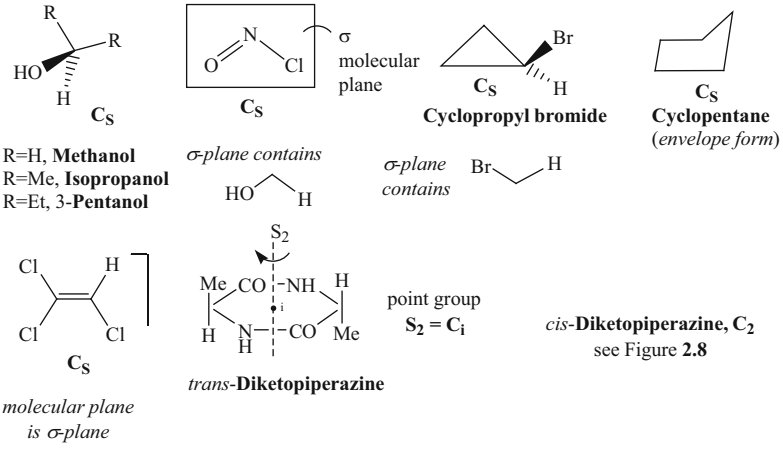
Table 2.2 Point group notations of achiral molecules

C_s	S_n	C_{nv}	C_{nh}	D_{nd}	D_{nh}	T_d
One σ only	(no σ) *n* even	*Cn* + *n* σ_v only	$C_n + \sigma_h$ no σ_v	$C_n + n\,C_2$ (Ir) + *n* σ_v but no σ_h	$C_n + n\,C_2$ (1r) + *n* $\sigma_v + \sigma_h$	4 C_3 + 3 C_2 + 6 σ

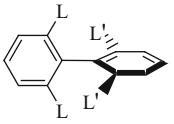
Table 2.3 Symmetry number and order of point groups

Pt. group	C_1	C_n	D_n	C_S	S_n^{Ψ}	C_{nv}; C_{nh}	$C_{\infty v}$	D_{nd}; D_{nh}	T_d	O_h
Order	1	*n*	2*n*	2	*n*	2*n*	∝	4*n*	24	48
Symmetry number, σ	1	*n*	2*n*	1	*n*/2	*n*	1	2*n*	12	24

S_n^{Ψ} includes $S_2 = C_i$, $\sigma = 1$, order 2



S_4 see Figure **2.5**
One more example :



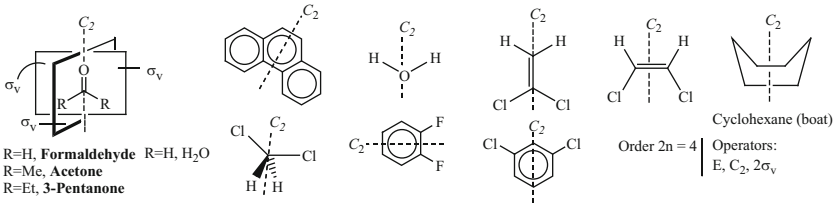
Here S_4 includes S_2 (C_i). For S_4 order is 4
Operators: E, S_4^1, S_4^2 (C_2), S_4^3

Symmetry number σ = 2

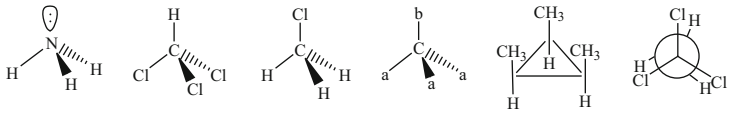
Fig. 2.11 (continued)

(ii) $\mathbf{C_{nv}} = C_n + n\ \sigma_v$; no σ_h *Each example contains a C_2 (shown) and two σ_v planes, as shown in the 1st example.*

(a) $\mathbf{C_{2v}} = C_2 + 2\ \sigma_v$ *Other examples*:



(b) $\mathbf{C_{3v}} = C_3 + 3\ \sigma_v$ Examples**:**



Order is 6: E, C_3, 3 σ_v's, C_3^2

(c) $\mathbf{C_{4v}} = C_4 + 4\ \sigma_v$

all *cis*

(d) $C_{5v} = C_5 + 5\ \sigma_v$

the ring is taken as planar

(e) $C_{\infty v} - C_\infty + \infty\sigma_v$

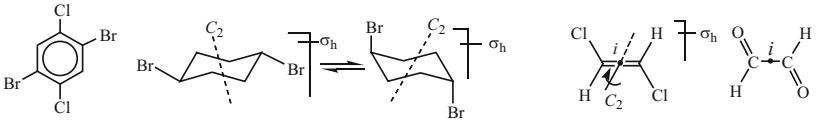
Conical symmetry. H-Cl; C=O; H-C≡N; H-C≡C-Cl

(iii) $\mathbf{C_{nh}} = C_n + \sigma_h$; no σ_v.

(a) $\mathbf{C_{2h}} \rightarrow$ order is 4: E, C_2^1 σ_h, i

$\sigma = 2$

Examples of point group $\mathbf{C_{2h}} = C_2 + \sigma_h$



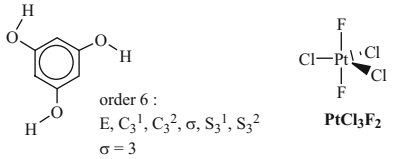
trans-**1,4-Dibromocyclohexane**

σ_h *passing through 2C-Br bonds*

C_2 *passing through the mid points of opposite bonds shown*

s-trans-**1,3-Butadiene**

(b) $\mathbf{C_{3h}} = C_3 + \sigma_h$



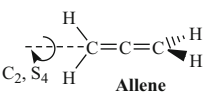
This particular conformation has point group $\mathbf{C_{3h}}$

Fig. 2.11 (continued)

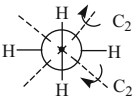
(iv) $\mathbf{D_{nd}} = C_n + n\,C_2\,(\perp r) + n\,\sigma_v\,(\sigma_d)$; no σ_h

(a) $\mathbf{D_{2d}} = C_2 + 2\perp r\,C_2 + 2\,\sigma_d$

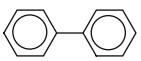
also S_4 (prin. C_2)



Allene



(also see **Figure 2.3**)

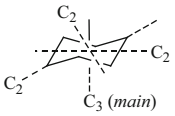


Biphenyl

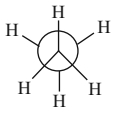
Spirane

Order 8 :
E, $3C_2$ (mutually $\perp$r), S_4^1, S_4^3, $2\sigma_d$
$\sigma = 4$

(b) $\mathbf{D_{3d}} = C_3 + 3\,C_2\,(\perp r) + 3\,\sigma_d$



Cyclohexane (chair)



Staggered Ethane

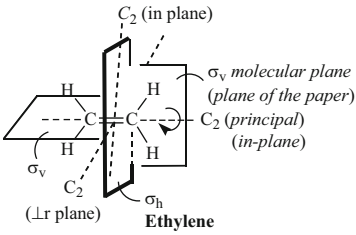
Order 12 ; $\sigma = 6$
E, C_3^1, C_3^2, 3 C_2^1,
S_6^1 (*coincident with* C_3)
S_6^5, i, $3\sigma_d$

See **2.3.2**

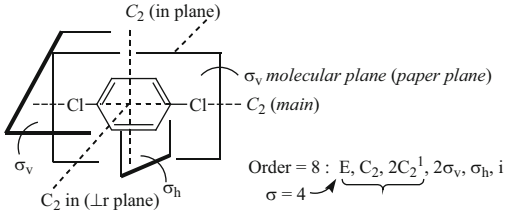
(v) $\mathbf{D_{nh}} = C_n + n\perp r\,C_2 + n\,\sigma_v + \sigma_h$

(a) $\mathbf{D_{2h}} = C_2 + 2\,C_2\,(\perp r) + 2\,\sigma_v + \sigma_h$

Examples:



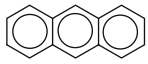
Ethylene



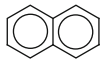
Order = 8 : E, C_2, $2C_2^1$, $2\sigma_v$, σ_h, i
$\sigma = 4$

$\mathbf{D_{2h}} = \underbrace{C_2 + 2 \text{ orthogonal } C_2}_{D_2} + 2\,\sigma_v + \sigma_h$

(also see **Figure 2.3**)

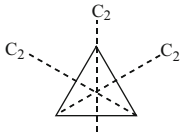


Anthracene



Naphthalene

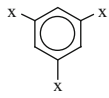
(b) $\mathbf{D_{3h}} = C_3 + 3\,C_2\,(\perp r) + 3\,\sigma_v + \sigma_h$



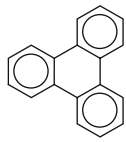
Cyclopropane

C_3 axis $\perp r$ to the ring plane
3 C_2 axes in the ring plane
3 σ_v planes each containing a C_2 axis, the ring plane is σ_h

BF_3,



,



Triphenylene

(c) $\mathbf{D_{4h}} = C_4 + 4\,C_2\,(\perp r) + 4\,\sigma_v + \sigma_h$

Order = 16 : $\underbrace{\text{E, } C_4^1, C_4^2, C_4^3, 4\,C_2^1}_{\sigma = 8}$, $4\,\sigma_v$, σ_h S_4^1, I, S_4^3

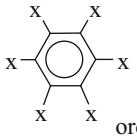
cyclobutane

Pt Cl_4 PtX_4
square planar

Fig. 2.11 (continued)

(d) D_{6h}

Benzene



$\mathbf{D_{6h}} = C_6 + 6\,C_2 + 6\,\sigma_v + \sigma_h$

$\sigma = E, C_6^1, C_6^2, C_6^3, C_6^4, C_6^5, 6\,C_2^1 = 12$

order = $\sigma + 6\,\sigma_v, \sigma_h, S_6^5, S_6^4, S_6^3, S_6^2, S_6^1 = 24$

sym. no. ($i = S_6^3$)

order = 24, σ = 12

sym. no.

(vi) $D_{\infty h}$ E, C_∞ , $\propto C_2$ ($\perp$r C_∞ axis), $\propto \sigma_v$, σ_h cylindrical H–H, O=C=O, HC≡CH

(vii) T_d

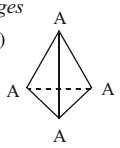
Pt gr. of regular of tetrahedron

A = achiral group like H, Me, Cl etc.

E, 3 C_2 *(mutually* $\perp$*r) passing through pairs of opposite edges*

4 C_3 → *passing though each apex and the center of opp. face (containing A-C bond)*

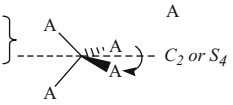
(i)



6 σ_d → *Each* σ_d *contains each A–C–A plane;*

∴ 4 C_2 *= 6 combinations*

$3S_4$ → *coincident with* C_2 *axes*



σ = 12; E, 4 C_3^1, 4 C_3^2, 3 C_2^1 = 12. order = 12 + $\underline{6\,\sigma_d + 3\,S_4^1 + 3\,S_4^3}$ = 24

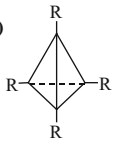
Underlined ones are additional operations

(ii)

Adamantane

Chiral center in the center, not at any carbon

(iii)



R = *t*-Bu, synthesised by Maier in 1978

R = H, could not be synthesised as yet

(viii) O_h octahedral symmetry

cubic pt. gr.

Sym. operations : *E*, 3 C_4 (mutually perp.) + 4 C_3, 3 S_4, 3 C_2 (coincident with 3 C_4 axes) 6 C_2, 9 σ, 4 S_6 (coincident with $4C_3$ axes)

Other examples:

σ = *1 + 9 + 8 + 6* = 24

order = 24 x 2 = 48

(double by the existence of symmetry planes

$[PtCl_6]^-$; $[Co(NH_3)_6]^{3+}$

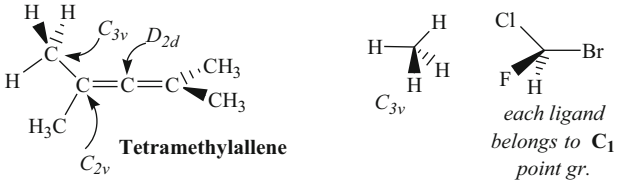
Cubane

first synthesised by Eaton *in 1964*

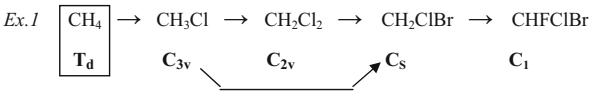
Fig. 2.11 Examples of some achiral compounds belonging to different achiral point groups

2.2.7 Local Symmetry (or Site Symmetry). Desymmetrization

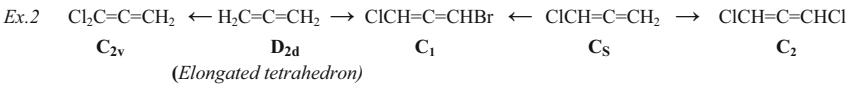
Local symmetry shows the symmetry properties of atoms or groups within a molecule (Fig. 2.12).



Desymmetrization results from successive substitution on a symmetrical lattice framework



Desymmetrization results in distortion of the framework, small but real, as demanded by the symmetry of the system.



Ex.3

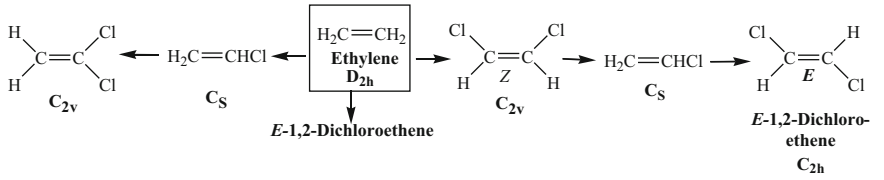


Fig. 2.12 Local symmetry and desymmetrization illustrated

2.2.8 Optical Isomerism. Optical Rotation

2.2.8.1 Optical Activity Due to Chiral Molecular Structure

A chiral compound in the solid, fused, gaseous (rarely) liquid or dissolved state, *e.g.*, lactic acid, tartaric acid, glucose etc., exhibits optical activity, *i.e.*, rotates the plane of polarization of an incident beam of polarized monochromatic light. During the early nineteenth century the French physicist Jean Baptiste Biot (1774–1862) discovered that many natural organic compounds exhibit optical activity. Since this optical rotation occurs in the liquid, dissolved or gaseous phase, it must be a molecular phenomenon. Here, the optical activity is entirely due to the *dissymmetry of the molecular structure*. The original molecule and its nonsuperposable mirror images are known as *enantiomers, enantiomorphs* (this name is taken from crystallography), or optical antipodes, one of which is dextrorotatory (rotates the plane of polarization in the clockwise direction by a positive (+)-angle) and the other levorotary (rotates in the anti-clockwise direction by a negative (−)-angle). For the measurement of optical rotation, earlier polarimeters have now been replaced by digital polarimeters.

2.2.8.2 Optical Activity Due to Crystalline Structure

Some crystals, *e.g*., *quartz, sodium chloride, benzil*, etc. are optically active in the crystalline state only, *i.e*., rotate the polarized light. Quartz is the first substance shown by Biot in 1812 to be optically active. It exists in two hemihedral nonsuperposable mirror image forms, one of which is *dextrorotatory* and the other *levorotary*. Here, the optical activity is a property of the chiral crystal and not of the molecule, as the rotation disappears when the crystal is melted or dissolved to give the achiral molecules. Such pairs of nonsuperposable crystals are said to be *enantiomorphous*.

(See also Appendix B, 1821/1822, 1948, 1950)

2.2.8.3 Dependence of Rotation (α) on Concentration and Cell Length. Value of α

The observed angle of rotation of the plane of polarization is denoted by α, which can be recorded in a range of $-90°$ and $+90°$. For example, distinction can't be made between α and $\alpha \pm n\ 180°$; the plane when rotated by $\pm 180°$ or its integer n, the new plane will coincide with the old one. Thus theoretically, no difference appears between $+40°$, $+220°$ ($40° + 180°$), $+400°$ ($40° + 2 \times 180°$), or $-140°$ ($40° - 180°$). Hence, rotation must be measured at least at two different concentrations to get α unequivocally. Since α is proportional to concentration, if the original solution is diluted to 1/10, α would be $+4°$, $+22°$, $+40°$, $-14°$, respectively. Again α being proportional to length (discovered by Biot) can be unambiguously determined by using smaller cells, *e.g.,* of 0.25 dm length for the original concentrations, when observed α will be $+10°$, $+55°$, $+100°$ (equivalent to $-80°$), or $-35°$, respectively, again all clearly distinguishable.

2.2.8.4 Dependence of Sign of [α] of Polar Compounds on Solvent, Concentration, and pH

The rotation of compounds, especially the polar ones, is affected by the solvent because of its participation in solvation and association phenomena. It has been reported by Winther [1, 2] in 1907 that the specific rotation $[\alpha]_D$ (Sect. 2.2.9) of (+)-nicotine (Chap. 18) is positive in polar solvents like formamide (maximum positive specific rotation) and water, $[\alpha]_D$ decreasing with increase of concentration. It remains almost same in MeOH and EtOH and increases in benzene, *o*-, *m*-, and *p*-xylenes, and mesitylene with increase of concentration. Whereas, the rotation of natural nicotine in ethylene bromide and chloroform at lower concentrations (>0.6 g/g) was observed to be negative. Specific rotation of nicotine at infinite dilution of the solvent (meaning no solvent) obtained by extrapolation of the specific rotations in solvents of different polarities has been found to be ~8 units

(Sect. 2.2.9); this is known as *intrinsic rotation* $\{\alpha\}$. As the concentration of the solute increases, solute–solute interactions are maximized, which can differ greatly from one solvent to another. In polar solvents solute–solute association effect may be suppressed by competition with concentration-independent solvent association, which appears to increase the rotation.

Reversal of the sign of the rotation of (+)-nicotine takes place in less polar solvents like $CHCl_3$ and ethylene bromide at much less concentrations. Other examples: 2-methyl-2-ethylsuccinic acid displays positive rotation in $CHCl_3$ containing 0.7 % EtOH at >6.3 %, no rotation (null) at ~6.3 %, and negative rotation at <6.3 % concentration; but no reversal is observed in alcohol solvents, pyridine, diglyme, and acetonitrile.

Rotation of acids and bases is dependent upon the pH, *e.g.*, (*S*)-(+)-lactic acid is dextrorotatory in water, but its sodium salt is levorotatory. Another example: L-Leucine is levorotatory in water but dextrorotatory in aqueous hydrochloric acid.

2.2.9 *Specific Rotation. Molecular Rotation. Units (Fig. 2.13)*

The rotation per unit length in dm and unit concentration in g/ml is called *specific rotation* expressed as follows:

Specific rotation $[\alpha]_D^t = \frac{\alpha}{l.c} = \frac{\alpha.100}{l.c'}$ where α is the observed rotation, l = length of the cell in dm, c = conc. in g/ml, t = temperature, c' = conc. in g/100 ml, d = density of the neat liquid

(Rotation is measured at the wavelength of Na-D line (589 nm)

For liquids $[\alpha]_D^t = \frac{\alpha}{l.d}$

A new term "molecular rotation" [M] or [ϕ] also called molar rotation is defined to compensate for the effect of differing mol. wts. as follows : $[M] = [\varphi] = \frac{[\alpha]_D^t.M}{100^a} = \frac{\alpha.M}{l.c.100^a} = \frac{\alpha}{l.\frac{c}{M}(100\,ml)^{-1}} = \frac{\alpha}{l.c''}$

where M = mol. wt., c′′ = mole per 100 ml

[a]The division by 100 is arbitrary in order to keep its numerical value on the same approximate scale as that of specific rotationl.

Note: *Thus, optical rotation is proportional to the number of molecules encountered. Hence, for a compound with mol. wt. 100, [ϕ] = [α]*

Units or dimensions of [α] and [ϕ]

$[\alpha] = \frac{\alpha}{l.c}$ Thus *units of* $[\alpha]$ are $\frac{degree}{dm.g\,cm^{-3}} = \frac{degree}{100\,cm.g.cm^{-3}} = \frac{degree}{100\,cm^{-2}\,g} = 10^{-1}$ deg. $cm^2\ g^{-1}$

So, while α is given in degrees, [α] should not be expressed in degree alone and should always be given without the units (understood to be 10^{-1} deg $cm^2\ g^{-1}$), as has been done in this book [*cf.* references in further reading (iii) and (v)].

$Again, [\varphi] = \frac{[\alpha]M}{100} = \frac{\alpha}{l.mole\ per\ 100\ ml}$

∴ *Units of molar rotation* are $\frac{degree}{10\,cm.mole.10^{-2}cm^{-3}} = \frac{degree}{10^{-1}cm^{-2}\,mole} = 10\ deg\ cm^2\ mole^{-1}$

$[\phi]$ *is also expressed* ***without any unit****, understood to be 10 deg* $cm^2\ mole^{-1}$

Fig. 2.13 Specific and molecular rotations and their units

2.2.10 *Fischer Projection. Flying Wedge Formulas. Tetrahedral Representations of Cabcd*

A common representation of a tetracoordinated compound with one chiral center was first proposed by Emil Fischer [3] in 1891, which is much easier than tetrahedral representation. While writing a Fischer projection formula of an organic compound having one or more chiral center/s, each sp^3 carbon atom in the chain is written as a cross +. Thus, the *agreed modes of projection* of the three-dimensional molecule on two-dimensional plane (projection plane, PP) to give the Fisher projection (FP) formula are as follows:

(1) The asymmetric (or dissymmetric) carbon should be in the projection plane.

The main chain must be in the vertical plane and pointing away from the observer, so that the vertical or up and down bonds are below the projection plane extending toward rear. The other two sideways substituents (ligands) of each carbon must be in the horizontal plane (above the PP) and pointing forward toward the observer.

Corollaries

1. One pair of ligands when exchanged gives the enantiomer.[1]
2. Two exchanges give another FP formula of the same enantiomer.
3. Ligands may be rotated in groups of three.

Limitations

1. FP formula being two-dimensional cannot be lifted out of the P.P. and turned over.
2. FP formula may be rotated in the projection plane by even multiple of 90° (*i.e.*, 180°), but not by odd multiple of 90° (*i.e.*, 90° or 270°).

Three-dimensional (flying wedge) and tetrahedral representations of enantiomers with one chiral center, C_{abcd}, and their two-dimensional representations as Fischer projection formulae are exemplified in Fig. 2.14.

There are 12 ways of writing the Fischer projection formula of an enantiomer of a compound with one chiral center, as depicted in Fig. 2.16. For detailed discussion on (D,L) and (*R*,*S*) nomenclatures, see Sect. 2.6.1 and 2.6.2.

The best way to confirm whether the two-dimensional (Fischer projection) or three-dimensional (flying wedge or tetrahedral) representations are identical or mirror images is to specify their (*R*,*S*) nomenclature (Fig. 2.6.2). In Fig. 2.14 all

[1] It may be noted that a tetrahedron is the only skeleton in which every transposition of ligands is equivalent to reversal of the ligated assembly, in which the tetrahedral atom is at the center of the tetrahedron and the four bonds of that atom are directed toward the four vertices of the tetrahedron carrying the four ligand atoms.

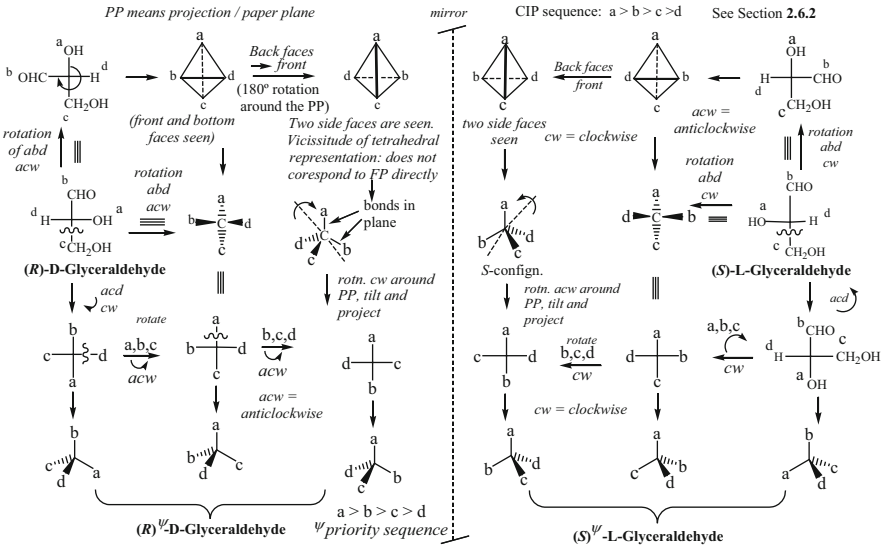


Fig. 2.14 Fisher projection, flying wedge, and tetrahedral representations of (*R*)-D-glyceraldehyde and (*S*)-L-glyceraldehyde

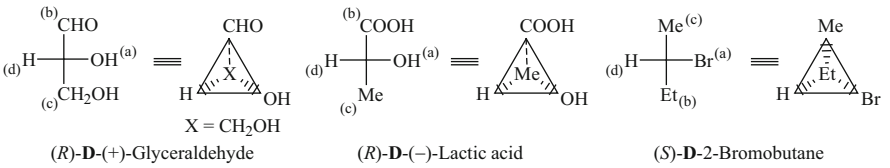
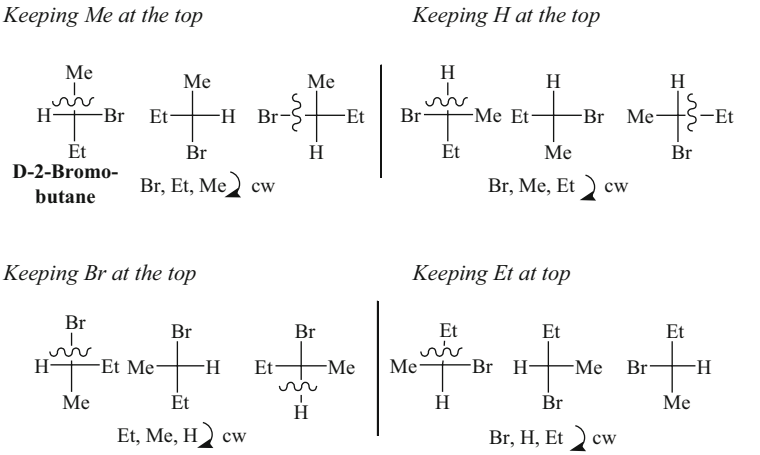


Fig. 2.15 A different tetrahedral representation of a few compounds, Cabcd (one enantiomer)

representations on the left side or right side of the mirror are of (*R*)-glyceraldehyde or (*S*)-glyceraldehyde. The names are given under the Fischer projection formulas specifying the (D,L) nomenclature. Each of the 12F.P. formulae of (*R*)-C_{abcd} (Fig. 2.16) can give many more flying wedge formulas by tilting and rotating to different extents and can be confirmed by specifying the absolute configuration according to CIP rules (Sect. 6.2.1).

The tetrahedral representation of (*R*)-C_{abcd} can also be written as shown in Fig. 2.15, tilting the bottom side of the F.P. formula downwards so that the bottom ligand goes to the rear side of the triangle (front face) made by the other three ligands; the other three faces of the tetrahedron goes to the rear side. A few other common compounds with one chiral center are also shown in this figure.

For a chiral compound with one chiral center there are factorial four (4!) = 4 × 3 × 2 = 24 ways of writing the Fischer projection formula, this being the permutations of four ligands among four sites. Of these, 12 FP formulas will correspond to the *R*-enantiomer and the remaining 12 to the (*S*)-enantiomer.



***Note**: Each F.P. is having (S-) and **D**-absolute configuration. The **D**-configuration is revealed only from the first F.P. formula.*

Fig. 2.16 12 Fischer projection formulae of (*S*)-**D**-2-bromobutane

The 12 Fischer projection formulas for (*S*)-**D**-2-bromobutane are depicted in Fig. 2.16.

2.3 Conformation of Simple Acyclic Molecules

The term *conformation* of a molecule (excepting the diatomic ones) signifies any one of the infinite number of momentary arrangements of its atoms in space that result from rotation around single bonds and also from twisting around bonds. The conformations of a molecule that correspond to the minimum energy in its potential energy diagram are known as conformers or *conformational isomers*. Thus, any point on the curves (Figs. 2.20, 2.21, 2.22) corresponds to some conformation of the concerned molecule. Conformations are not superposable upon each other.

Conformational analysis involves the interpretation or prediction of the physical (including spectral) properties, thermodynamic stabilities, and reactivities of substances in terms of the conformation or conformations of their molecules.

2.3.1 Dihedral Angle. Torsion Angle. Torsional Strength

The *dihedral angle* θ (*theta*) denotes the angle between two planes containing A–B–C and B–C–D, respectively, in a nonlinear molecule, A–B–C–D, as shown in Fig. 2.17. It is best seen in a Newman projection formula (Fig. 2.17) in which the

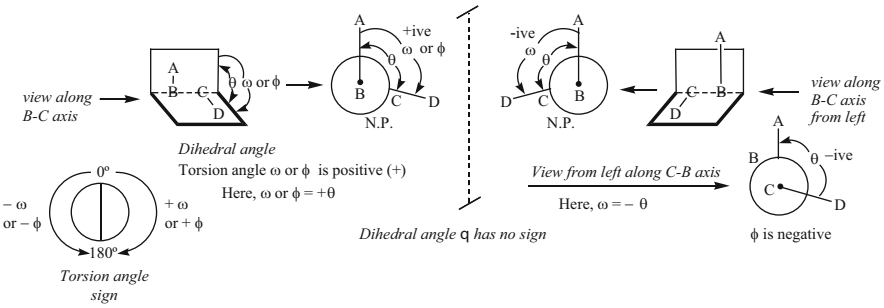


Fig. 2.17 Torsion angles and dihedral angles for two enantiomers

molecule is viewed from left along B–C axis; the dot in the front indicates the front atom B, and the circle indicates the back atom C. Here if B and C are tetracoordinated atoms, the remaining two bonds of each one are not shown. Thus, dihedral angle is a three-dimensional parameter of a molecule involving four atoms. The dihedral angle if given *a directional sense* becomes torsion angle denoted by Greek letter ω (omega) or ϕ (phi). If looking along the B–C axis *in either direction* the turn from A to D or D to A is clockwise, ω is positive; if the turn is anticlockwise, ω is negative. The torsion angle ω is best represented by Newman projection (N.P.) formula for each enantiomer (Fig. 2.17).

Torsional strain or Pitzer strain (V_ϕ) or potential energy (E) is caused by the rotational motion around the bond axis. Torsional strain is represented by the following equation:

$$V_\phi = \tfrac{1}{2}\, V_O(1 + \text{Cos}\, n\Delta\phi)\ \text{kcal/mol}$$

where $\Delta\phi$ is the displacement of the dihedral angle (torsional displacement), V_O is the torsional energy barrier or Pitzer strain, and n is the periodicity, *i.e.*, the number of times that a given conformation recurs during a complete revolution ($\Delta\phi = 360°$).

2.3.2 *Klyne–Prelog Nomenclature for Torsion Angles. Conformational Chirality*

A general method of nomenclature has been worked out by Klyne and Prelog [4] in 1960 to describe the steric relationship across a single bond in a molecule or a part of a molecule. The following rules are followed.

1. It has been mentioned earlier that in contrast to dihedral angle, torsion angle has a *directional property*, being mentioned as (+) when measured in a clockwise direction, and as (−) when measured in an anticlockwise direction. In a molecule

of the type A−B−C−D, the measurement is to be started from the *front substituent* A at 0° ending at the *rear substituent D* at 180° after rotation of the back substituent *D* around *B*−*C* bond.

2. *Conformation Selection Rule* (CSR): The two fiducial (reference) groups A and D are specified according to the *Conformation Selection Rule* by Cahn, Ingold, and Prelog [5] in 1966 as follows:

   (i) If all the atoms or groups (ligands) of the set on both carbons (front and back) are different, the ligand most preferred by the standard *sequence sub-rules* (Sect. 2.6.2) is fiducial.
   (ii) If two ligands of a set on one or both carbon/s are identical, the nonidentical (unique) group is fiducial, irrespective of the sequence rule.
   (iii) If all or two ligands of the set on carbons are identical, that which provides the smallest torsion angle is fiducial.

3. The fiducial group A at the front atom is preferably (but not necessarily) placed at the top of the Newman projection formula, and the torsion angle is named in terms of the three designations **a, b,** and **c** (Fig. 2.18). The circle is divided into six segments by combination of **a**, **b,** and **c**, as shown in **d** in the figure. The conformation of any molecule bears the designation of the torsion angle. *Synperiplanar* and *antiperiplanar* conformations are expressed with (±) sign because of probable variation or libration of ω around 0° and 180°, respectively, by upto +30° or −30°

The sign of a torsion angle in any conformation remains unchanged whether the molecule is viewed from the front or from the rear. Since the exact values of torsion angles are often not known, the (+) and (−) signs immediately show the direction of a torsion angle, and the symbols *sp*, *sc*, *ac*, *ap* in the Klyne–Prelog system show the range of a torsion angle. The system is applicable to any molecule A−B−C−D, whether B and C are tetrahedral or trigonal atom; this system may also be used to describe partial conformation of ring compounds and polymer chains. Figure 2.19 depicts a few examples of different types. **Example 1** illustrates the torsion angle nomenclatures of the conformers and also the higher dipole moment of active stilbene dichloride than that of its meso isomer based on the analysis of the three conformers in each case.

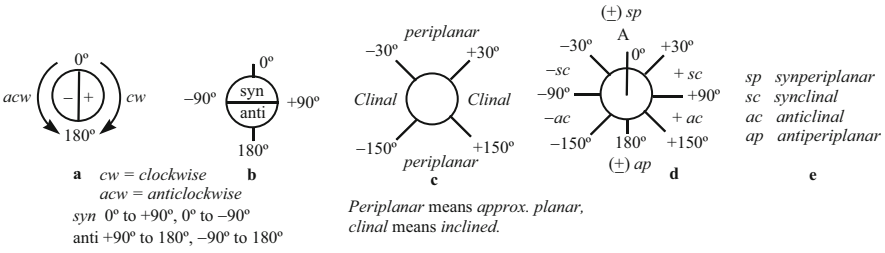


Fig. 2.18 Designation of conformation based on torsion angle ω or ϕ

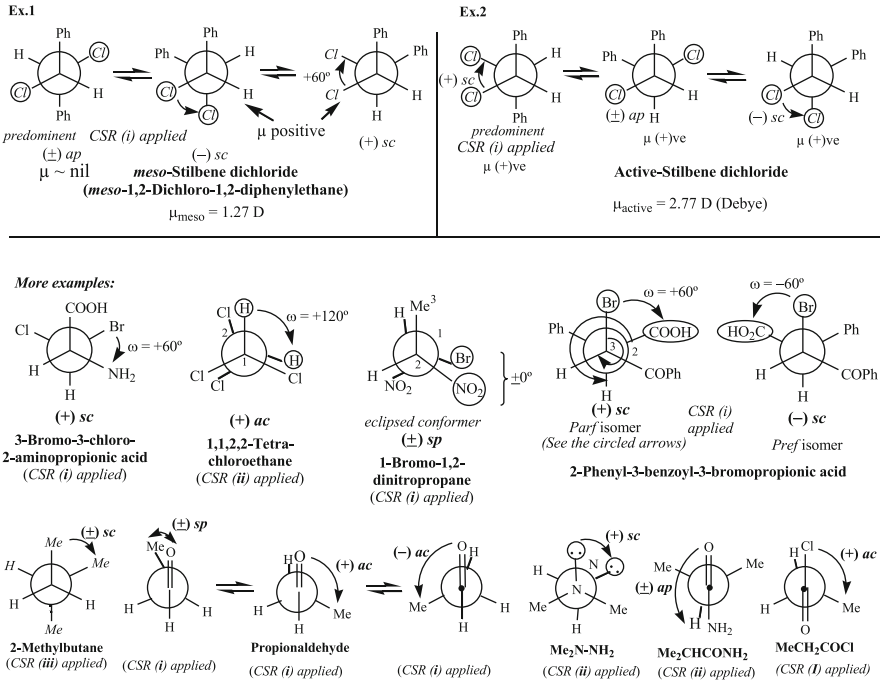


Fig. 2.19 A few examples of Klyne–Prelog nomenclature of torsion angles (*conformational chirality*)

2.3.3 *Torsional Strain Curve (Potential Energy Diagram) of Ethane*

The change of V_ϕ with ϕ or better with $\Delta\phi$ in case of ethane with the conformations having maximum and minimum energies is represented graphically in Fig. 2.20. The energy difference between the eclipsed conformer of maximum torsional strain or potential energy and the staggered conformer of minimum energy, the so-called *torsional or rotational energy barrier*, is only about 3 kcal/mol (12.5 kJ/mol). Hence, rotation around C–C single bond, though not free, is quite facile. Here, the greater the periodicity, the smaller the torsional barrier. For example, in case of nitromethane ($MeNO_2$) periodicity $n = 6$; the torsional energy barrier is only 0.006 kcal/mol.

Recent studies have shown that the high torsional energy of the eclipsed form of ethane and hence *the torsional energy barrier* is not due to van der Waals repulsive steric interaction between the two eclipsed H atoms (the distance between them is 2.3 Å = 0.23 nm, whereas the van der Waals radii of two H atoms is 1.83 Å), or not due to electrostatic interaction between weakly polarized C–H bonds, but it *is due to torsional strain caused by unfavorable overlap interaction between the C–H bond orbitals*. In case of the staggered form (the distance between two nearest H

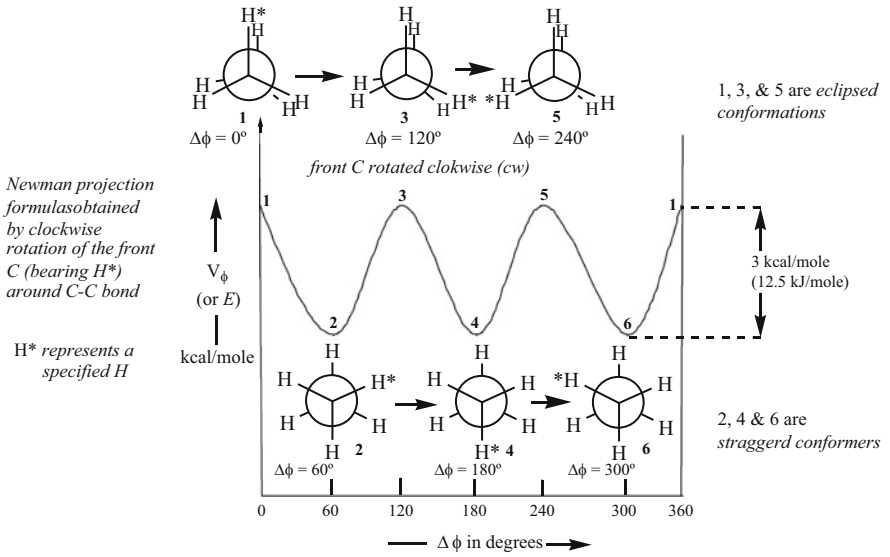


Fig. 2.20 V_ϕ as a function of Δ_ϕ in ethane ($H_3C–CH_3$)

atoms on C1 and C2 is 2.5 Å) favorable interaction between the bonding–antibonding orbitals makes its torsional strain 3 kcal/mol less than the eclipsed conformation and makes it more stable. This energy difference leads at 25 °C to the fact that for each 160 staggered ethane molecules, there is only one molecule of eclipsed ethane, *i.e.*, in negligible proportion.

2.3.4 Torsional Strain Curve of Propane

The torsional strain curve of propane is similar to that of ethane with a slightly higher torsional strain barrier (3.36 kcal/mol = 14 kJ/mol). The small difference between the energy barriers of ethane (Fig. 2.20) and propane (Fig. 2.21) indicates clearly that the torsional energy does not originate from steric effects, as already mentioned. The eclipsing of H with CH_3 in propane is hardly more unfavorable than the eclipsing of H and H in ethane, despite the bulkiness of the methyl group. In case of propane also the periodicity n is 3.

2.3.5 Torsional Strain Curve of Molecules ACX_2CX_2B, n-Butane

Torsional strain curve (V_ϕ as a function of $\Delta\phi$) of a molecule AC(XY)C(XY)B becomes more complex when a given conformation does not recur within a complete torsional displacement ($\Delta\phi = 360°$), *i.e.*., n (periodicity) = 1. The

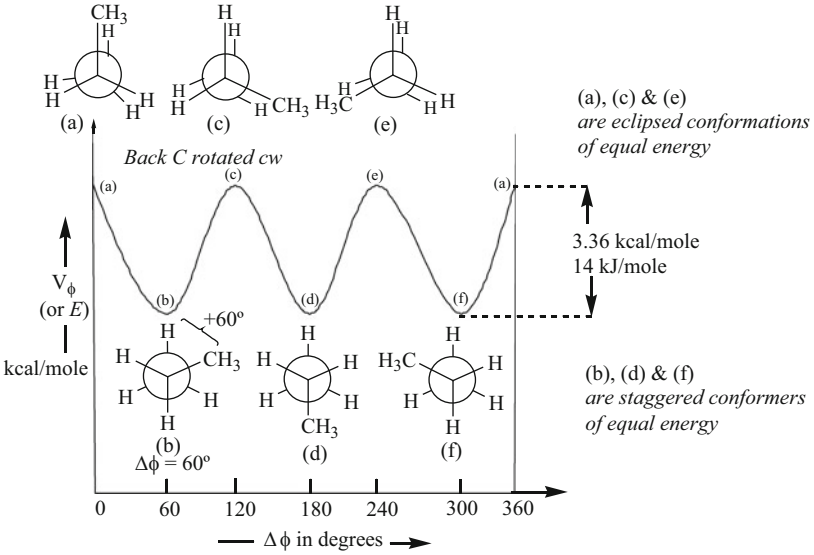


Fig. 2.21 V_ϕ as a function of Δ_ϕ in propane ($H_3C–CH_2–CH_3$)

torsional strain curve of the molecules of butane, $CH_3CH_2CH_2CH_3$, (where A = B = CH_3 and X = Y = H) giving the Newman projection formula of the maximum and minimum energy conformations, is displayed schematically in Fig. 2.20. The diagram shows two types of conformations having maxima: (**a**) and (**c**) = (**e**). The conformation (**d**), called *anti*, represents the energy minimum corresponding to the lowest valley (minimum); the energies of the other conformers (**b**) and (**f**), called *gauche*, corresponding to the other valleys (minima) are measured relative to that of this conformer (**d**), as shown in Fig. 2.22. The conformers, (**c**) and (**e**), representing the steric interaction energy of two CH_3 groups at $\Delta\phi = 60°$ or 300° are destabilized by 0.8-0.9 kcal/mol (~ 3.3 kJ/mol) at room temperature relative to the *anti* conformer (**a**).

This energy difference corresponds to about 1 mol of butane as the *gauche* conformer for every 2 moles of butane as the *anti* conformer. This may be calculated for the equilibrium [gauche] ⇆ [anti], as follows:
$\Delta G° = \Delta H° - T\Delta S° = -0.8$ kcal/mol$-(-$TRln2) = (0.8−0.41 = 0.39 kcal/mol at 27 °C, T = 300 °K, and
Rln2 = 1.38 cal/deg/mol.
Putting the value of $\Delta G°$ in the equation $\Delta G = -$RTlnK
K = [anti]/[gauche] ≈ 2

Thus, it is possible to predict the most stable conformer of a long chain $(-CH_2)_n$, which possesses repeated butane chain, as a *zigzag* planar arrangement of the chain.

The torsional strain curve of other molecules having the same molecular formula ACX_2CX_2B will be of same pattern as that of butane. Only the energy barriers will be different. In *n*-propyl chloride, $CH_3CH_2CH_2Cl$ (A = CH_3, B = Cl, and X = H),

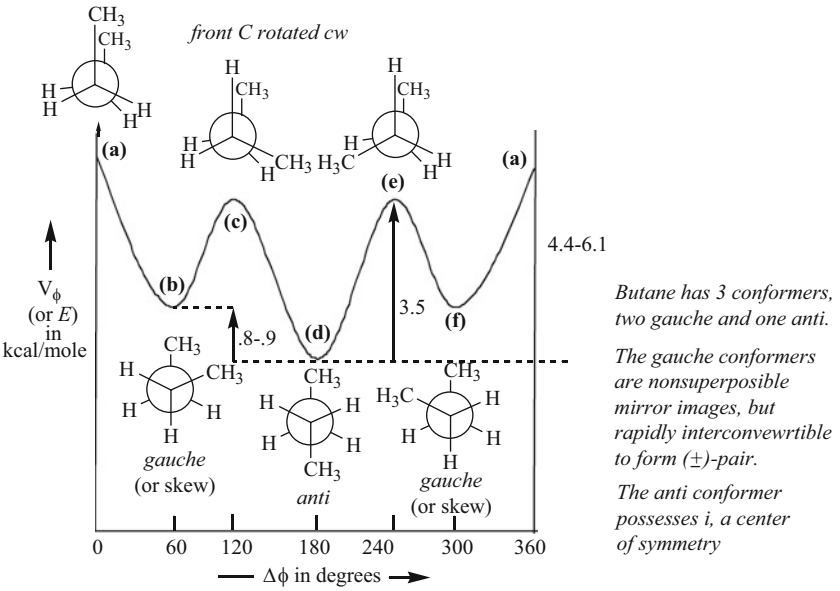


Fig. 2.22 V_ϕ or E of butane as function of Δ_ϕ (displacement of torsion angle)

and 1,2-dichloroethane (A = B = Cl, X = H) the *anti* and gauche conformers have approximately the same energy, whereas in 1,1,2,2-tetrachloroethane and 1,1,2,2-tetrabromoethane (A = B = H, X = Cl or Br), the *gauche* conformer is known to be more stable than the *anti* conformer by ~1.0 kcal/mol, perhaps due to the stabilizing *gauche effect of the two halogen atoms*.

2.4 Configuration. Relative Configuration. Absolute Configuration

Molecules with the same molecular formula and same constitution or bonding connectivities may still be different. The arrangement of the ligands (atoms or groups) in space around the dissymmetric part of a molecule—in the simplest case around an asymmetric center or around the rigid part of a molecule like a double bond—is termed as *configuration*. If there is any dissymmetry in the molecule (lacking a reflection symmetry)—possessing a chiral point group (Sect. 2.2.5)—the molecule will be nonsuperposable on its mirror image and the two mirror image forms (enantiomers) will exhibit opposite optical rotation. When molecules differ only in relative orientation of ligands in space, stereoisomerism results. Stereoisomers having same bond connectivity but different absolute configuration are often called configurational isomers. Most of the natural products and biologically active

molecules occur in specific stereoisomeric forms; their chemical and biological behaviors are controlled by their absolute configuration and also conformation.

In 1780 Scheele isolated (−)-lactic acid [= (−)-α-hydroxypropionic acid] from sour milk. Berzelius isolated (+)-lactic acid from muscle tissues in 1807 (Fig. 2.23). Actually, in 1848 Berzelius established that although they have same structure and same properties, they were not same, but enantiomers (nonsuperposable mirror images) exhibit opposite optical rotation. *Configuration is a theoretical concept* related to the molecular architecture expressed by a three-dimensional or projection formula, *while rotation is an experimental property*, measured in a polarimeter. Configuration is more fundamental than rotation and remain unchanged as long as any bond to the chiral center is not broken; but specific rotation changes with concentration and with solvent; even the sign of rotation may sometimes change with some solvent at different concentrations (*vide* Sect. 2.2.8). Again (+)-lactic acid when dissolved in aqueous sodium hydroxide, the resulting solution is levorotatory and would have to be called a solution of (−)-sodium lactate, although the configuration remains unchanged.

The simplest way to explain relative and absolute configuration is to state that two enantiomers (*e.g.*, of lactic acid) have got the same relative configuration, but have different absolute configurations.

Absolute configuration is specified by (D,L) notation (Sect. 2.6.1) in some cases and by (*R*,*S*) notation universally (Sect. 2.6.2), to be discussed in the respective sections. In a compound with several asymmetric centers, configuration at each center is to be specified in order to specify its configuration completely.

The arrangement of groups (or ligands) about the rigid double bond or ring (*i.e.* the configuration) is expressed as *cis* or *trans* (*Z* or *E*) for double bond compounds (Sect. 2.6.2.6) and *cis* or *trans* for cyclic compounds (Sect. 2.12.4).

The Fischer projection and tetrahedral representations of two common chiral molecules are shown in Fig. 2.23).

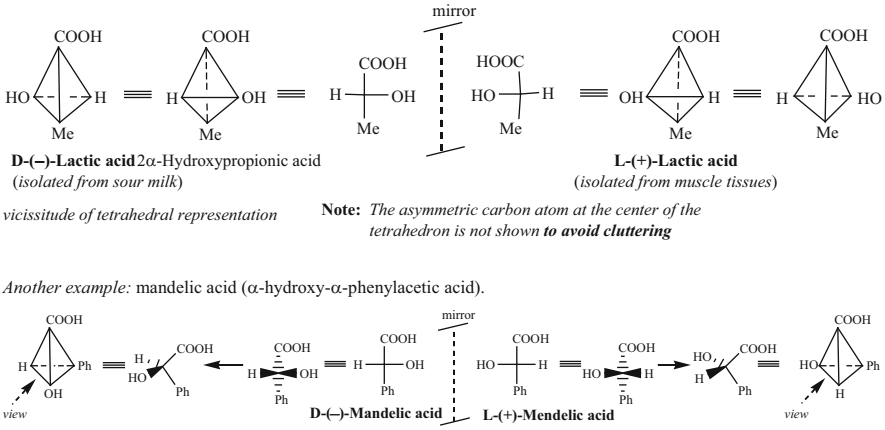


Fig. 2.23 The enantiomers of lactic acid and mandelic acid. Each pair has same relative configuration but different absolute configuration

2.5 Relationship Between Two Molecules of Same Molecular Formula. Homomers, Constitutional Isomers, Stereoisomers, Enantiomers, Diastereomers, Configurational/Conformational Enantiomers/ Diastereomers

Relationship between two molecules of same molecular formula giving rise to different stereochemical or structural terms is delineated schematically with examples in Fig. 2.24. The definition of each term, expressed in bold face, is understood from this figure.

2.6 Configurational Nomenclature

Two different methods are in use for completely specifying the absolute configuration of a chiral center in a molecule.

2.6.1 Fischer's D,L Nomenclature

This oldest system of nomenclature of chiral compounds was introduced by Emil Fischer [3] in 1891, while working with carbohydrates. Rosanoff [6] modified the system in 1906 and suggested the *following conventions* for a *projection* nomenclature of D,L system:

(i) As in Fischer's system, the molecule is written with the longest carbon chain placed vertically.
(ii) The C1 carbon or the most highly oxidized end of the chain is placed at the top, following Fischer's convention, *e.g.*, COOH > CHO > CH_2OH (according to IUPAC also).

Some examples of the use of D,L nomenclature and its extension in some cases are delineated in Fig. 2.25).

(a) The system works well for compounds $RCHXR^1$, when X is a hetero atom (Fig. 2.25). The molecule is D if X is on the right, and L if X is on the left. While writing the Fischer projection phenyl or aryl group should be at the bottom.
(b) In compounds of the type RR^1CXR^2, this system is applicable; D means that *X* is on the right, and the small alkyl group R^1 is to the left. The enantiomer of D is L.

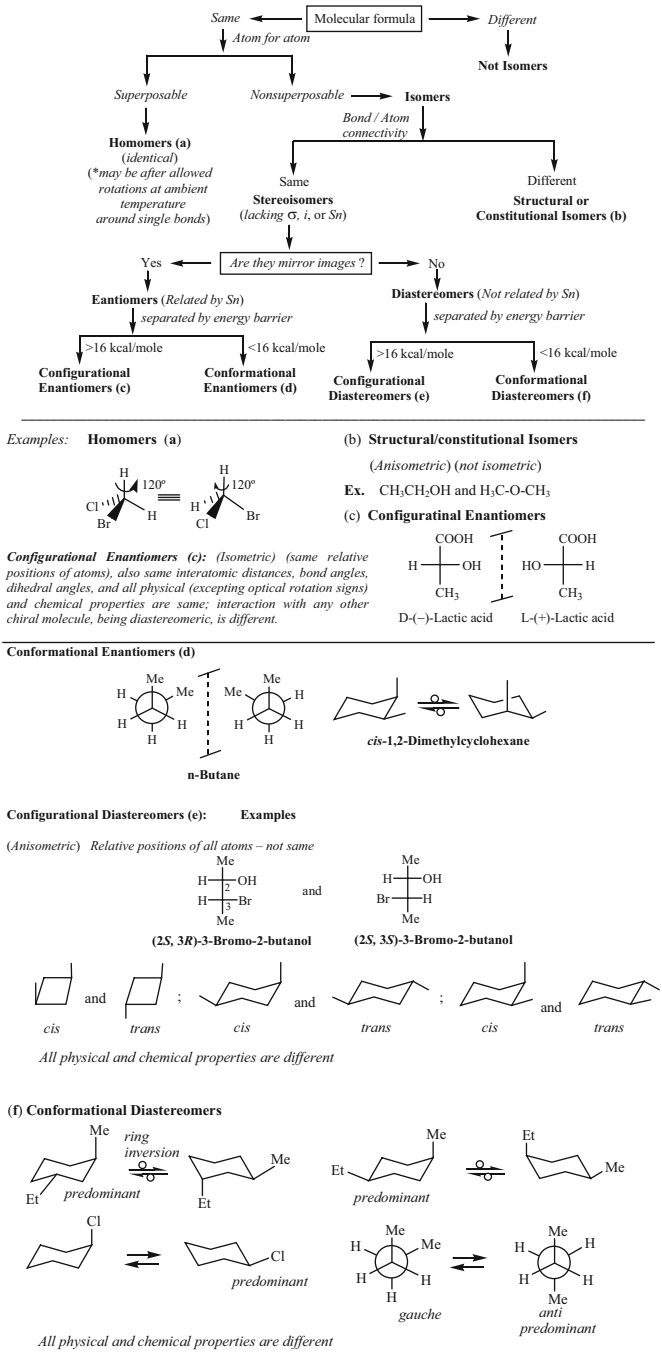


Fig. 2.24 Relationship between two molecules of same molecular formula: different terms with examples

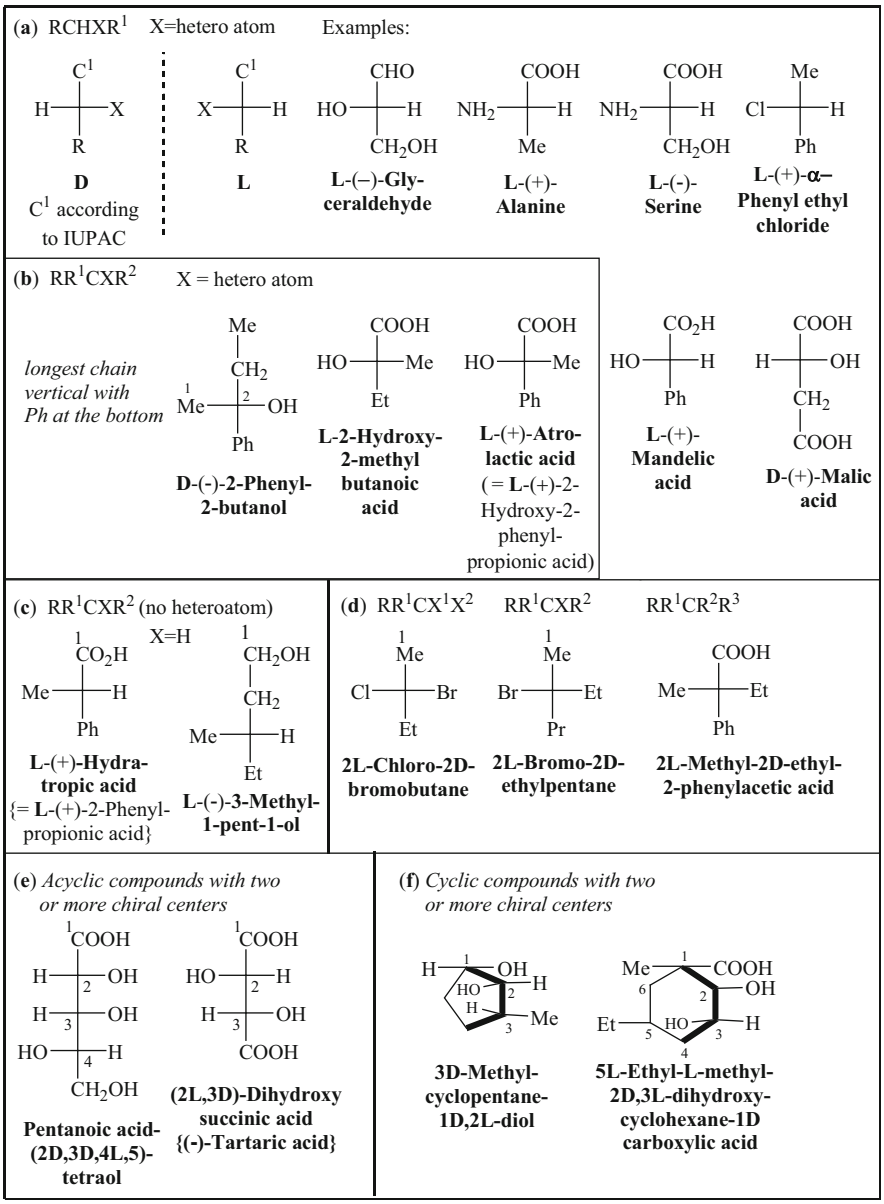


Fig. 2.25 Configurational nomenclature. Use of D and L

(c) If there is no hetero atom, then the smallest alkyl group (usually Me) becomes the fiducial group; if it is on the right it is D, if on the left it is L.
(d) When the chiral center carries two different substituents of comparable electronegativity, D- and L- may be used separately for each substituent to specify the configuration. Thus, the system may be applicable to compounds of the types $RR^1CR^2R^3$, having asymmetric quaternary carbon, *e.g.*, 2L-methyl-2D-ethyl-2-phenylacetic acid.
(e) For compounds containing more than one asymmetric carbon and (f) for compounds possessing asymmetric carbons in rings the *D,L* nomenclature has been extended by Klyne [7], *e.g.*, 3D-methylcyclopentane-1D,2L-diol. Rings are oriented with the edge of the lowest numbers toward the viewer, the main chain being numbered from the top bearing the more oxidized carbon [6] or C1 (Fig. 2.25).
(g) In carbohydrate chemistry, D,L nomenclature is assigned on the basis of configuration of the last chiral center of the chain written vertically counting from the top in the Fischer projection, more oxidized carbon being at the top (Fig. 2.26) [6].

It is to be noted that D,L *(like R,S) nomenclature has no genetic relationship*. Thus, the COOH group of D-hydratropic acid when converted to NH_2 gives L-1-phenylethylamine, although no bond to the chiral center is broken (Fig. 2.27).

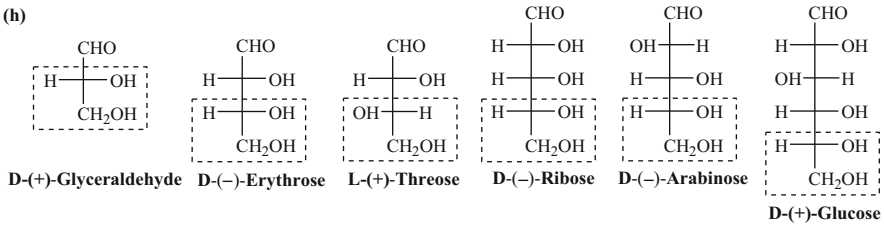


Fig. 2.26 Configurational (D,L) nomenclature, Glyceraldehyde, tetroses, pentoses, and Glucose

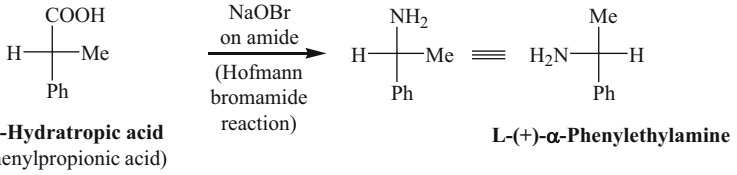


Fig. 2.27 D,L nomenclature has no genetic relationship. Illustration

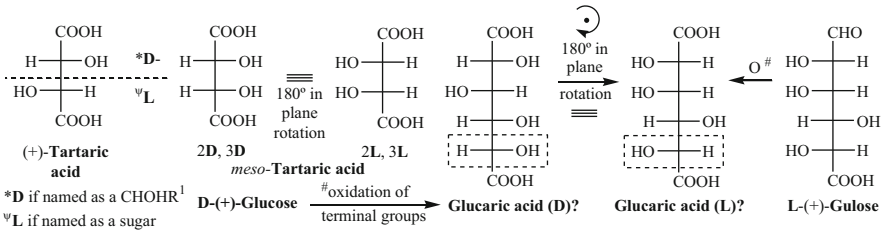


Fig. 2.28 A few ambiguities of D,L nomenclature

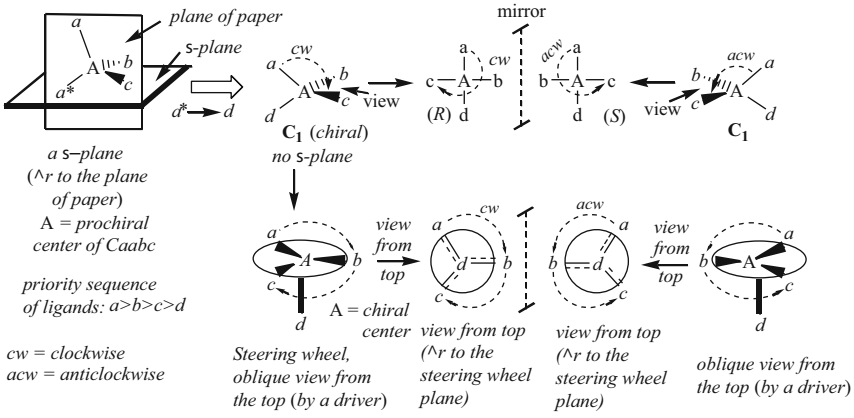
Ambiguities/Shortcomings of **D,L** ***Nomenclature*** (Fig. 2.28)

(i) As already mentioned, too many arbitrary conventions are to be remembered for **D,L** nomenclature of different types of compounds.
(ii) This system cannot be conveniently used for polycyclic compounds.
(iii) Ambiguity arises for **D** and **L** designation of tartaric acid diastereomers (Fig. 2.28).
(iv) The oxidation product of **D-**(+)-glucose may be termed both **D**-glucaric acid and **L**-glucaric acid which is also obtained by oxidation of **L**-(+)-gulose.

2.6.2 R,S *Nomenclature for Absolute Configuration*

The **D,L** nomenclature of Fischer (Sect. 2.6.1) applies to the Fischer projection. In spite of the shortcomings/ambiguities already discussed, the Fischer nomenclature has proved particularly useful for sugars or amino acids. However, one should avoid confusion between *d, l* (dextrorotatory or levorotatory) and **D,L** (absolute configuration). A *d* compound may have the absolute configuration either **D** or **L**, *e.g.*, (*d*)-**L**-alanine and (*d*)-**D**- glyceraldehyde, which are now expressed as **L**-(+)-alanine and **D**-(+)-glyceraldehyde.

As **D,L** nomenclature is not generally useful to some types of compounds with chiral centers mentioned earlier. Cahn, Ingold, and Prelog (CIP) developed *R-S* nomenclature, [5, 8, 9] generally applicable to chiral compounds of all three different types having (i) *a center of chirality*, (ii) *an axis of chirality*, and (iii) *a plane of chirality*. Each of these chirality elements requires a particular method of nomenclature. We will now discuss the CIP (Cahn–Ingold–Prelog) rules for compounds containing one or more chiral centers. Compounds of the types (ii) and (iii) will be discussed in Sects. 2.16 and 2.17, respectively.



When d is on the rear side of view

(i) clockwise (cw) arrow of a→ b→ c defines R-configuration, and

(ii) anticlockwise (acw) movement of a→ b→ c defines S-configuration

Fig. 2.29 Prochiral center. *R*,*S*-Designation of a chiral center (*cf.* steering wheel)

2.6.2.1 *R*,*S* Nomenclature. Center of Chirality

A center of chirality is usually associated with a nonplanar tetracoordinated atom "A" bonded (or ligated) to four different ligands (atoms or groups) or an asymmetric carbon atom, A*abcd*. The latter could be derived from an *achiral* or a *prochiral precursor* A*aabc* by changing a ligand *a* to a new ligand *d* (different from the existing ligands). This precursor possesses a plane of symmetry containing A–b and A–c bonds (Fig. 2.29); the chirality of A*abcd* (point group C_1) is the consequence of the destruction of the plane of symmetry of A*aabc*.

2.6.2.2 Specification of Center/s of Chirality

Two CIP rules are involved: (i) CIP *sequence rule* and (ii) CIP *chirality rule*.

(i) ***CIP Sequence rule:*** Ligands are sequenced by comparing them at each step in bond-to-bond exploration in branched ligands *along the branch path of highest precedence*. This rule will be illustrated by examples.

The following "Standard Sub-rules" are used for finding out the priority sequence of all possible types of compounds—*each to exhaustion in turn* (Fig. 2.30).

Sub-rule 0: Nearer end of axis or side of plane precedes the further end (applicable to chiral axis or chiral plane).
Sub-rule 1: Higher atomic number precedes lower.
Sub-rule 2: Higher atomic mass number precedes lower, *e.g.*, T > D > H $^{14}C > {}^{13}C$
Two ligands varying only by isotopes produce marginal chirality and hence small optical rotation
Sub-rule 3: *Seq cis* (*Z*) precedes *seq trans* (*E*)
Sub-rule 4: *Like pair precedes unlike pair,* > stands for 'precedes' or 'has priority over'; M = minus helicity
RR or *SS* > *SR* or *RS*; P = plus helicity
MM or *PP* > *MP* or *PM*; *RM* or *SP* > *RP* or *SM*
MR or *PS* > *MS* or *PR*; *r* > *s* (in case of achirotopic but stereogenic center)
(Like and unlike pairs are gleaned from the sub-rule 5)
Sub-rule 5: *R* > *S*; *M* > *P*
The priority sequence in the decreasing order, a > b > c > d is assigned to the four ligands of the chiral center.

Fig. 2.30 CIP standard sub-rules for ascertaining the priority sequence of ligands

(ii) ***CIP Chirality rule*** (Fig. 2.29): The path of the sequence of precedence/priority *a*→ *b*→ *c* is followed from the preferred side of the model (containing the three preferred ligands a, b, and c), *i.e.*, remote from the ligand of the lowest precedence *d*. If the path turns *right* (traces clockwise, which is the entire sense of direction of abc) the element is assigned chiral label "*R*" (*rectus,* Latin for right). If the said path turns *left* (traces anticlockwise, which is the entire sense of direction of *abc*) then the stereogenic center is assigned the chiral label "*S*" (*sinister*, Latin for left).

One can imagine that *a, b*, and *c* are placed on a steering wheel of a car, and *d* is placed on the shaft (the axis of the wheel is A-d); if from a position above the wheel one rotates the wheel on the right side (in the clockwise sense a→b→c), the car moves to the *right*, and the absolute configuration is called *R*, whereas in the opposite case, the car moves to the *left* and the absolute configuration is called *S*.

It is evident from Fig. 2.29 that in the Fischer projection formula, if *d* (the least priority group) is at the *bottom* or *top*: *a*→*b*→*c* makes a clockwise movement in the *R*-configuration; *a*→*b*→*c* makes an anticlockwise movement in the *S*-configuration.

R,S-configurational specification of some common compounds is shown in Fig. 2.31.

Corollaries of the Chirality Rule

1. If *d* is on the *right* or *left* side of the Fischer projection formula, or *above* the plane containing the two in-plane bonds in *the flying wedge* formula, the

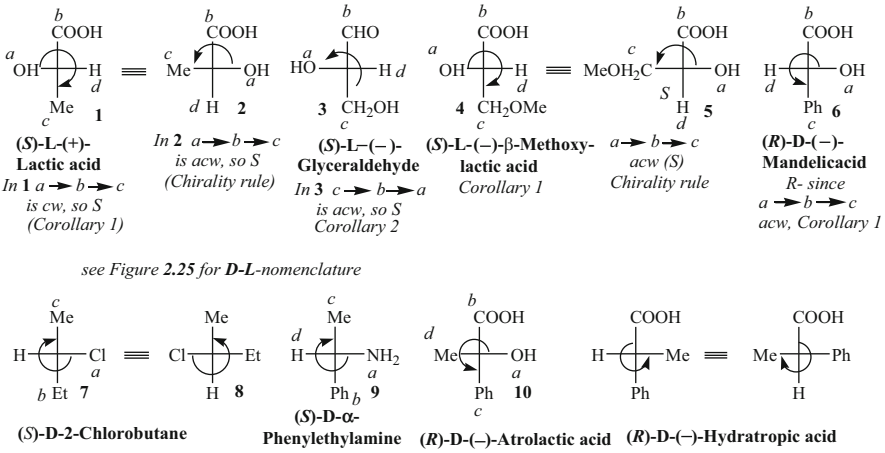


Fig. 2.31 *R-S*-configurational designation of some common chiral compounds. Use of corollaries (**1**) and (**2**) also

anticlockwise movement from $a \rightarrow b \rightarrow c$ (or $1 \rightarrow 2 \rightarrow 3$) will define *R*-configuration, and *clockwise* movement will define *S*-configuration.

2. In the above case, clockwise movement from $c \rightarrow b \rightarrow a$ (or $3 \rightarrow 2 \rightarrow 1$) will define *R*-configuration, and anticlockwise movement from $c \rightarrow b \rightarrow a$ (or $3 \rightarrow 2 \rightarrow 1$) will define *S*-configuration.

The sequence sub-rules are applied considering different aspects, as needed:

1. Sub-rule 0 gets the topmost priority but is applicable to axially dissymmetric compounds to be discussed later (Sect. 2.16).
2. If the atoms of two ligands attached to the asymmetric tetracovalent atom (like C, N, P, Si, etc.) are the same, their respective states of substitution are considered. If the second atom gives no choice, third, etc., is to be considered, following *along the branches with closer atoms of higher atomic number*.
3. If the two atoms directly linked to the asymmetric atom are different, the atoms of higher atomic number gets precedence, *e.g.*, $I > Br > Cl > S > P > Si > F > O > N > C$
4. If the atoms of two ligands attached to the asymmetric atom are same, that with more substituents of higher atomic number gets precedence

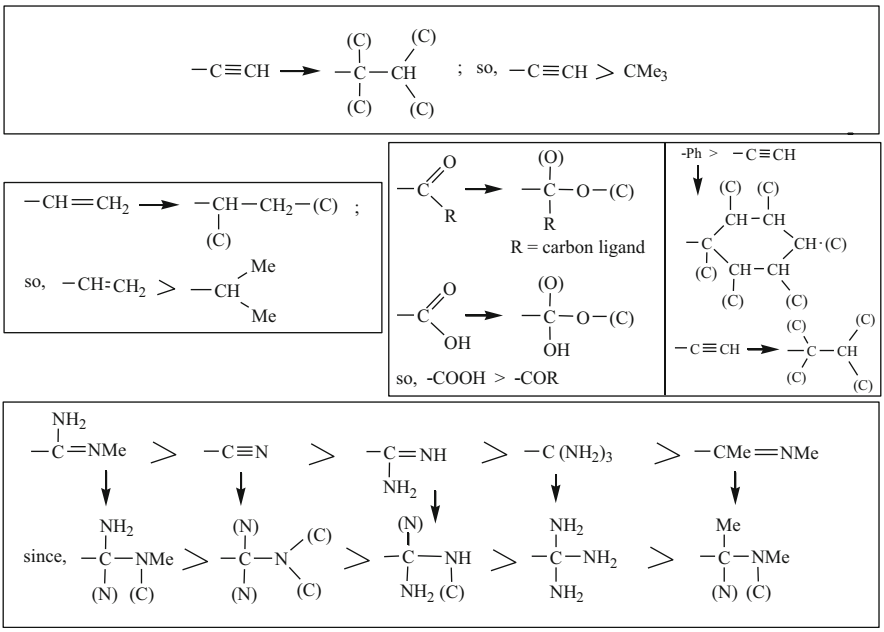


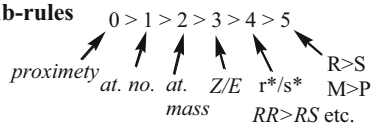
Fig. 2.32 Multiple bonded atoms treated as 4-coordinated ones for determining priority sequence

5. If the ligands attached to the asymmetric center are composed of only C and H atoms, then *the ligand with less number of H atom gets priority*.
   For example: $-CR^{1R2R3} > -CHR^{1R2} > -CH_{2R}{}^{1} > CH_3$
   (here R^1, R^2, R^3 may be same or different alkyl or aryl substituent)
6. *Multiple bonded atoms* are treated as four-coordinated ones by adding replica atoms of the same type (duplications or triplications) which are bracketed to signify that they are surrounded by phantom atoms of atomic number zero. This is illustrated by some examples given in Fig. 2.32.
   Table 2.4 contains some of the most common groups in the decreasing order of precedence obtained by applications (2–6) of the sequence sub-rules 1 and 2.
7. A few more examples of *R-S* designation of compounds with several asymmetric atoms (written as Fischer projection or flying wedge formulas) have been included in Fig. 2.33.

Table 2.4 Descending order of priority of some common ligands according to the sequence rules

1. $-I$	25. $-NH_2$	49. $-Ph$
2. $-Br$	26. $-CX_3$	50. $-C{\equiv}CH$
3. $-Cl$	27. COX	51. $-CMe_3$
4. $-PR_2$	28. $-CO_2CMe_3$	52. $-CH{=}CH{-}Me$
5. $-SO_3H$	29. $-CO_2Ph$	53. $-C_6H_{11}$
6. $-SO_2R$	30. $-CO_2CH_2Me$	54. $-CH(Me)Et$
7. $-S(O)R$	31. $-CO_2Me$	55. $-CH{=}CH_2$
8. $-SR$	32. $-CO_2H$	56. $-CHMe_2$
9. $-SH$	33. $CONH_2$	57. $-CH_2Ph$
10. $-F$	34. $-COPh$	58. $-CH_2C{\equiv}CH$
11. $-OSO_2R$	35. $-COMe$	59. $-CH_2{-}CMe_3$
12. $-OCOR$	36. $-CHO$	60. $-CH_2CH{=}CH_2$
13. $-OR$	37. $-CR_2OH$	61. $-CH_2CHMe_2$
14. $-OH$	38. $-CH(OH)R$	62. $-CH_2CH_2Et$
15. $-NO_2$	39. $-CH_2OR$	63. $-CH_2CH_2Me$
16. $-NO$	40. $-CH_2OH$	64. $-CD_2Me$
17. $-N^+R_3$	41. $-CN$	65. $-CHDMe$
18. $-NR_2$	42. $-CH_2NH_2$	66. $-CH_2Me$
19. $-NHCOPh$	43. $-C_6H_4Me(o)$	67. $-CD_3$
20. $-NHCOR$	44. $-C{\equiv}CMe$	68. $-Me$
21. $-NHCH_2Me$	45. $-C_6H_4NO_2(m)$	69. $-T$
22. $-NMe_2$	46. $-C_6H_4Me(m)$	70. $-D$
23. $-NHR$	47. $-C_6H_4NO_2(p)$	71. $-H$
24. $-N^+H_3$	48. $-C_6H_4Me(p)$	72. Electron pair

2.6.2.3 Priority Sequence of the Application of the CIP Sub-rules



Sub-rule O (proximity rule) enjoys the topmost priority in cases of axial chirality.

Few *examples of the priorities of the sub-rules are provided in Fig.* 2.34.

2.6.2.4 Modification of Sub-rule 3

The sub-rule 3 has been modified by Prelog and Helmchen [10] in 1982 by an alternative proposal. The modified sub-rule and some examples illustrating it are given in Fig. 2.35.

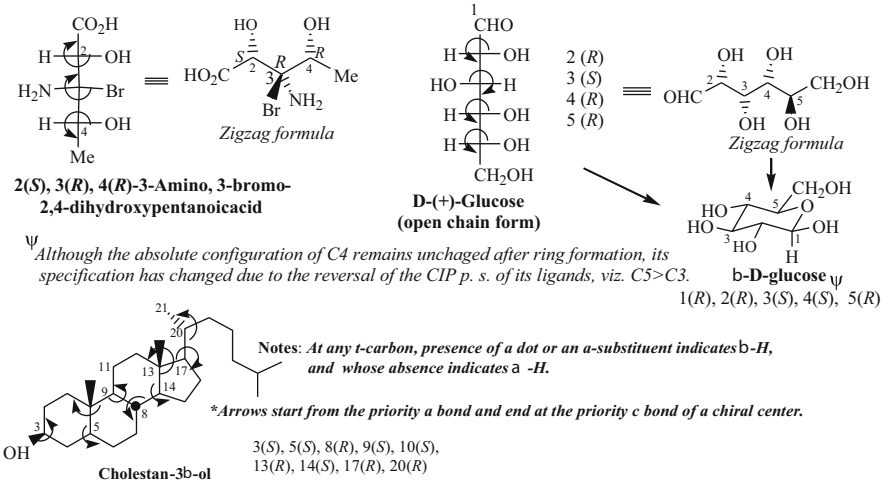


Fig. 2.33 *R-S* Designation of compounds with multi-chiral centers and monocyclic and polycyclic compounds

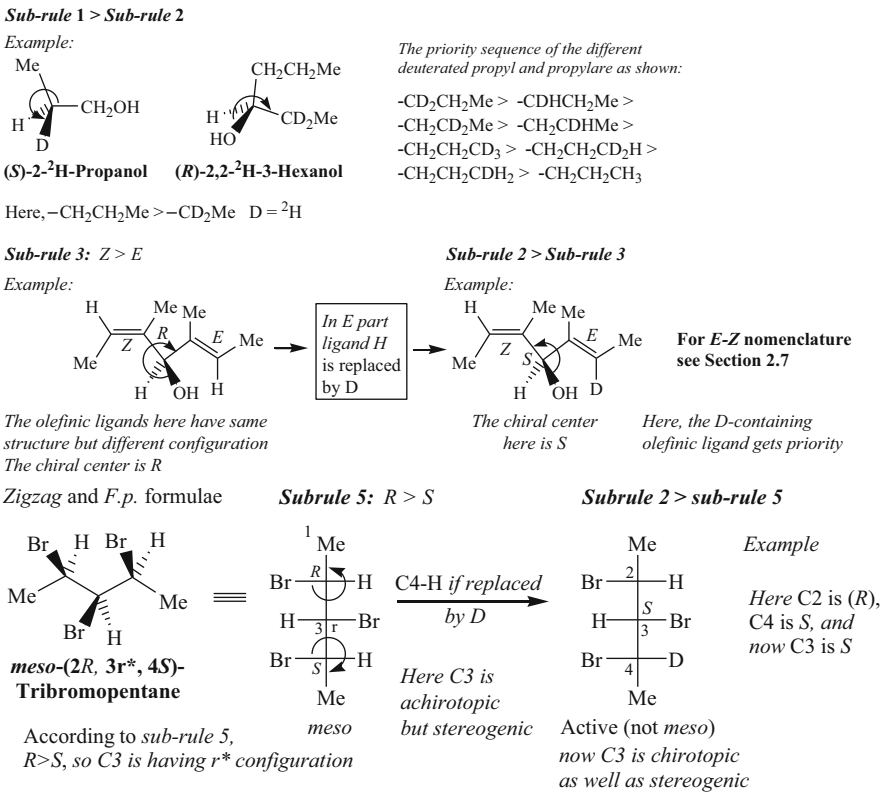
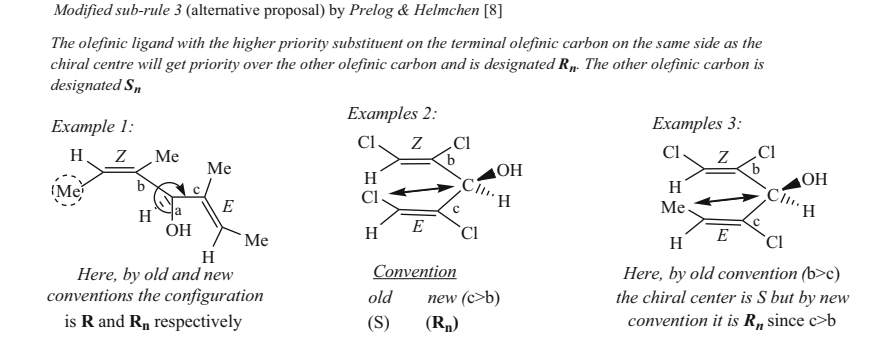
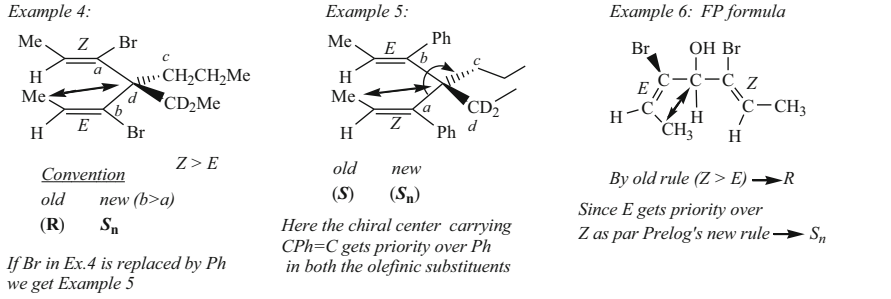


Fig. 2.34 Priority of some sub-rules over others. *R-S*-nomenclature of compounds with multi-chiral (here 3) centers from zigzag formula



Notes: *Old convention*: Z>E

New convention: In either example 2 or example 3, the lower part of the olefinic substituent carrying Cl in example 2 and Me in example 3 on the same side of the double bond as the chiral center, gets priority over the upper olefinic substituent, which has Z-configuration.



Note: *Intervening CH_2 or CH_2's will not change the chirality specification*

Fig. 2.35 Modification of sub-rule 3 by Prelog and Helmchen. Several examples to show chirality specifications

2.6.2.5 R* and S* Nomenclature

When *R*-*S* nomenclature is applied, in a molecule with multiple chiral centers both the relative and the absolute configurations are fixed, as has been illustrated in many chiral molecules so far. For examples, 2(*R*)-bromo-3(*S*)-hydroxybutane refers to the enantiomer (**1**) (Fig. 2.36).

It often happens that *a pure enantiomer is reported with known relative configuration but unknown absolute configuration*. In such cases the *R*-*S* system of nomenclature is modified as follows (IUPAC Commission 1976) [11]. The atoms are numbered such that the chiral center carrying the highest priority ligand, *e.g.*, C-Br in (**1**) or (**2**) or C-I in (**3**) is given the lowest number (lowest locant). The molecule is written in such a way that the lowest chiral locant gets the *R* configuration. The other chiral centers are then assigned in the usual way, and each descriptor is asterisked (pronounced as *R*-star or *S*-star, indicating thereby that they represent relative configuration). Thus, 2(*R**)-bromo-3(S*)-hydroxybutane or

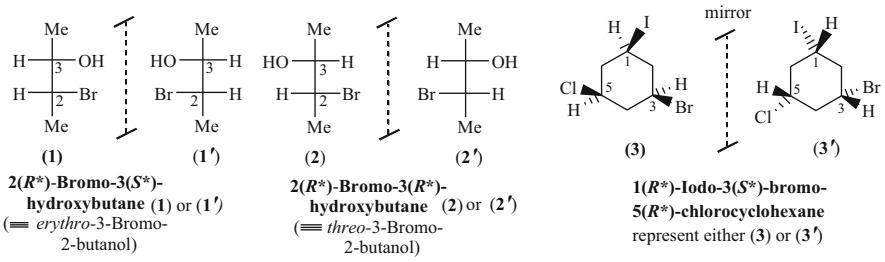


Fig. 2.36 *R**-*S** Nomenclature of some compounds of known relative configuration

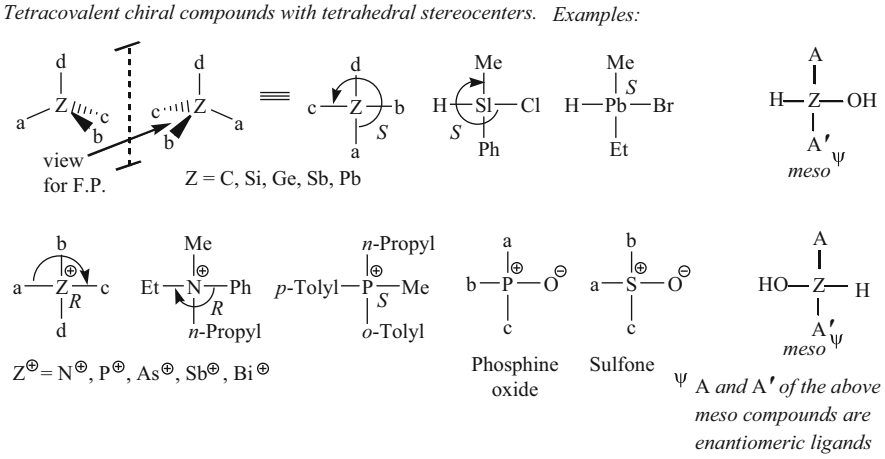


Fig. 2.37 Tetrahedral stereocenters of tetracovalent chiral compounds

simply *erythro*-3-bromo-2-butanol represents either compound (**1**) or its mirror image (**1′**); 2(*R**)-bromo-3(*R**)-hydroxybutane (or threo-3-bromo-2-butanol) represents either (**2**) or (**2′**); and 1(*R**)-Iodo-3-(*S**)-bromo-5(*R**)-chlorocyclohexane represents either (**3**) or (**3′**).

2.6.2.6 Specification of Other Tetracovalent Chiral Atoms

If four different achiral ligands are attached to a tetracovalent atom other than carbon, *e.g.*, Si, Ge, As^+, P^+, N^+, the resulting compounds also possess stereogenecity (*R*,*S*) as well as chirotopicity; they will have non-superposable mirror images and hence they are optically active. However, such tetracovalent atom carrying two chiral ligands of same structure but of opposite configuration will be still *stereogenic* (*r*,*s*) but *achirotopic*, making the compound *meso* (Fig. 2.37).

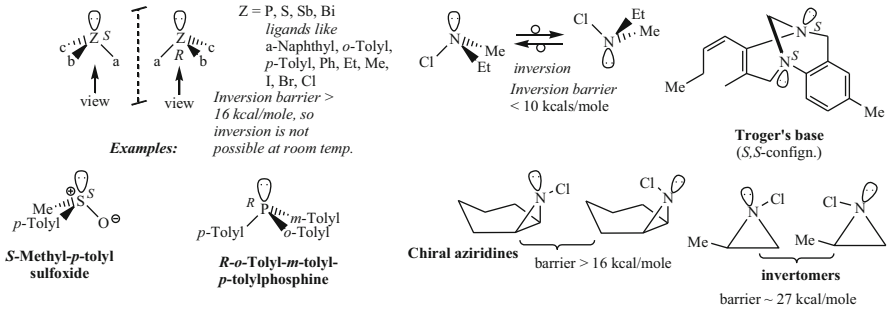


Fig. 2.38 Pyramidal stereocenters of tricovalent chiral compounds

2.6.2.7 Specification of Tricovalent Chiral Compounds (*with pyramidal stereocenter*)

Tricovalent chiral compounds with P, As, Sb, Bi, etc. at the stereocenter are incapable of inversion of the pyramidal stereocenter to form their mirror images (due to their high energy barrier), and hence exhibit optical isomerism. The barriers to nitrogen inversion are usually far too low to permit isolation of the two stereoisomers. Since the inversion of the sp^3 hybridized nitrogen pyramid involves an sp^2 hybridized transition state, increase of *p* character of the bonds to N slows down the rate of inversion as in substituted aziridines (internuclear angle 60°–90°); the rate of inversion is also slowed down by electronegative substituent like Cl. These two factors cooperate in substituted aziridines to increase the energy barrier between the two isomers, which are also called *invertomers* (Fig. 2.38), permitting their isolation. In Troger's base (Fig. 2.38) the N atoms are present at the bridgeheads and hence pyramidal inversion is not possible without bond cleavage.

2.6.3 Stereochemistry of Alkenes. E,Z *Nomenclature [12–14]*

(a) **Stereochemistry**. The C–C σ bond strength is about 83 kcal mol^{-1}, and the strength of the π bond is only 62 kcal mol^{-1} due to its less favorable lateral overlap; addition of these two numbers gives the generally accepted total energy of a C = C double bond as 145 kcal mol^{-1}, much more than the rotational barriers in alkanes (*e.g.*, 3.6 kcal mol^{-1} for C2–C3 bond in butane). During the process of rotation of the *Z* to the *E* isomer or *vice versa*, the *p* orbitals of the two olefinic carbon atoms become orthogonal, with no overlap. Thus, the π-bond is completely broken in the transition state.

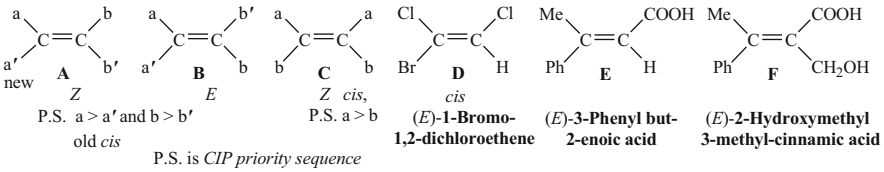
The bond length of C = C in unstrained unconjugated ethenes ranges from 1.335 to 1.35 Å (133.5–135 pm), but is extended in conjugated alkenes in which C = C bond is weakened. The bond angle of substituted olefins is not 120° and

varies. Electron diffraction showed that H–C–H angle of ethane is 117.8°. In propene the C = C−C angle is 124.3° and the C = C−H angle is 119°. In *cis*-2-butene the C = C−C angle is 125.8°. The Me−C−Me angle in 2-methylpropene, Me−C(Me) = CH_2, is 115.3°. Apparently, R−C−R′ angle in RR′C = C is generally smaller than 120°, while R−C = C angle is larger.

(b) **Nomenclature.** *Cis-trans* isomerism in substituted olefins, earlier called geometrical isomerism, is now regarded as a type of diastereomerism, since *cis* and *trans* isomers are optically inactive (if all olefinic ligands are achiral) stereoisomers and are not enantiomers. Since this isomerism owes its existence to the presence of a π-bond, it is called *π-diastereomerism*, to distinguish it from σ-*diastereomerism*, exhibited by cyclic compounds. This terminology, however, is not commonly used.

For molecules of the type C_{ab}=C_{ab} or C_{ab}=C_{ac}, the terms *cis* and *trans* are unambiguous. But if all four substituents are different, this nomenclature leads to ambiguity. This problem is solved by arranging the pair of ligands at each olefinic carbon in CIP sequence. If the groups of higher priority are on the same side the configuration is *seq-cis*, later replaced by the symbol *Z* (from the German *zusammen* meaning "together"); if they are on the opposite sides, the configuration is *seq-trans*, replaced by the symbol *E* (from the German *entgegen* meaning "opposite" [12]. Examples illustrating application of the rules for assignment of *E* and *Z* are shown in Fig. 2.39.

The *E-Z* Nomenclature is always applicable and unequivocal, including cases where *cis* and *trans* nomenclature becomes ambiguous (Fig. 2.40). The *E-Z* system is useful for unambiguous nomenclature [13, 14] of oximes (such as **F′** and **G**) and of compounds containing non-cumulated (conjugated) double bonds (such as **H** and **I**), and of cumulenes with odd number of cumulated double bonds with two = C_{ab} as terminal groups (such as **J**), as illustrated in Fig. 2.40. The compounds **F′** and **G** are *E* and *Z* isomers rather than *anti* and *syn* isomers (old nomenclature), respectively. In case of compounds **H** and **I**, the *locants must be used in conjunction with the E,Z-descriptors*. In the cumulenes with odd number of double bonds (planar, achiral) (three double bonds in case of **J**), the two ligands in each terminal carbon are in the same plane (cf. olefin) and hence *E-Z* nomenclature is applicable to them also. Thus, the cumulene **J** is a *Z*-isomer.



Notes: • *The descriptors (E) and (Z) are always italicized and is placed in parenthesis in front of the name*
• *Structure* **D** *reveals that E and Z do not always correspond to trans and cis respectively. Compound* **E** *and* **F** *have (E) stereochemistry, but they cannot be assigned cis or trans.*

Fig. 2.39 (*Z*)- and (*E*)-alkenes. Unambiguous nomenclature for any substituted olefin

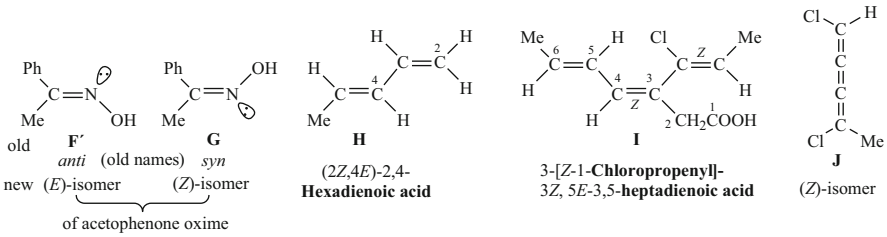


Fig. 2.40 Additional examples of *E*-*Z* nomenclature

2.7 Projection (Fischer, Newman, Sawhorse) and Perspective (Flying Wedge and Zigzag) Formulas of Molecules with Two or More Chiral Centers. Working out Stereoisomers

2.7.1 Molecules with Two Unlike (Unsymmetrical) Chiral Centers (AB Type)

Many natural products, *e.g.*, steroids, terpenoids, alkaloids, and carbohydrates contain two or more chiral centers, the stereochemistry of which should be thoroughly understood. An acyclic molecule containing two or more chiral carbons is constitutionally unsymmetrical if the two end groups are nonequivalent, $R(Cab)_n R^1$, where $n \geq 2$. Interconversion of Fischer projection, Newman projection, sawhorses, and flying-wedge formulas of $RCabCabR^1$ is depicted in a tabular form in Fig. 2.41. The conventions followed in writing the sawhorse and flying-wedge formulas will be revealed from their careful inspection.

Designation of Diastereomers. Several systems of designation of AB type diastereomers (Fig. 2.42) are now known of which a few are discussed below:

2.7.1.1 *Erythro* and *Threo* Nomenclature

A compound containing two adjacent nonequivalent chiral centers, $RCabCabR^1$, *i.e.*, of AB type, gives rise to two diastereomeric (±)-pairs, in total 4 stereoisomers (Fig. 2.42), *e.g.*, the aldotetroses, erythrose and threose (Fig. 2.42), and 3-bromo-2-butanol (Fig. 2.43), each one contains two nonequivalent chiral centers.

Erythro isomer: Examining different two-dimensional (Fischer projection, Newman, and sawhorse) and three-dimensional (flying wedge) formulas of an enantiomer of the *erythro* isomer of $R^1CabCabR^2$ in Fig. 2.41, one can define an *erythro* isomer in one of the following alternative ways:

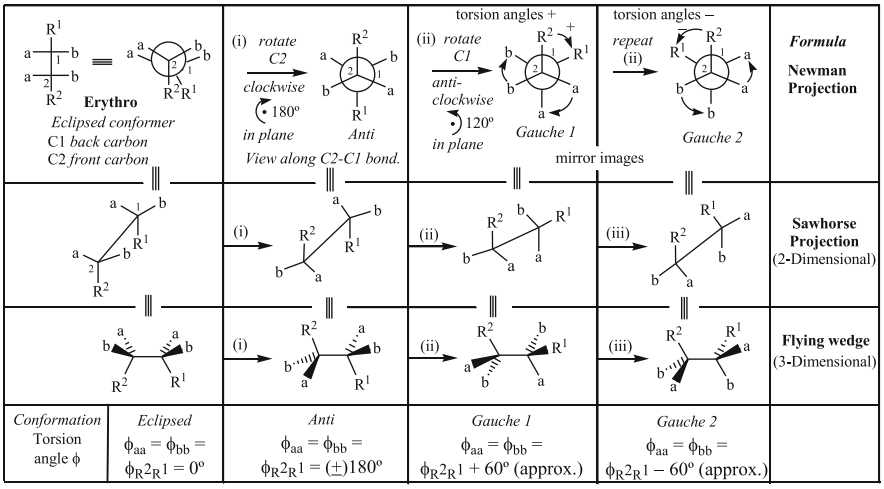


Fig. 2.41 *I*nterconversion of Fischer, Newman, sawhorse, and flying-wedge formulas of *erythro*-$R^1CabCabR^2$

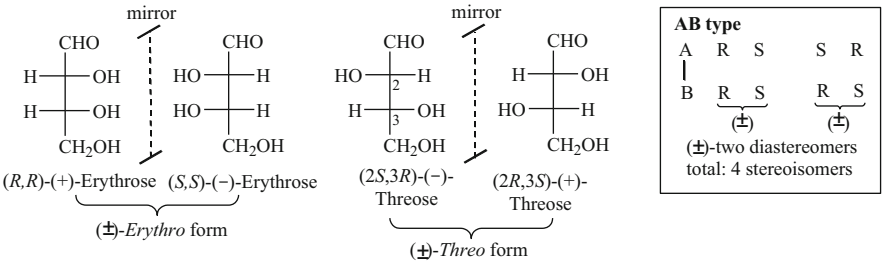


Fig. 2.42 Fischer projection formulas of (±)-erythrose and (±)-threose (AB type)

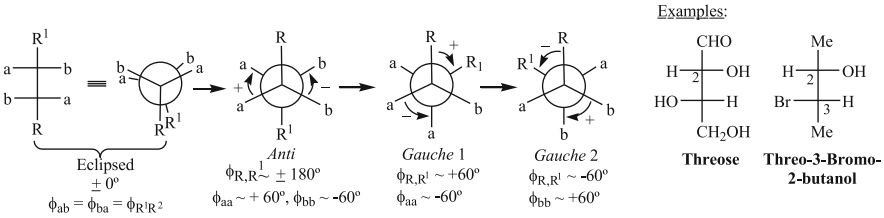


Fig. 2.43 One enantiomer of *threo*-$R^1CabCabR^2$; Fischer and Newman projection formulas; Conformers. Examples.

(i) "At a glance" nomenclature is possible in the Fischer projection (F.p.) formula having the main chain vertical in which the *erythro* diastereomer will *have both horizontal pairs of matched like (or similar) groups (or ligands)*, (*e.g.*, two OH groups and two H's in erythrose) *on the same side*, *i.e.*, *they are*

eclipsed (Torsion angle $\phi \approx \pm 0^\circ$); the other pair of ligands (CHO and CH_2OH in erythrose) will also be eclipsed.

(ii) In the Newman projection, sawhorse, and flying wedge formulas of the eclipsed conformer ($\equiv$ F.p. formula), *at least two pairs of like (or similar) ligands are eclipsed* ($\phi \approx \pm 0^\circ$) (*vide* first column of Fig. 2.41).

(iii) In the *anti* conformer (second column of Fig. 2.41) *at least two pairs of like (or similar) or identical ligands are antiperiplanar* ($\phi \approx \pm 180^\circ$).

(iv) In any of the two gauche conformers (third and fourth columns of Fig. 2.41), *each pair of the two like (or similar) or identical ligands will be gauche* having $\phi \approx +60^\circ$ (third column) or -60° (fourth column).

Threo isomer: The diastereomer of *erythro* $RCabCabR^1$ is called *threo* isomer. It will have (by default) the following characteristics. Figure 2.43 shows only the Fischer and Newman projection formulas, from which sawhorse and flying formulas can be written (*cf.* Fig. 2.41).

The different formulas of *threo* isomer results by *exchange of any two ligands* (*e.g.*, a and b) *at any one chiral center* (C1 or C2) of the *erythro* isomer in the Fischer, Newman, and sawhorse projection or flying wedge formulas (*cf.* Fig. 2.43).

(v) In the F.p. formula (having the main chain vertical) the *threo* diastereomer *will have both horizontal pairs of unlike (or dissimilar) ligands* (*e.g.*, the OH and H in threose) *on the same side*, *i.e.*, they are eclipsed ($\phi \approx \pm 0^\circ$). Hence, both pairs of like (or similar) ligands will be on the opposite side.

(vi) In the Newman projection formula (sawhorse and flying wedge formulas are not shown) of the eclipsed conformer ($\equiv$ F.p. formula), at least two pairs of unlike (or dissimilar) ligands at the chiral carbons will be eclipsed, having torsion angle $\phi = \pm 0^\circ$.

(vii) In the *anti* conformer of Newman projection formula, two pairs of like (or similar) ligands *will not be antiperiplanar* having $\phi = \pm 180^\circ$.

(viii) In either gauche conformer the *torsion angle* ϕ *between two pairs of like* (or similar) *ligands will be of opposite sign*.

The above statements for recognizing the *threo* isomer are illustrated in Fig. 2.43. The *erythro–threo* nomenclature is possible for compounds of the type $RCabCacR^1$. Of course, the easiest way to identify the *erythro* and *threo* isomers is to examine their Fischer or Newman projection formulas.

Threo diastereomer: (*compare with the first row of Fig.* 2.41)

The ambiguity in threo–erythro nomenclature arises in cases **A** and **B** (Fig. 2.44), when at least all of R, R^1, x, and y are alkyls or aryls, when matching of the two pairs of ligands at the two chiral centers is not possible; examples are cases **C** and **D**. However, this nomenclature in cases **A** and **B** is still possible if x and y are ligands with hetero atoms such as OR, NR^1R^2, or halogen, and R and R^1 are alkyl or aryl groups (examples **E**, **F**, **G**).

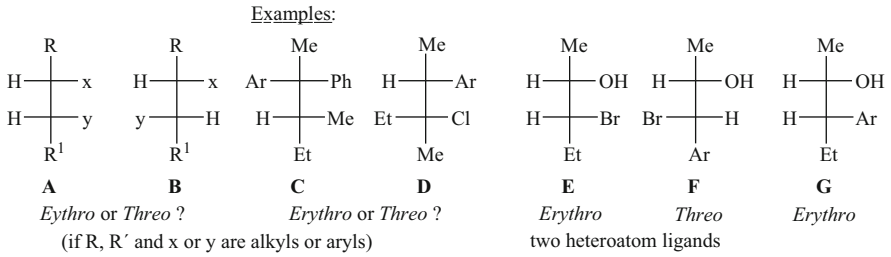


Fig. 2.44 Some cases where ambiguity arises (**a**–**d**) or does not arise (**e**, **f**, **g**)

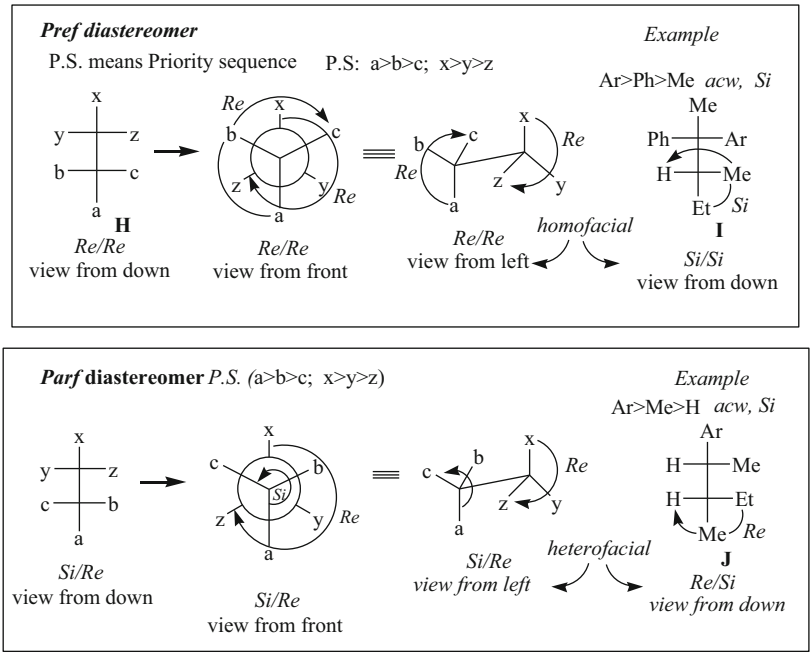


Fig. 2.45 Delineation of *pref* and *parf* diastereomers (one enantiomer of each)

2.7.1.2 "*Pref*" and "*Parf*" Nomenclature

In cases like **A** and **B** (Fig. 2.44), and also in the general case **H** (Fig. 2.45), when only one or no pair of ligands on the two chiral carbons can be matched the ***pref–parf*** nomenclature, developed in 1982 by Carey and Kuehne [15], can be used conveniently. The original version of this nomenclature has been simplified in the following way: Three ligands at each chiral center (case **H**) are to be ordered by CIP priority sequence rules as a > b > c and x > y > z. One does this by viewing the ligands from any one side of the F.p. formula joining the two chiral centers, say,

from bottom/down side. If the three ligands on each chiral center appear in the clockwise (*Re*), or anticlockwise (*Si*) order, when viewed from any end, the relative configuration may then be specified as *Re*/*Re* (or *Si*/*Si*) for one diastereomer and as *Re*/*Si* (or *Si*/*Re*) for the other. The former case is denoted by Carey and Kuchne system as ***pref*** (***p***riority ***ref***lective) and the latter case is denoted by ***parf*** (***p***riority ***a***nti***r******ef***lective) (Fig. 2.45).

For an AB system usually *erythro* isomer corresponds to the *pref* isomer, but the *erythro–threo* nomenclature has no correspondence with the *pref–parf* nomenclature. For example, compound K (Fig. 2.46) appears to be *erythro* isomer by convention, but *parf* according to *pref–parf* system (Fig. 2.46). In fact, *pref–parf* nomenclature is used only when *threo–erythro* nomenclature is not possible. For *pref–parf* nomenclature of compounds having more than two chiral centers, *e.g.*, (**E**) and (**F**), see Fig. 2.49.

An ***advantage*** of the ***pref–parf*** system is that it can be used for specifying relative configuration of two noncontiguous chiral centers, assuming that they are directly linked, disregarding the intervening achiral centers (Fig. 2.47).

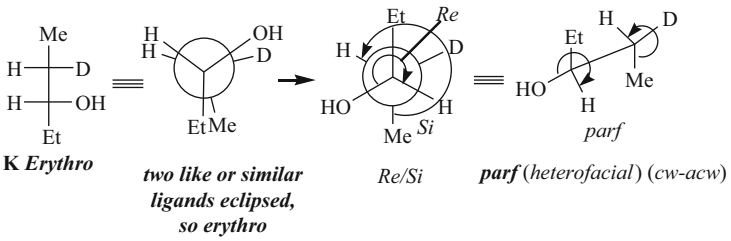


Fig. 2.46 No correspondence between *erythro–threo* and *pref–parf* nomenclatures

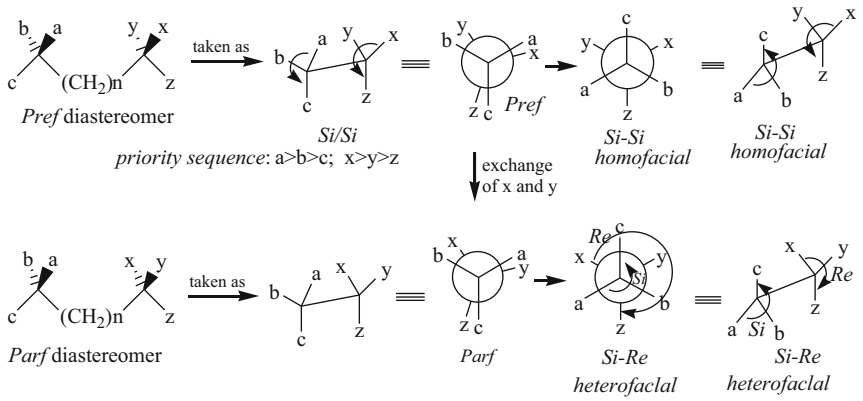


Fig. 2.47 *Pref–parf* specification of two diastereomers having two noncontiguous chiral centers

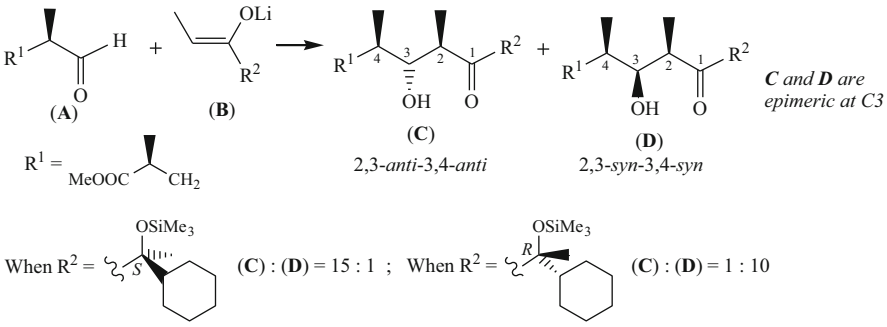


Fig. 2.48 Stereoselective formation of chiral aldols with multiple chiral centers and *syn, anti* designations

2.7.1.3 *Syn* and *Anti* System

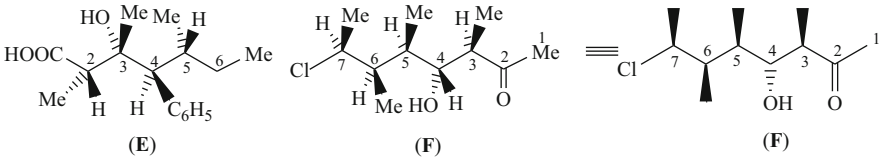
This simple system of nomenclature of relative configuration has been introduced by Masamune [16] for aldol type compounds containing multiple chiral centers. Thus, the chiral aldehyde (**A**) was transformed stereoselectively into the diastereomers 3,4-*anti*-aldol (**C**) and into 3,4-*syn*-aldol (**D**) in different ratios using Li-enolates of different ketones (**B**) (Fig. 2.48). The longest carbon chain is written in a zigzag fashion. If two substituents (usually alkyl and hydroxyl) are on the *same side of the carbon chain plane*, the prefix *syn* is used. If they are on the *opposite side*, the prefix *anti* is used, as illustrated in Masamune's stereoselective aldol formation (Fig. 2.48).

2.7.1.4 *Like* (*l*) and *Unlike* (*u*) System

Prelog and Helmchen [10] have proposed a similar system of designation for the relative configuration of acyclic molecules with multiple adjacent chiral centers, in terms of the *R,S* designation of the adjacent chiral centers. If the adjacent chiral centers of the diastereomer are of like chirality (*R*R**) or of unlike chirality (*R*S**), they are termed *l* (like) or *u* (unlike) respectively, starting from the lowest numbered chiral center (locant). Here R*R* and R*S* refer to relative stereochemistry (Sect. 2.6.2.4). This is illustrated by compounds (**E**) and (**F**) containing four and five consecutive chiral centers (Fig. 2.49) which are represented by prefixes *u u u* and *l l l u*, respectively.

2.7.1.5 Brewster's System of Nomenclature

A general stereochemical notation, balanced in its emphasis in geometry (here relative configuration) and topography (here absolute configuration), has been developed in 1986 by Brewster [17] from the *R-S* (R*S*) system "by use of *ul*



(**E**) (**F**) (**F**)

2*S*, 3*R*, 4*S*, 5*R*
u *u* *u* (Prelog)
Represents (**E**) or its enantiomer (**E′**).
S (2*l*, 3*u*, 4*l*, 5*u*) Represents (**E**) (Brewster).
R(2*l*, 3*u*, 4*l*, 5*u*) Represemts (E') Brewster).
2,3-*pref*, 3,4-*pref*, 4,5-*parf* {*Si/Si, Si/Si, Si/Re*}
Represents (**E**) or (**E′**).

3*R*, 4*R*, 5*R*, 6*R*, 7*S* (CIP)
l *l* *l* *u* (Prelog)
Represents (**F**) or its enantiomer (**F′**).
R (3*l*, 4*l*, 5*l*, 6*l*, 7u) Represents (**F**) (Brewster).
S (3*l*, 4*l*, 5l, 6*l*, 7*u*) Represents (**F′**).
3,4-*parf*, 4,5-*parf*, 5,6-*parf*, 6,7-*parf* (*Re/Si, Si/Re, Re/Si, Si/ Re*) Represents (**F**) or (**F′**).

Fig. 2.49 Prelog's and Brewster's stereochemical notations for acyclic compounds having multiple chiral centers

notation" of Prelog et al. and "the concept of external referencing." Thus, the absolute configuration of the compounds (**E**) (Fig. 2.49) may be designated as *S* (2 *l*, 3*u*, 4 *l*, 5*u*), where *S* represents the absolute configuration of the lowest locant (here C2), which is written outside the parenthesis (external reference), while *l* or *u* notation (being like or unlike the external reference) for each chiral center is placed inside the parenthesis. The chirality of each chiral center of the enantiomer of (**E**), designated (**E**′), is defined by *R* (2 *l*, 3*u*, 4 *l*, 5*u*), which means that the nomenclature depicts the chirality as a property of the whole molecule. Thus, topography (absolute configuration) at each center is readily recoverable: *RS* (2 *l*, 3*u*, 4 *l*, 5*u*) represents a racemic mixture (**E**) and (**E**′). A partially resolved mixture consisting of 80 % (**E**) and 20 % (**E**′) is represented by [80*S*, 20*R*) (2 *l*, 3*u*, 4 *l*, 5*u*). Any unknown chirality (say at C3) in compound (**E**) may be indicated by putting an *x* as in *S* (2 *l*, 3*x*, 4 *l*, 5*u*), which means that the chirality (absolute configuration) of C3 is not known.

2.7.2 *Molecules with Two Like (Symmetrical) Chiral Centers (AA Type)*

Molecules with two like chiral centers are of AA type and are *constitutionally symmetrical* having two diastereomers: one (±)-pair and one *meso* isomer (Fig. 2.50). It may be noted that if any one ligand on any chiral carbon (C2 or C3) is substituted by a new ligand (different from the existing ones), *meso* isomer becomes *erythro*, and active isomer becomes *threo*. The *anti* forms of (−)-tartaric acid and (+)-tartaric acid, each with **C2** point group, are shown in the figure.

Optical inactivity of meso-tartaric acid. The optical inactivity of the meso-tartaric acid may be explained in terms of its three staggered conformers, *viz*., the

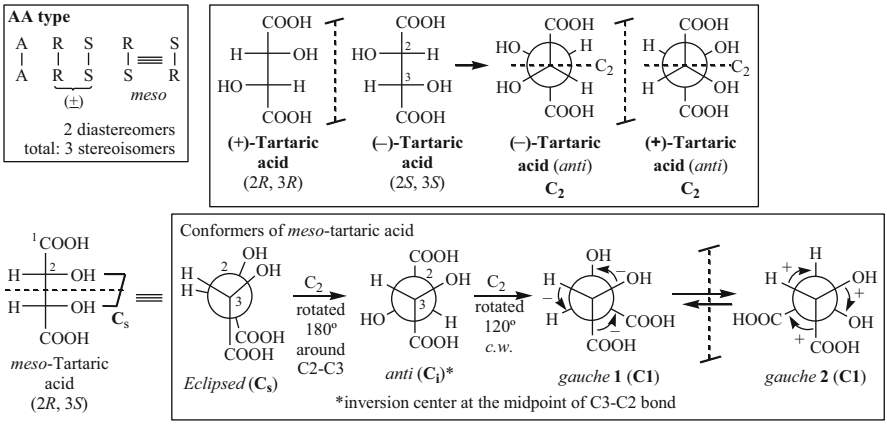


Fig. 2.50 Stereoisomers of tartaric acid (AA type). Optical inactivity of *meso*-tartaric acid

anti conformer and two gauche conformers which constitute over 99 % of the total number of molecules present in a sample of *meso*-tartaric acid. The eclipsed form shown in Fig. 2.50 along with the two other eclipsed forms obtained by rotation of any chiral carbon of the eclipsed form (say C2) around C2–C3 by 120° and 240° (*cw* or *acw*) constitute much less than 1 % of the total number of molecules present. The *anti* conformer with $\mathbf{S_2}$ ($\equiv \mathbf{C_i}$ point group) is optically inactive. The gauche 1 and gauche 2 conformers although optically active (each with $\mathbf{C_1}$ point group) are *equally populated* (statistically symmetrical) *enantiomers,* as evident from the same but opposite torsion angle signs between the like ligands in each of them—negative in gauche **1** and positive in gauche **2**. *Thus, the combined effect of all molecules is to make meso-tartaric acid optically inactive*.

The terminology *homochiral* (*RR* or *SS*) for optically active stereoisomers and *heterochiral* (*RS* or *SR*) for *meso* stereoisomers has been adopted [18].

2.7.3 *Molecules with Three Unlike Chiral Centers (ABC Type)*

An aldopentose, CHO−(CHOH)$_3$CH$_2$OH, a constitutionally unsymmetrical molecule belonging to ABC type, has three chiral centers and exists as eight (2^n, where n = number of chiral centers) stereoisomers involving four diastereomers, each one having its enantiomer (Fig. 2.51). Of the eight stereoisomers, the one with (2*R*, 3*R*, 4*R*) absolute configuration is shown in its zigzag and Fischer projection formulas in Fig. 2.51.

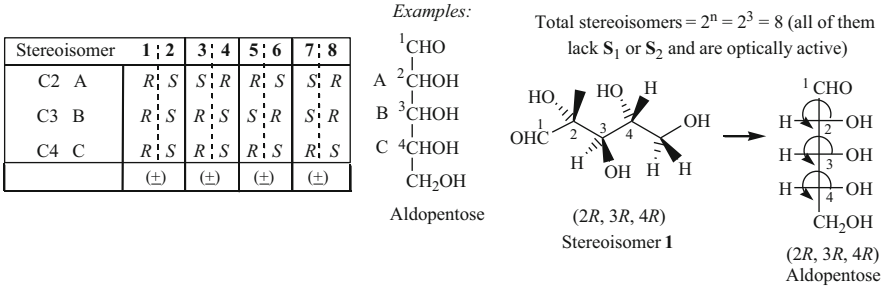


Fig. 2.51 Stereoisomers of ABA type with an example

2.7.4 *Constitutionally Symmetrical Molecules Having Three Chiral Centers (ABA Type)*

An acyclic molecule containing three or more chiral centers is constitutionally symmetrical if the chiral atoms equidistant from the geometrical center of the molecule are identically substituted. The two end groups of such a molecule are necessarily equivalent. Let us take n as the number of chiral centers in such a molecule. Thus, a constitutionally symmetrical molecule, say 2,3,4-trihydroxyglutaric acid, belongs to ABA type (Fig. 2.52) and exists as $2^{n-1} = 2^{3-1} = 4$ stereoisomers. Two of them (stereoisomers **1** and **2**) are enantiomers, and the remaining two, i.e., stereoisomers **3** and **4** are optically inactive *meso* forms. The latter two (**3** and **4**) are diastereomeric with each other and also with the two enantiomers **1** and **2**.

Monomethyl ester (**B**) [half ester of (**A**)] (Fig. 2.52) becomes constitutionally unsymmetrical and belongs to ABC type like an aldopentose (Fig. 2.51), and exists as four diastereomers, each having a (±)-pair, making eight stereoisomers in total (Fig. 2.52).

2.7.5 *Stereogenecity and Chirotopicity (Fig. 2.52)*

The status of the C2, C3, and C4 centers of the stereoisomers **1**, **2** (enantiomers), and **3** and **4** (Fig. 2.52) will now be discussed in terms of the observations of Mislow and Siegel [19] in 1984. Usually, a chiral center has two distinct attributes or characters:

1. *Stereogenecity* refers to bond connectivity—whether permutation or exchange of a pair of ligands gives a stereoisomer. (*R*-*S*)-Designation is associated with stereogenecity.
2. *Chirotopicity* is determined by local symmetry, *i.e.*, whether S_n axis is present locally. A chirotopic atom resides in a chiral environment. Thus, all the five

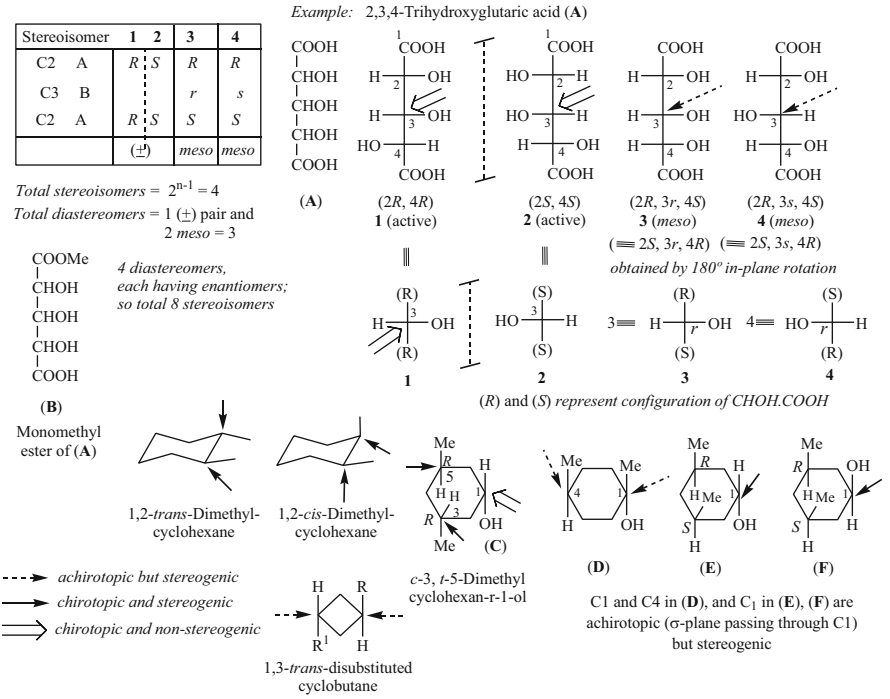


Fig. 2.52 Stereoisomers of compounds of ABA type. Stereogenecity and chirotopicity

atoms in bromofluorochloromethane (BrCl FCH) are chirotopic and belong to $\mathbf{C_1}$ point group, although only the C atom is a stereogen. Three categories of centers are possible:

(i) *Chirotopic and stereogenic*. In most cases, the chiral centers, *e.g.*, C2 and C4 of isomers **1**–**4**, are *chirotopic* as well as *stereogenic*, i.e., the two attributes overlap. The C1 and C2 of *cis*- and *trans*-1,2-dimethylcyclohexanes are stereogenic as well as chirotopic.

(ii) *Chirotopic but nonstereogenic*. The C3 centers of enantiomers **1** and **2** are chirotopic since they are devoid of any Sn axis (plane or center of symmetry), and no local symmetry is present. But they are nonstereogenic since two ligands of C3 in each are identical in both structure and chirality (*R, R*) or (*S, S*). Consequently, exchange of H and OH, for that matter, any two different ligands, does not give any isomer. Another example is the C1 atom of *c*-3,*t*-5-dimethyl-cyclohexan-*r*-1-ol (**C**) (Fig. 2.52), since the two faces (α-) and (β-) are exchangeable by *C2* operation, and exchange of H and OH does not give any new stereoisomer.

(iii) *Achirotopic but stereogenic* [19]. In the meso isomers **3** and **4,** C3 is achirotopic but stereogenic—thus again showing that stereogenecity and

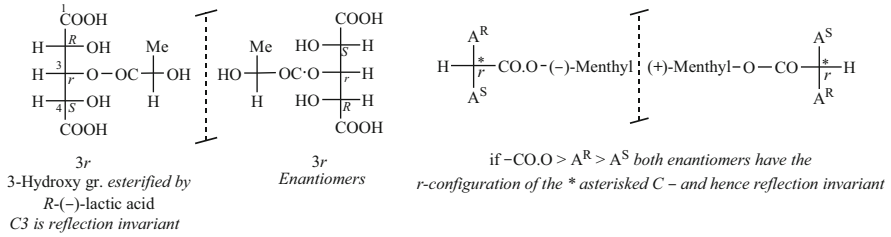


Fig. 2.53 Examples of reflection invariant carbon, which is *chirotopic* as well as *stereogenic*

chirotopicity are two distinct properties and can be delinked. Exchange of H and OH at C3 of **3** gives the other *meso* isomer **4**. C3 in both **3** and **4** can be given configurational descriptors: *r* to **3** and *s* to **4**, since the priority sequence $R > S$. C3 of the two molecules **3** and **4** are invariant to reflection; their configuration is denoted by lower case symbols, *r* and *s*. Even if C3 is made chiral by esterifying OH with *R*-lactic acid, the configurational specification *r* or *s* remains invariant to reflection (Fig. 1.53). In classical stereochemistry such an achirotopic but stereogenic center is called *pseudoasymmetric* and may be designated as C*RS*bc where *R* and *S* represent two enantiomorphous ligands [19].

Some more examples of molecules belonging to the category (iii) are 1,3-disubstituted cyclobutane and the three dimethylcyclohexanols (**D**), (**E**), and (**F**) (Fig. 2.53).

Some examples of compounds with *reflection invariant carbon being chirotopic as well as stereogenic* are depicted in Fig. 2.53.

2.7.6 *Molecules with Four (ABCD Type) or More Unlike Chiral Centers in a Chain*

An aldohexose, OHC−(CHOH)$_4$−CH$_2$OH, a constitutionally unsymmetrical molecule belonging to ABCD type, has four chiral centers and exists as $2^n = 2^4 = 16$ (where *n* is the number of chiral centers) stereoisomers involving *eight diastereomers*; each one is optically active and has its enantiomer. Figure 2.54 depicts the D-enantiomer of each of the eight pairs of diastereomeric aldohexoses (ABCD type).

Much of the early development of stereochemistry was stimulated by investigations of various sugars. It is, therefore, pertinent to depict in Fig. 2.55 the aldohexoses, only the *D*-enantiomers (C5 OH on the right side possessing *R*-configuration) of the eight diastereomers. Of these aldohexoses D-(+)-glucose occurs mostly as plant natural product glycosides. D-Gulose and D-idose are levorotatory; all other D-aldohexoses are dextrorotatory.

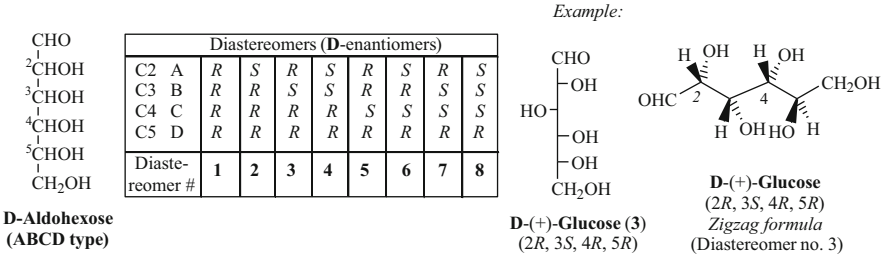
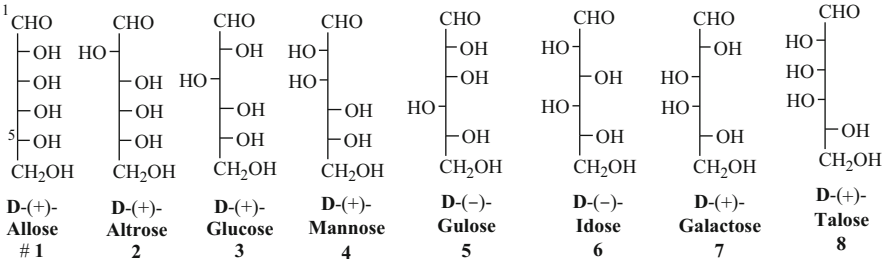


Fig. 2.54 Eight diastereomers of ABCD type (only D-(+)-Glucose shown)



Compare **3** *with that of the* **Figure 2.54**

Fig. 2.55 Diastereomeric D-aldohexoses

2.7.7 *Constitutionally Symmetrical Molecules with Four or More Like Chiral Centers in a Chain (ABBA, ABCBA, etc. Types)*

A constitutionally symmetrical molecule has been defined in Sect. 2.7.4. Such a molecule with four chiral centers (the first and second being similar to the 4th and 3rd chiral centers, respectively) belongs to ABBA type, *e.g.*, tetrahydroxyadipic acids (or hexaric acids), which exist as six diastereomers (four racemic pairs and two *meso* forms) and hence ten stereoisomers (Fig. 2.56).

Stereoisomers of compounds with even number of constitutionally symmetrical chiral centers in a chain. In general, in the series of the type Cabd $(Cab)_{n-2}$ Cabd, where n is the number of chiral centers and n is **even**, there are 2^{n-1} optically active stereoisomers and $2^{(n-2)/2}$ *meso* forms. So, in case of hexaric acid, $n = 4$ and total number of stereoisomers $= 2^{n-1} + 2^{(n-2)/2} = 2^3 + 2^{2/2} = 8 + 2 = 10$. Perhydrophenanthrene (Sect. 2.15.2) also belongs to ABBA.

Stereoisomers of compounds with odd number of constitutionally symmetrical chiral centers in a chain. In the series of the type Cabd $(Cab)_{n-2}$ Cabd where n is the number of chiral centers and n is **odd**,

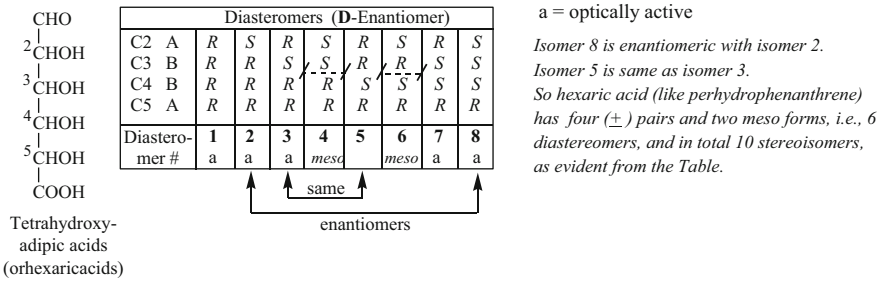


Fig. 2.56 Stereoisomers of hexaric acid (ABBA type)

total number of stereoisomers $= 2^{n-1}$, of which number of *meso* forms $= 2^{(n-1)/2}$.

Thus, when $n = 3$, total number of stereoisomers $= 2^{n-1} = 2^2 = 4$, of which number of meso forms $= 2^{(n-1)/2} = 2^{(3-1)/2} = 2$; so number of optically active isomers $= 4–2 = 2$ ($1 \pm$ pair) (see Fig. 2.47).

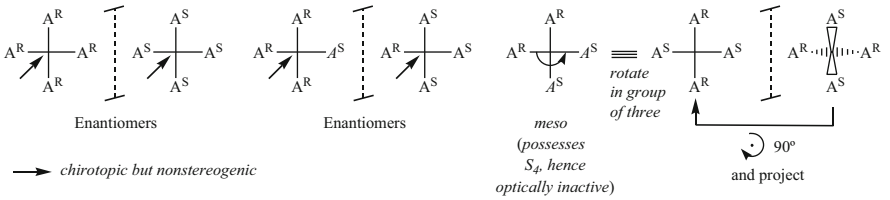
The next higher homologue with n odd, $HOOC\text{-}(CHOH)_5COOH$ (heptaric acid) is of the ABCBA type. Here $n = 5$, hence total number of stereoisomers $= 2^{5-1} = 2^4 = 16$, of which number of the meso forms $= 2^{(5-1)/2} = 2^2 = 4$. Hence, number of optically active isomers $= 16–4 = 12$. So there will be 6 racemic pairs and 4 meso forms, *i.e.*, 10 diastereomers. All $2^n \div 2 = 2^5 \div 2 = 16$ diastereomers of ABCDE system can be written down following the system in Fig. 2.54. The six racemic and four meso isomers of ABCBA system can also be ascertained from the former system (ABCDE) (*cf.* Fig. 2.56).

2.7.8 Chiral Compounds with Asymmetric Carbon Atoms in Branched Chains

On rare occasions one encounters molecules in which chiral carbon atoms cannot be aligned in one chain. We depict the cases (**1**) and (**2**) in Fig. 2.57. Cases (**2**)–(**5**), where two or more of the chiral ligands attached to the central carbon atom are alike, are more complicated as delineated in Figs. 2.57 and 2.58.

2.8 Chirality and Dimension. One-, Two-, and Three-Dimensional Chiral Simplexes

Some simple concepts of stereochemistry (in the molecular level) may be conveniently revealed on the basis of "*chiral simplex*" (applicable to *1D*, *2D*, or *3D*), introduced by Prelog and Helmchen [10]. The term means the simplest structure



An example of such a meso compound possessing a four-fold alternating axis of symmetry (S_4) and no other element of symmetry, prepared and shown to be optically inactive [18]*, is given below*

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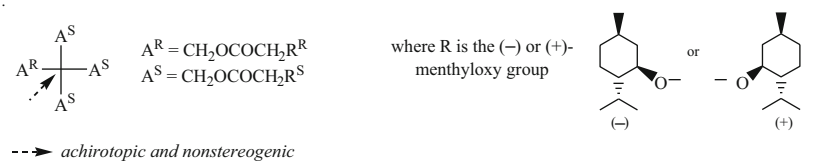
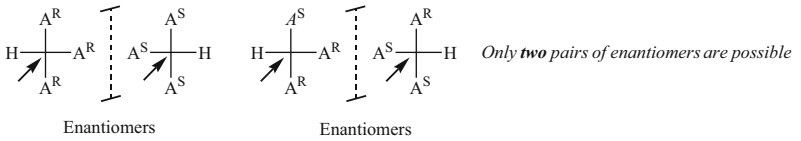


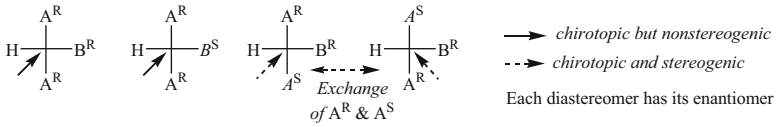
Fig. 2.57 The stereoisomers of cases CA*B*D*E* and CA^*_4. A molecule possessing S_4

(**3**) Case $CH(A^*)_3$.



Central carbon of each stereoisomer is nonstereogenic but chirolopic (⟶)

(**4**) Case $CH(A^*)_2B^*$. *Here **four** enantiomeric pairs (four diastereomers) and **eight** stereoisomers are possible as shown below*



One enantiomer each of four enantiomeric pairs

(**5**) Case $C(A^*)_2(B^*)_2$. *Here **five** enantiomeric pairs and **ten** stereoisomers are possible as shown below:*

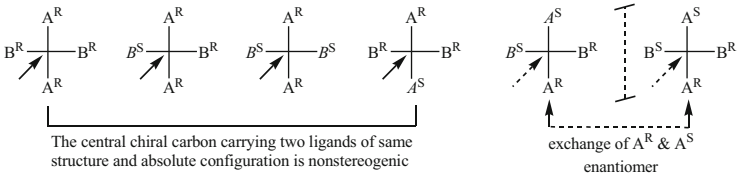


Fig. 2.58 The streoisomers of cases $CH(A^*)_3$, $CH(A^*)_2B^*$, and $C(A^*)_{2(}B^*)_2$

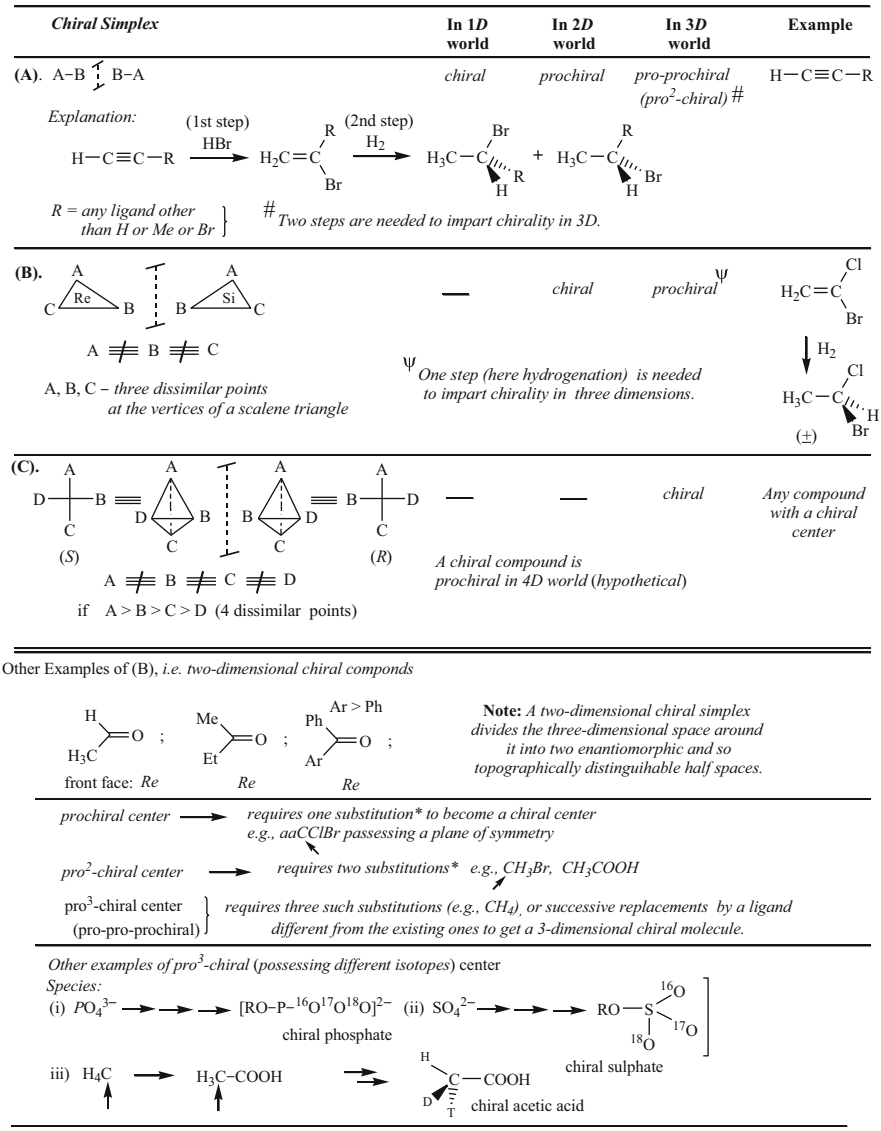


Fig. 2.59 One-, two-, and three-dimensional chiral simplexes. Pro-, pro^2-, and pro^3-chiral molecules

that could be chiral in *1D*, *2D*, or *3D* world, as illustrated in Fig. 2.59 with examples.

A linear molecule, with two dissimilar points, like A–B (case **A**) is a one-dimensional chiral simplex, *i.e.*, chiral in one dimension (*y*-axis) since its mirror image cannot be superposed on it without the help of a second dimension,

i.e., by its rotation around the orthogonal x axis. So a linear molecule A–B is prochiral in a two-dimensional world, since one more dimension (*Z* axis) is necessary to make it chiral in three dimensions. The molecule A–B is pro-prochiral in the three-dimensional world since two steps are necessary to convert it to a chiral molecule in our *3D* world.

Likewise, a dissimilarly substituted trigonal tricoordinated carbon (case **B**) is chiral in two dimensions (two-dimensional world) and prochiral in tetrahedral terracoordinated three dimensions.

By the same token, a dissimilarly substituted tetrahedral tetracoordinated carbon is chiral in three dimensions, *e.g.*, any chiral compound with a chiral center. However, one may assume that any chiral compound in three dimensions, which is a three-dimensional chiral simplex, would have been prochiral in an imaginary four-dimensional world, had there been any fourth dimension (*cf.*, time). Some examples of two-dimensional chiral simplex and of pro-, pro^2-, and pro^3-chiral molecules/species are illustrated in Fig. 2.59.

2.9 Prochirality and Prostereoisomerism. Topicity of Ligands and Faces: Homotopicity. Enantiotopicity. Diastereotopicity. Nomenclature [21–25]

2.9.1 Introduction

A nonstereogenic center can be changed to a stereogenic center by replacement of any one of the two *apparently identical ligands* by a different one. Two examples, propionic acid (**1**) or phenylacetic acid (**2**), are given in Fig. 2.60. In each case, the prostereogenic (also prochiral) C2 center is converted to a stereogenic (also chiral) center by replacement of one *homomorphic* ligand (here a hydrogen atom) by a ligand other than those present in the molecule. The term *homomorphic* originates from Greek *homos* meaning "same" and *morphe* meaning "form" [23, 24]. Homomorphic ligands are identical only when separated from the rest of the molecule. Replacement of the other homomorphic ligand by the same new ligand will lead to

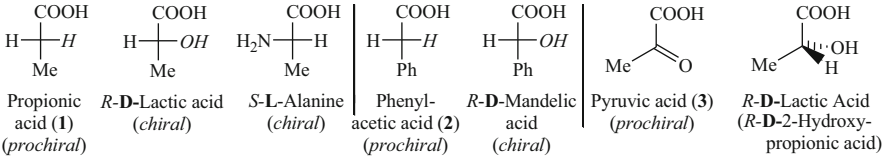


Fig. 2.60 Prochiral (prostereogenic) [(**1**), (**2**), and (**3**)] and chiral (stereogenic) molecules

the corresponding enantiomer. In such cases the topicity (Greek *topos* meaning "place" or "physical surrounding") of the two *homomorphic* ligands (here H's at C2), in other words, their special relation with the rest of the molecule is different; they reside in enantiomeric environments. Such ligands are called *stereoheterotopic*, in this case more specifically *enantiotopic*.

By the same token the two faces (front and rear of the molecular plane) of pyruvic acid (**3**) (Fig. 2.60) are enantiotopic, since the two faces are in enantiomeric environments; thus, addition of hydride (or any nucleophile) to the two faces in turn gives rise to enantiomers, *viz*., (*R*)- and (*S*)-2-hydroxypropionic acid (*R*- and *S*-lactic acid).

Molecules having stereoheterotopic ligands or faces exhibit *prostereoisomerism* (if they can be reacted upon). Prostereoisomerism is attributed to such a center carrying stereoheterotopic ligands, for example, to C2 of *prostereogenic* and *prochiral* compounds (**1**) and (**2**), or to a center carrying stereoheterotopic faces, *e.g*., pyruvic acid (**3**).

The principle of stereoselective synthesis (Sect. 2.11.1) is based on the different behavior of stereoheterotopic ligands and faces in the chemical reactions. The concept of stereoheterotopicity has been discussed in 1967 [22], that of prochirality in 1966 [21], and the topic has been reviewed in detail by Eliel in 1982 [25].

Two criteria, *viz*., (i) substitution or derivatization (in case of ligands) or addition (in case of faces) and (ii) a symmetry criterion are used to determine the topic relationship of homomorphic ligands and faces, as illustrated by several examples of each of homotopic ligands (Fig. 2.61), homotopic faces (Fig. 2.62, enantiotopic ligands (Fig. 2.63), enantotopic faces (Fig. 2.64), diastereotopic ligands (Fig. 2.66), and diastereotopic faces (Fig. 2.67). Any of the two criteria is sufficient to determine the topicity in each case.

2.9.2 Homotopic Ligands

The first example of homotopic ligands is of Za_2b_2 type, belonging to the $\mathbf{C_{2V}}$ point group (Fig. 2.61). The homotopic ligands are recognized by either of the two criteria already stated. Two examples of homotopic ligands are illustrated, recognized by both substitution and symmetry criteria, in this Figure. Four more examples of homotopic ligands of simple molecules like chloromethane, ethylene, allene, and acetic acid are also included indicating the homotopicity of the homomorphic ligands by symmetry criteria.

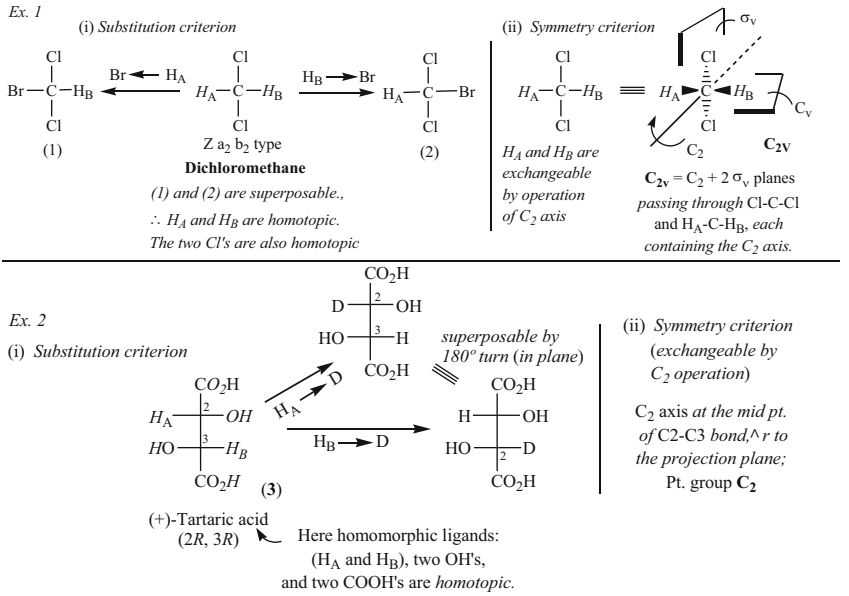


Fig. 2.61 Homotopic ligands recognized by substitution and symmetry criteria

2.9.3 Homotopic Faces

Homotopic faces are recognized by the two criteria as illustrated in Fig. 2.62 with the help of four examples.

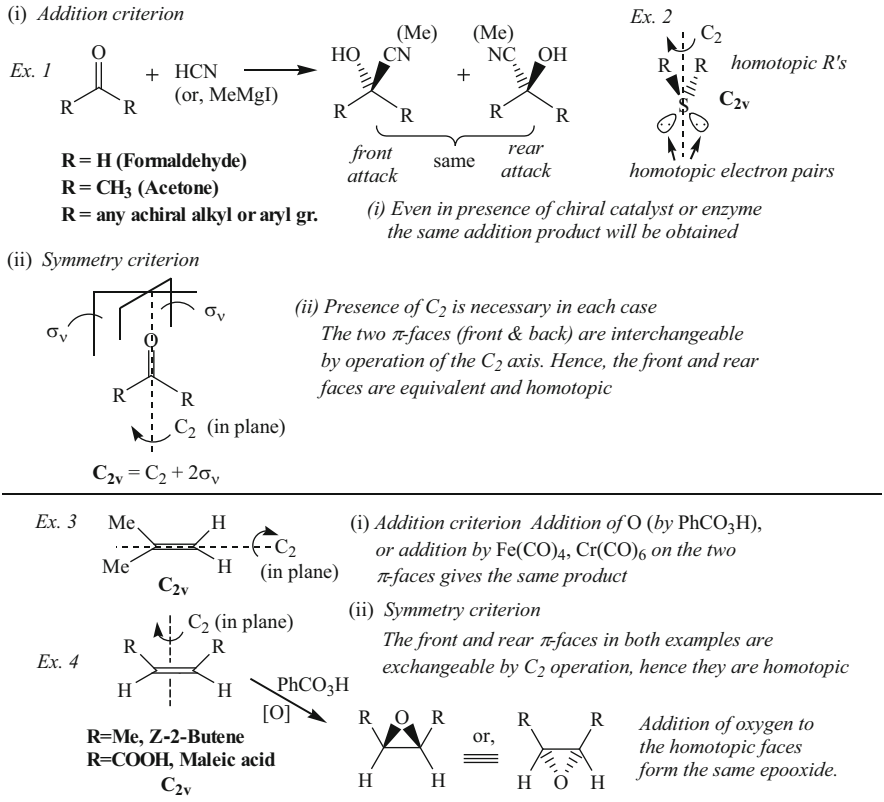


Fig. 2.62 Homotopic faces recognized by addition and symmetry criteria

2.9.4 Enantiotopic Ligands

Enantiotopic ligands are recognized by any one of the two criteria as illustrated in Fig. 2.63 with six examples.

2.9.5 Nomenclature of Geminal Enantiotopic Ligands. Pro-R and Pro-S

In compounds **1**–**3** of Fig. 2.63, of the two homomorphic ligands H_AH_B or H_CH_D (attached to the same carbon) if replacement of any one, say H_A, by an atom of higher priority, but of lower priority than the other two ligands (in such case by D), gives rise to (*S*) configuration of the new chiral molecule, H_A is then termed as ***pro-S*** and is expressed as H_S. H_B in that case, by default, will be termed as ***pro-R*** and is expressed as H_R. H_A and H_B may be replaced by D to give the (*S*) and (*R*) configuration, respectively, of the resulting new chiral molecules. This terminology

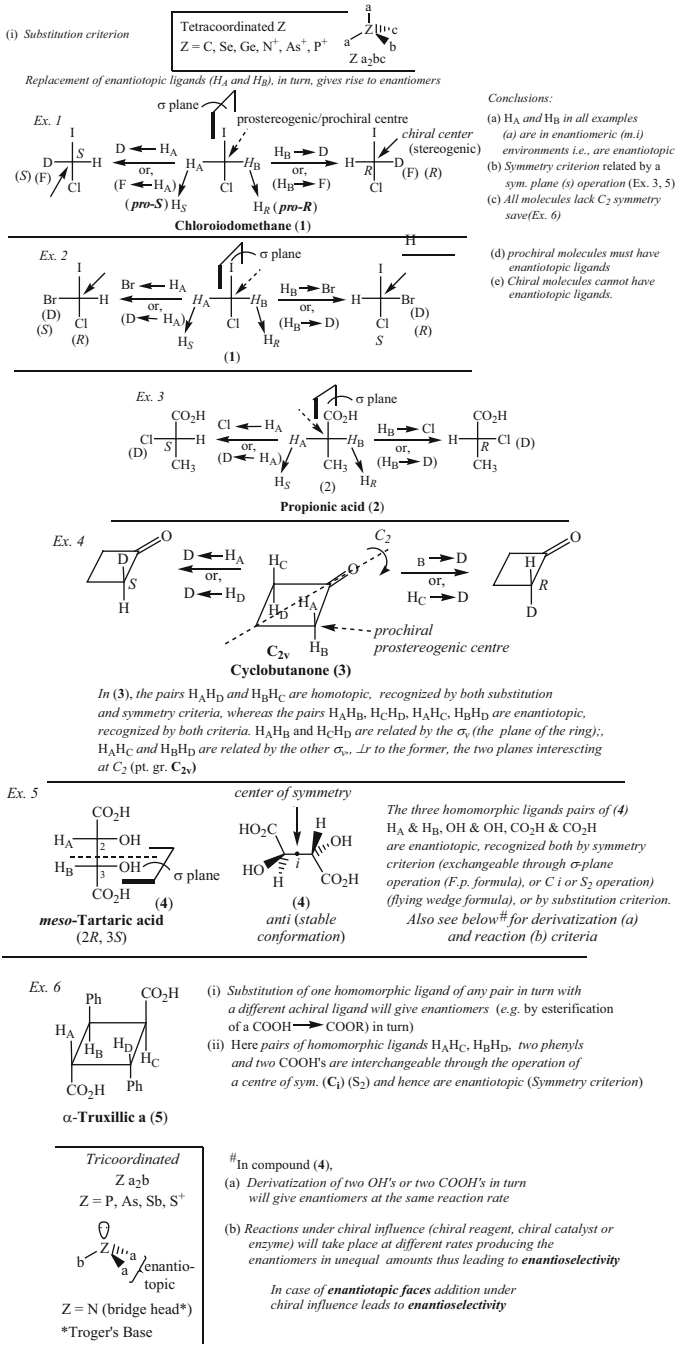
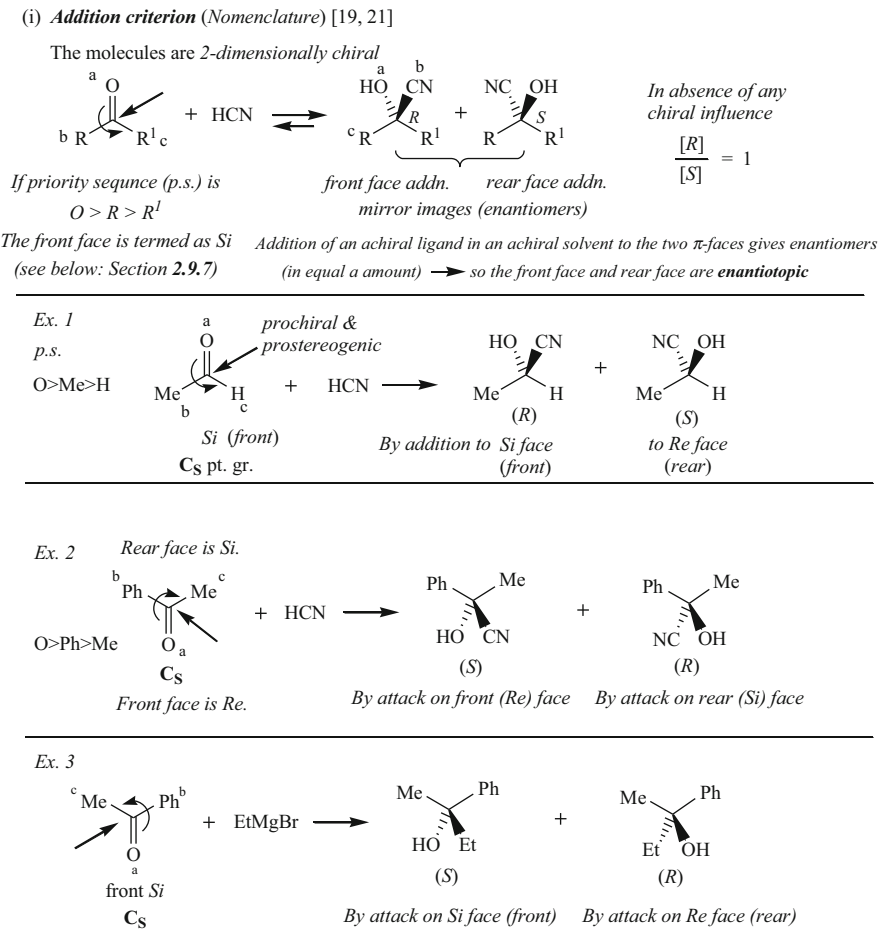


Fig. 2.63 Enantiotopic ligands recognized by substitution and symmetry criteria

is applicable to any enantiotopic homomorphic germinal ligands (attached to the same carbon) [21].

2.9.6 Enantiotopic Faces

Enantiotopic faces are recognized by any one of the two criteria as illustrated in Fig. 2.64 with three examples.



(ii) ***Symmetry criterion***

a) If the π-faces are exchangeable by operation of the symmetry plane, they are enantiotopic

b) In each above example, and always if the ligands are achiral, the plane of the molecule is the symmetry plane; so such molecules must belong to $\mathbf{C_S}$ *point group*

c) Enantiotopic faces are not exchangeable by C_2 *operation*

Fig. 2.64 Enantiotopic faces. Addition and symmetry criteria, and nomenclature

2.9.7 *Nomenclature of Enantiotopic Faces*

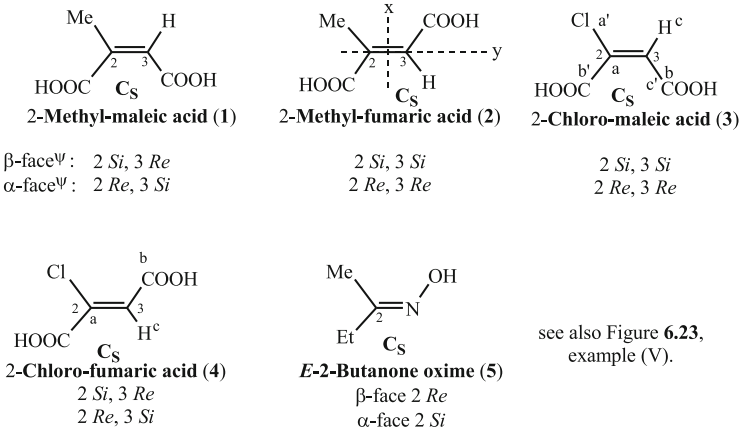
The π-faces of a trigonal atom (olefinic/carbonyl carbon) having three different ligands will be enantiotopic and are two-dimensionally chiral (Sect. 2.8).

If one looks at the plane of a π-face, and finds that the CIP priority sequence of the three dissimilar ligands is clockwise, the face is called *Re*. If the sequence is anticlockwise, the face is called *Si*, these being the first two letters of ***Re****ctus* and ***Si****nister*, respectively [21]. Examples 1–3 are shown in Fig. 2.64. If one face is *Re*, the other face will be *Si*.

In case of a double bond containing two specifiable trigonal atoms, each face may be uniquely defined by two symbols, one for each specifiable atom, as illustrated by the compounds in Fig. 2.65. The π-faces of an oxime are also specified in this Figure. The α- and β-faces of each olefin excepting maleic acid being exchangeable only by a σ-plane (*xy* or paper plane) operation and not by any C_2 operation, each olefin produces enantiomeric epoxides or complexes with the double bond in equal amount in absence of any chiral influence (see also Fig. 2.73).

2.9.8 *Diastereotopic Ligands*

Substitution criterion. If the sequential replacement of two homomorphic ligands in a molecule by a different achiral ligand gives rise to diastereomeric products, those two ligands are said to be diastereotopic. Such ligands are generally distinct both



Notes: [ψ] *β- and α-faces specify front and rear faces respectively when the structures are written in the xy (paper) plane, as shown. After rotation around in-plane x-axis (vertical) or, around in-plane y-axis (horizontal) the β face becomes α and vice-versa but (Re, Si) specification of a specified face remains unaltered.*

Fig. 2.65 Nomenclature of enantiotopic π-faces of some unsymmetrically substituted olefins

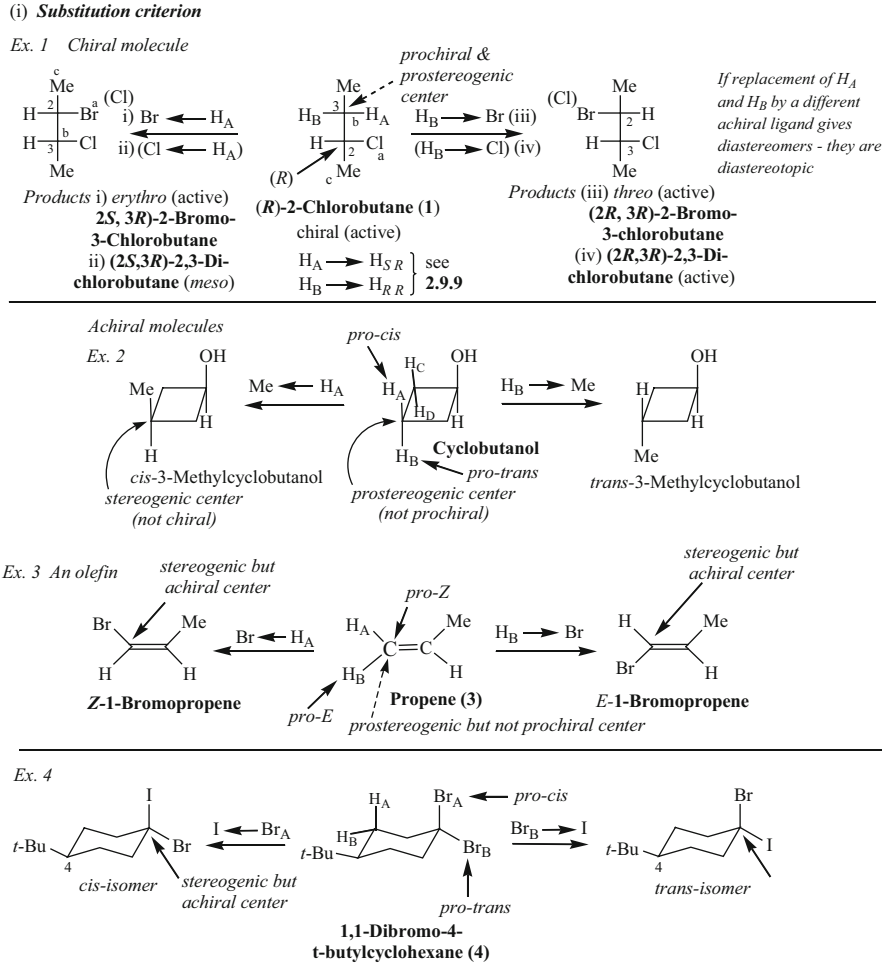


Fig. 2.66 Diastereotopic ligands. Nomenclature

chemically and spectroscopically. They react at unequal rates and their NMR signals will be different (anisochronous).

Symmetry criterion. Diastereotopic ligands must not be related (exchangeable) by a C_n or S_n axis. The molecule (**1**), depicted in Fig. 2.66, is devoid of any symmetry element; the molecules (**2**), (**3**), and (**4**) (Fig. 2.66) although contains a plane of symmetry, its operation does not interchange the ligands.

2.9.9 Nomenclature of Diastereotopic Ligands

A *chiral* molecule containing a pair of homomorphic diastereotopic geminal ligands at a prochiral center must have a chiral element (center, axis, or plane) also. To designate the diastereotopic pair of ligands, one ligand is arbitrarily given preference over the other to treat the prochiral center as a chiral one. Now applying CIP rules if the center becomes (*R*), then that particular homomorphic ligand is called ***pro-R*** (hypothetical configuration symbol) and is expressed as the ligand subscripted with *R*, *i.e.*, the ligand is termed $(\text{ligand})_R$. Let us take (*R*)-2-chlorobutane (**1**) as an example (Fig. 2.66, first row). The hydrogen atoms H_A and H_B at C3 are diastereotopic. If preference is given to H_A over H_B in the sequence rule, the hypothetical configurational symbol for C3 of (**1**) would be *S*. The configurational symbol of H_A thus becomes H_{SR} after adding to the subscript *S* a further subscript *R*, the symbol of the absolute configuration of the chirality present (in this case at C2). By default H_B is H_{RR}. This procedure is applicable for any such homomorphic pair of ligands at a prochiral center, *e.g.*, COOH, COOH or Ph, Ph or Me, Me, etc. of a compound having a chiral element. This type of double indexing system [26] was first introduced in 1982 in a somewhat modified form. It has the advantage that it makes the diastereotopicity immediately obvious.

Ex. 2, Ex. 3, and Ex. 4 of Fig. 2.66 illustrate the nomenclature of different types of *achiral* molecules [*viz.*, cyclic compounds or olefins or cumulenes with odd number of double bonds (having successive planes of π bonds orthogonal to each other)] having diastereotopic homomorphic geminal ligands. The substitution products shown are also achiral since the original plane of symmetry is retained.

The substitution products of homomorphic geminal diastereotopic ligands may become chiral if the original symmetry plane is destroyed, *e.g.*, H_C and H_D at C2 of (**2**) and H_A and H_B at C3 of (**4**) in Fig. 2.66. Thus, C2 of (**2**) and C3 of (**4**) are prostereogenic as well as prochiral centers.

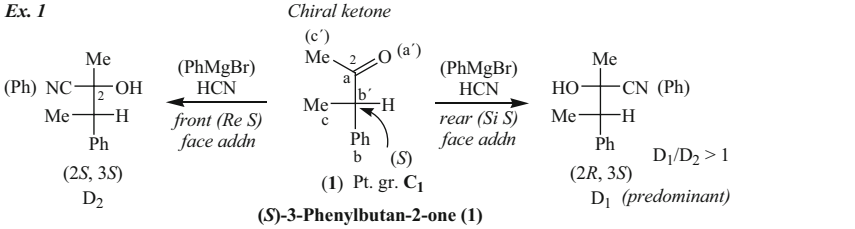
2.9.10 Diastereotopic Faces. Nomenclature

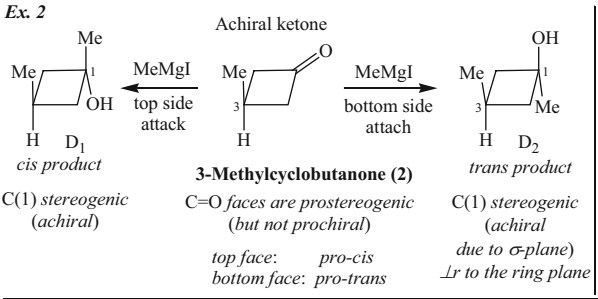
The diastereotopic faces are designated as follows. First, the face is designated as *Re* or *Si* as stated earlier in Sect. 2.9.7. To this symbol the specified absolute configuration of the chiral element present in the molecule is added. Thus, in case of a chiral compound (**1**) (Fig. 2.67), the front face becomes *ReS* by adding *S*, the absolute configuration of the chiral center present, to the *Re* face. Thus, the diastereotopic rear face is designated *SiS*.

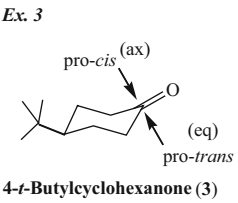
Diastereotopic nomenclature in cases of compounds with diastereotopic faces has been illustrated in Fig. 2.67 with examples of an achiral ketone 3-Methylcyclobutanone (**2**), achiral allene with diastereotopic faces 1-chloroallene (**3**), and a chiral sulfide (**4**). In each case the nomenclature of the diastereotopic faces is self-explanatory (*cf.* Ex. 1).

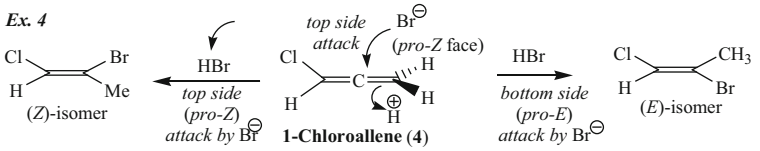
Diastereotopic faces are recognized by application of any one of the usual two criteria as illustrated in Fig. 2.67.

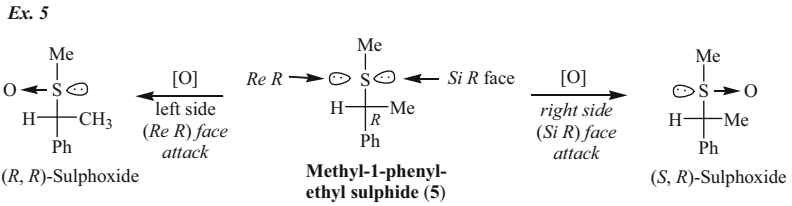
i) ***Addition criterion***: *Addition to diastereotopic π-faces gives diastereomers*











(ii) ***Symmetry criterion***: *The two π-faces of compounds* ***(1)*** *to* ***(4)*** *are not exchangeable by operation of any C_2 or σ plane or S_n axis, so they are diastereotopic. Same is the case with the two lone pairs of* ***(5)****, which are also diastereotopic.*

In each case, diastereoselectivity is observed because of the involvement of diastereomeric transition states.

Fig. 2.67 Diastereotopic faces. Nomenclature

2.9.11 *Interesting Examples of Topicities of Homomorphic Ligands [27]*

In Fig. 2.68 homotopicity, enantiotopicity, and the diastereotopicity of the CH_2OH groups in the *cases 1*, *2,* and *3,* respectively, are illustrated by selective oxidations as well as by symmetry criteria.

In *case 1*, like the CH_2OH groups 2-OH & 5-OH, as well as 3-OH & 4-OH, exchangeable by C_2 operation, are homotopic, and hence the homotopic OHs when derivatized would lead to the same derivative, and the corresponding acetates will also be homotopic.

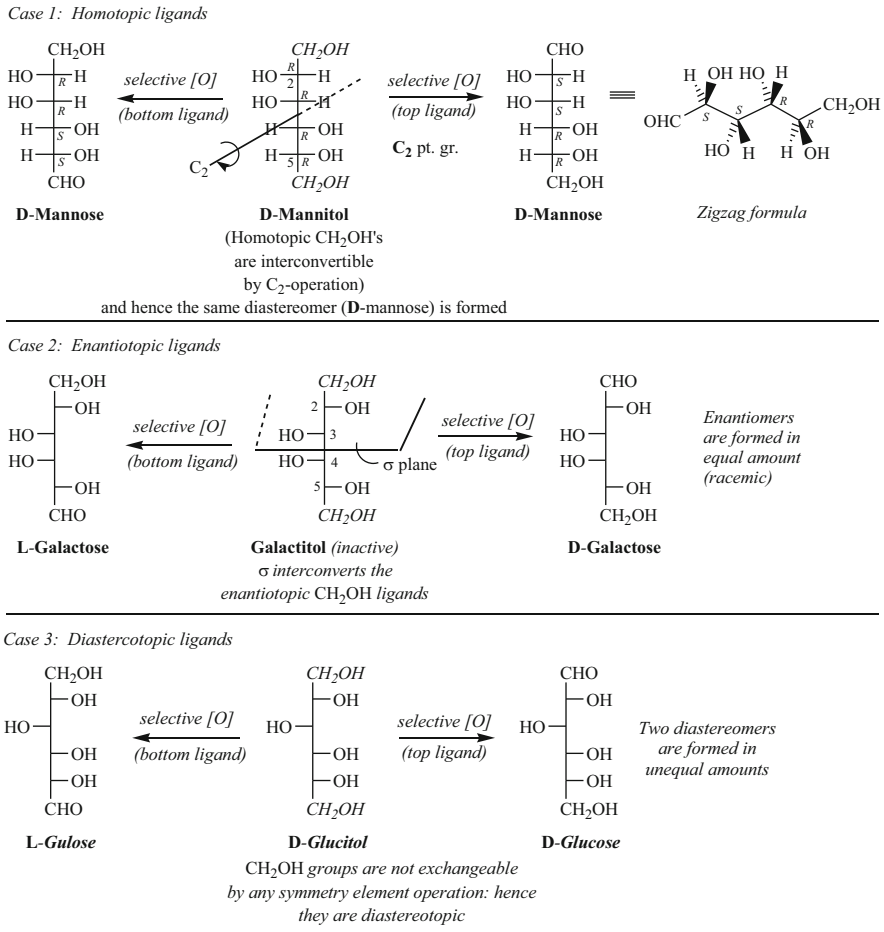


Fig. 2.68 Determination of topicity of CH_2OH groups of a few hexoses by selective oxidation

In *case 2*, like the CH_2OH groups 2-OH & 5-OH, as well as 3-OH & 4-OH are enantiotopic, and hence the corresponding acetates of the enantiotopic OH groups will lead to enantiomers.

In *case 3*, like the CH_2OH groups, any pair of OH groups are diastereotopic, and hence the corresponding acetates of any pair of diastereotopic OH groups will also be diastereomers.

2.9.12 Interrelation of Topicity of Ligands with Isomerism

Topicity of ligands is interrelated with isomerism in general. A classification diagram for topicity is drawn (Fig. 2.69), which may be compared with that drawn for isomerism (Fig. 2.24).

2.9.13 Molecules with Prostereogenic but Proachirotopic Center and Multi-Prochiral Centers

Some interesting examples of topicity and nomenclature are delineated in Fig. 2.70.

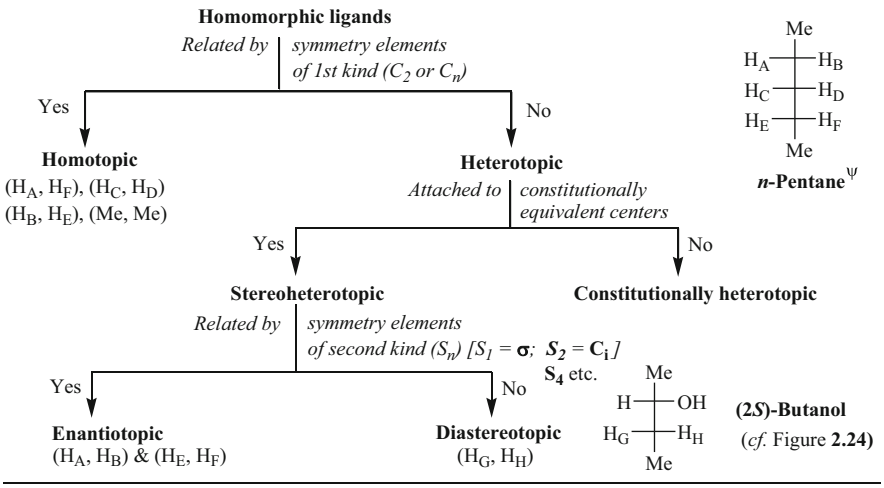


Fig. 2.69 Classification of homomorphic ligands based on topicity

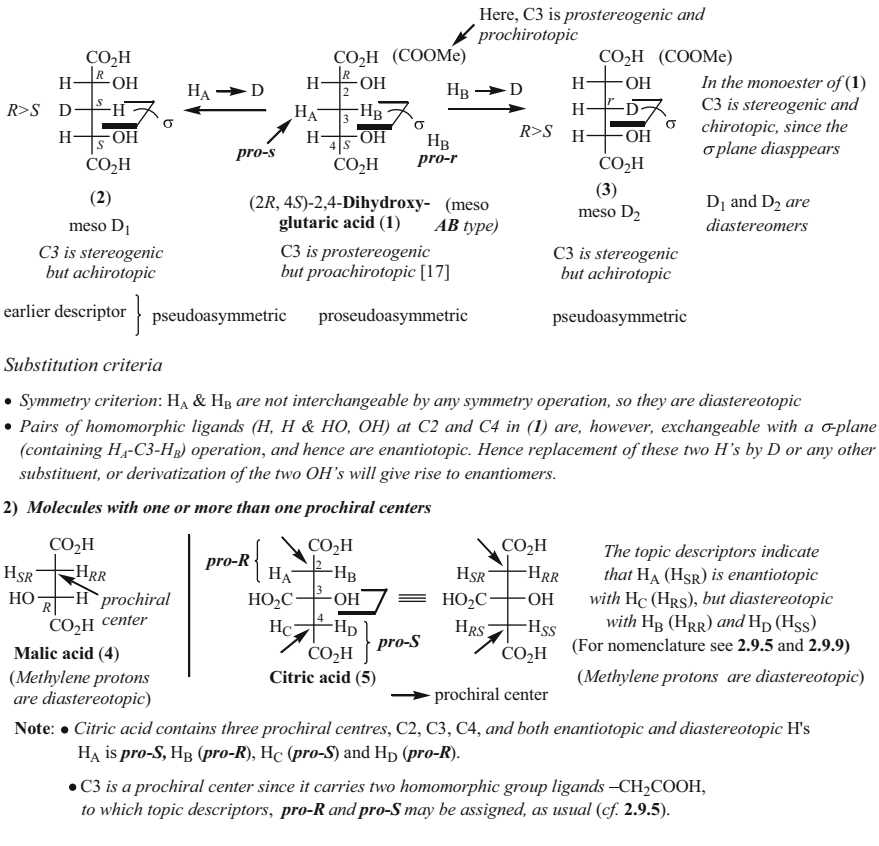


Fig. 2.70 Topicity nomenclature of methylene protons in two interesting examples

2.9.14 Topic Relationship of Ligands and Faces

Topic relationship of ligands and faces, so far discussed, are summarized in Fig. 2.71. This figure has much in common with the table given by Mislow and Raban in 1967 [22] and by Eliel in 1980 [28].

2.10 Stereoheterotopic Ligands and NMR Spectroscopy

NMR (like IR and UV) is an achiral probe. Nuclei that reside in different environments can be distinguished by NMR spectroscopy showing different chemical shifts. Such nuclei exhibiting nonequivalent chemical shifts are called *anisochronous*; they split. Nuclei showing same chemical shift are called isochronous, which do not split. The *isochrony* or *anisochrony* of nuclei is dependent on

Topic Relationship of Ligands and faces

Topicity	Substition/addition/ reaction criterion (by *achiral groups*)	Symmetry criterion	Behavioral difference
Homotopic	Identical product	**Ligands** related through C_n and **faces** by C_2 axis	No difference by any method
Enantiotopic	Enantiomeric products	**Ligands** related through σ, i or S_n **Faces** related by σ	Usually not distinguishable Distinguishable, in principle, in chiral media (NMR), by chiral reagents and enzyme
Diastereotopic	Diastereomeric products	Ligands and faces not related by any symmetry element	Distinguishable in principle by all physical and chemical methods

Fig. 2.71 Summary of topic relationship of ligands and faces

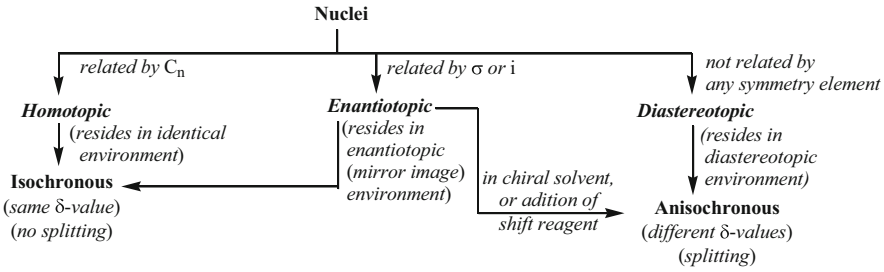


Fig. 2.72 Topicity. NMR chemical shift. Signal multiplicity

their topicity. The relationship between topicity, isochrony/anisochrony, and signal multiplicity are discussed in the form of a chart in Fig. 2.72. Anisochrony is often very small (1 ppm or less); a case, when it is undetectable, is called *accidental isochrony*. Use of higher field or different solvents in ^{1}H NMR or ^{13}C NMR may be helpful in increasing anisochrony.

A very instructive example is illustrated in Fig. 2.73.

2.10.1 Anisochrony Arising out of Diastereotopic Faces

Isochrony arising out of the homotopic faces of the maleic acid (−)-menthyl ester (**1**) and anisochrony arising out of the diastereotopic faces of fumaric acid (−)-menthyl ester (**2**) are much instructive [18] and are illustrated in Fig. 2.73.

The two olefinic protons H_A and H_B of the maleate (**1**), being exchangeable by the C_2 axis (in the molecular plane) operation, are *homotopic*, and hence are *isochronous*, and appear as a 2H singlet. Again, its front and rear π-faces are also interchangeable through the same C_2 axis operation and hence are homotopic. So the complexation by $Fe(CO)_4$ from the homotopic α- or β-face leads to the same complex (*cf.* epoxidation). But H_A and H_B of the $Fe(CO)_4$ complex being *diastereotopic* are *anisochronous* and have different chemical shift values. They

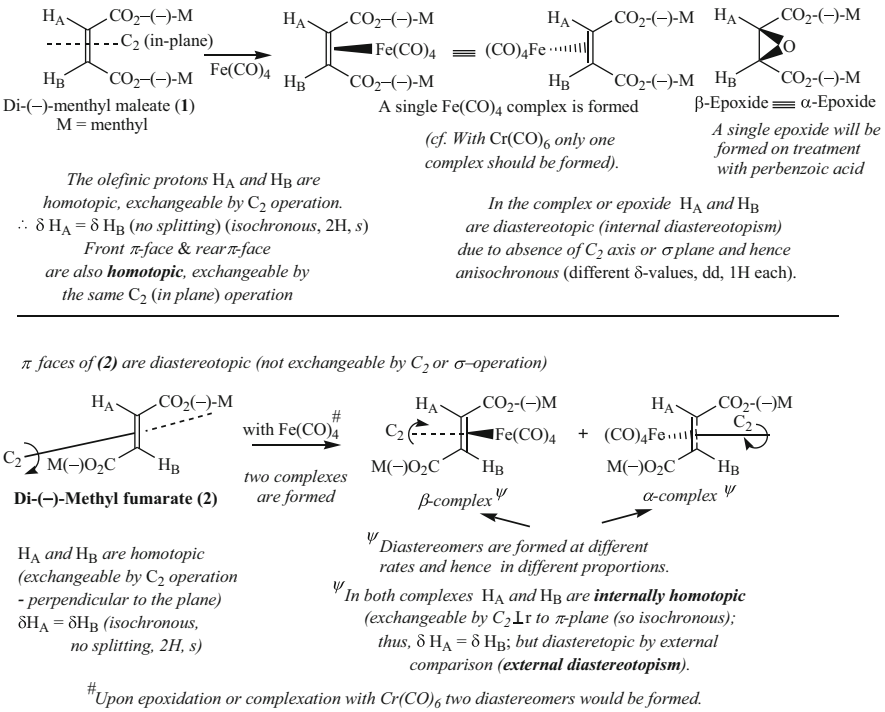


Fig. 2.73 Anisochrony arising out of diastereotopic faces. An instructive example

split and appear as a double doublet (dd) (1H each) in the NMR. Thus, the maleate (**1**) should form, on treatment with perbenzoic acid, a single epoxide of which H_A and H_B being diastereotopic should appear as a double doublet in the NMR.

In case of the fumarate (**2**) also, H_A and H_B, being exchangeable by C_2 (orthogonal to the π-plane) operation, are *homotopic* and appear as a 2H singlet. However, its two π-faces being not exchangeable by any symmetry operation are *diastereotopic*. So with $Fe(CO)_4$ two diastereomeric complexes are formed *at different rates and hence in different proportions*. In each diastereomeric complex, the olefinic protons are *internally homotopic* (exchangeable by a C_2 axis—orthogonal to the π-plane) and so isochronous. However, the two olefinic protons in one diastereomer are diastereotopic with the two olefinic protons of the other diastereomer *by external comparison*, so they are termed ***externally diastereotopic***. Consequently, the two olefinic protons of the two diastereomeric complexes show two 2H singlet peaks of different intensities.

The two olefinic esters (**1**) and (**2**) thus may be distinguished by NMR through complexation with faces of different topicities.

Although each of (**1**) and (**2**) show a 2H singlet (because in both compounds the two olefinic protons are homotopic), they may be distinguished by ^1H NMR through complexation (or epoxidation) with homotopic or diastereotopic faces, respectively.

2.11 Asymmetric Synthesis

2.11.1 Introduction. Principles of Stereoselection: Enantioselection. Diastereoselection

2.11.1.1 Lack of Stereoselection

In the absence of chiral influence by way of reactants, reagents, or solvents, a reaction of a starting material having a prochiral center generating a chiral center will produce equal amounts of enantiomers, i.e., a racemic product, since such a reaction proceeds through enantiomeric transition states (TS) of equal energy. Thus, the activation energies being same the reactions take place at the same rate. The principles of the *lack of stereooselection* and of *enantioselection* are illustrated with the help of energy diagrams (Figs. 2.74 and 2.75, respectively).

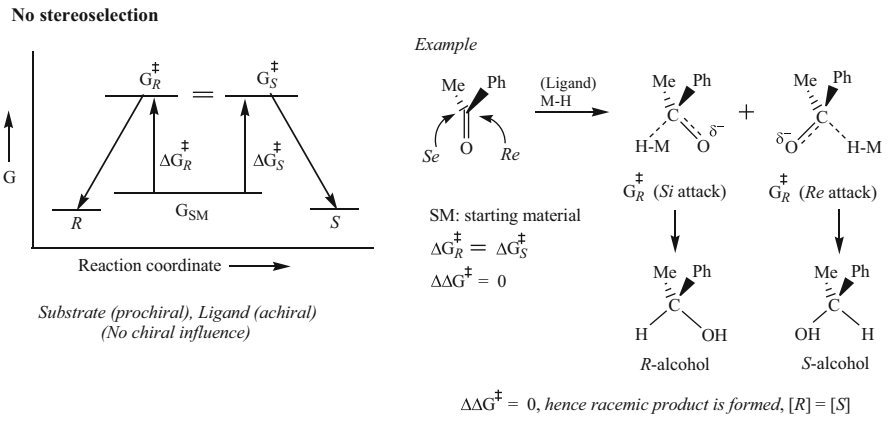


Fig. 2.74 Principle of absence of stereoselection. Energetics

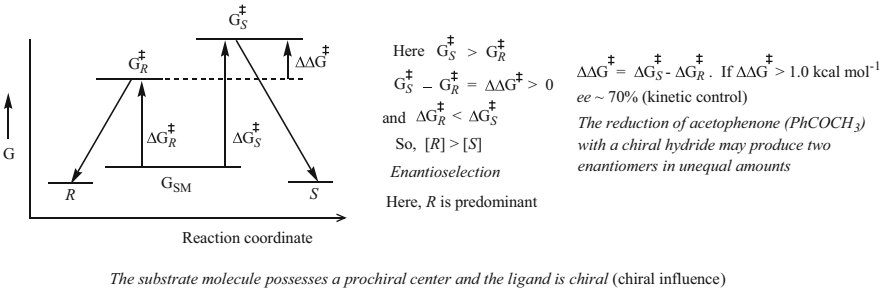


Fig. 2.75 Principle of enantioselection. Energetics

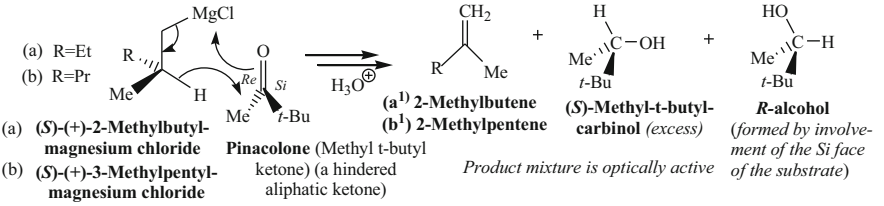


Fig. 2.76 Examples of first enantioselective Grignard reactions

2.11.1.2 Enantioselection

In 1950 Mosher and La Combe reported two papers [29, 30] on the first asymmetric Grignard reactions (Fig. 2.76) which are examples of enantioselection (Fig. 2.76). The existing chiral center (or centers) is said to bring about *asymmetric induction*. Thus, addition of a chiral nucleophile to the two enantiotopic faces of a prochiral carbonyl compound leads to enantioselectivity.

2.11.1.3 Diastereoselection

The concept of diastereoselection was first clearly outlined by Emil Fischer in 1894, based upon the conversion of one sugar to the next higher homologue via the cyanohydrin reaction. Thus, the substrate molecule should possess a prochiral center as well as at least one chiral center, and the reagent may be achiral, as in the reaction of a sugar with HCN. The principle of diastereoselection is illustrated with the help of an energy diagram (Fig. 2.77), which is self-explanatory. The expression for ΔG° in thermodynamic control and that for $\Delta\Delta G^{\neq}$ in kinetic control are shown. In case *the reaction is reversible*, the product which is formed at a slower rate but at the same time if it is of lower free energy (more stable) will be major, and the reaction is said to be of thermodynamic control. Like Fischer's cyanohydrin reaction, reactions to which Cram's rule and Prelog's rule are applicable are also examples of diastereoselection. Moreover, enantiomers have widely different reactivities especially in biological systems. The enantiomer may have different biological activities, and only a particular enantiomer may have the desired drug effect (Fig. 32.2). It is well known that only L-amino acids can participate in protein synthesis. Thus, diastereoselection is a result of involvement of diastereomeric transition states (with different free energies); consequently, the diastereomer, which is formed irreversibly via less activation energy and hence at a faster rate, is the predominant product of kinetic control.

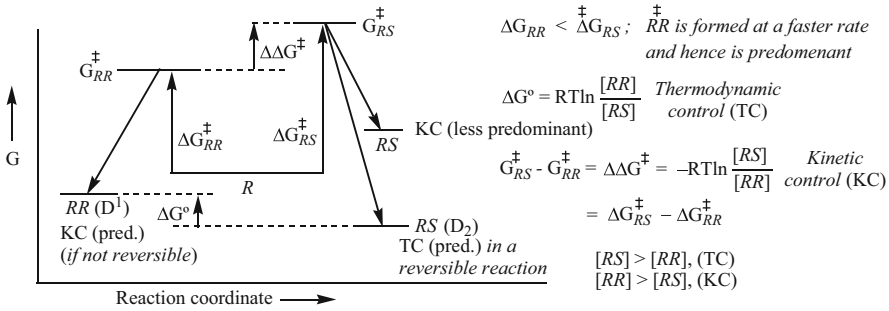
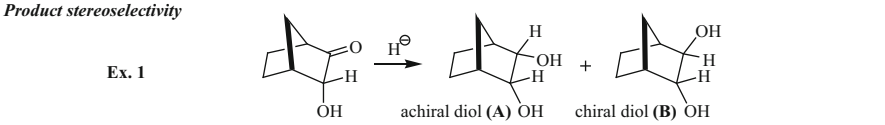


Fig. 2.77 Principle of diastereoselection. Energetics



*By use of an adequately bulky hydride reagent if the achiral diastereomeric diol (**A**) is formed exclusively, still it is an asymmetric synthesis, and the reaction is said to be 100% stereoselective as a result of 100% diastereoselection. This is an example of product stereoselectivity.*

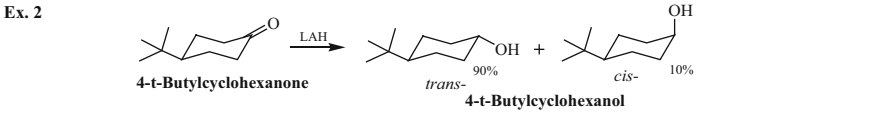


Fig. 2.78 Stereoselective reactions. Product stereoselectivity

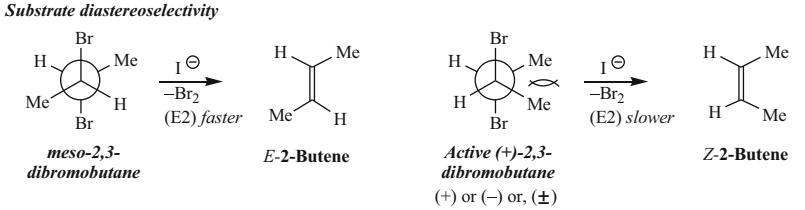
2.11.2 *Asymmetric Synthesis. Definition. Stereoselective and Stereospecific Reactions. Product/Substrate Stereoselectivity. Regioselectivity*

An *asymmetric synthesis* is a reaction in which a *prochiral unit* in a substrate molecule is converted by a reactant into a *chiral unit* in such a manner that the stereoisomeric products are produced in unequal amounts.

The prochiral unit in a substrate molecule must have enantiotopic or diastereotopic ligands or faces. In rare cases, only one stereoisomer is formed with the complete exclusion of the other stereoisomer, and the reaction is termed *100 % stereoselective*. One stereoisomer may be optically inactive also (Ex. 1 of Fig. 2.78). This is an example of *product stereoselectivity*.

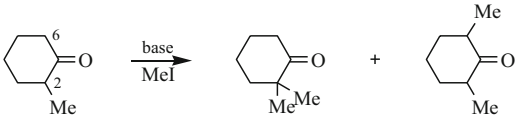
Example 2 provides another simple case of *product stereoselectivity* (Fig. 2.78).

When two diastereomers or two enantiomers react at different rates to give two stereoisomers (Fig. 2.79)—the reaction is termed *stereospecific*. Enzymes usually



This is a case of substrate selectivity or, better substrate diastereoselectivity. In some particular case one diastereomer may not react at all under the same condition.

Fig. 2.79 Stereospecific reactions. Substrate selectivity



2-Methylcyclohexanone **2,2-Dimethyl-cyclohexanone** (mostly) **2,6-Dimethyl-cyclohexanone** (a little)

The generated C2 anion is more reactive than that generated at C6, because of the inductive effect of the methyl group.

Fig. 2.80 An example of regioselectivity

react with one enantiomer only, and sometimes with both enantiomers in different ways (Fig. 32.2) and thus show total substrate enantioselectivity.

Regioselectivity. If a substrate is capable of reacting at more than one center (polydent molecules) and reacts at one center with a higher rate than the other centers, this is known as *regioselectivity* (Fig. 2.80).

2.11.2.1 Enantiomeric Excess. Diastereomeric Excess. Optical Purity

In a mixture of a pure enantiomer (*R* or *S*) and a racemate (*RS*), *enantiomeric excess* (*ee*) is the percent excess of the enantiomer over the racemate. Thus, p*ercent enantioselective excess* (*% ee*) or *percent diastereoselective excess* (*% de*) of *R* is illustrated by the following expression:

$$\%\ \text{ee or}\ \%\ \text{de of R} = \frac{[R]-[S]}{[R]+[S]} \times 100 = \%\ \text{R} - \%\ \text{S} = \%\ \text{stereoselectivity of R},$$

where R and *S represent* two enantiomers or configurations of the chiral center created in the two diastereomers.

Enantiomeric excess usually corresponds to the older expression, ***optical purity*****,** *op*, $([\alpha]_{\text{obs.}}/[\alpha]_{\text{max}}) \times 100$ %, *i.e.,* the absolute value of the ratio of the observed specific rotation of a sample made up of two enantiomers, which is otherwise chemically pure, to the corresponding specific rotation of one pure enantiomer,

expressed as percentage. The term $[\alpha]_{max}$ signifies the specific rotation of one enantiomerically pure sample. Currently, *ee* or *de* is usually measured by NMR and by chromatographic analysis, and hence the term optical purity is gradually becoming outmoded.

1,2-Addition of Achiral Nucleophiles to Chiral Ketones and Aldehydes (Diastereoselection) (Sects. 2.11.3–2.11.5).

2.11.3 Cram's rule

2.11.3.1 Cram's Open Chain Model

A carbonyl group attached to a chiral center (*e.g.*, RCOCLMS) (L, M, and S represent large, medium sized, and small ligands, respectively) undergoes nucleophilic addition with metal hydride or achiral organometallic reagents (*e.g.*, R^1MgX or R^1M) to produce two diastereomeric products of which one predominates. The relative configuration of the asymmetric center, created in the predominant diastereomer, and its absolute configuration—if the configuration of the chiral carbon already present in the substrate is known—is predicted by *Cram's rule* based on some arbitrary TS models [31–34]. The rule is essentially empirical but has excellent predictive value.

In the open chain model illustrated in Fig. 2.81, the substrate molecule is complexed with the reagent from the right side of the carbonyl π-plane to give (**A**), from which R^1 is transferred to the trigonal carbon from the right diastereotopic face (from the side of the small ligand S) (route **a**), in preference to the complex ($\mathbf{A}^1$) which transfers R^1 from the left diastereotopic face (route b).

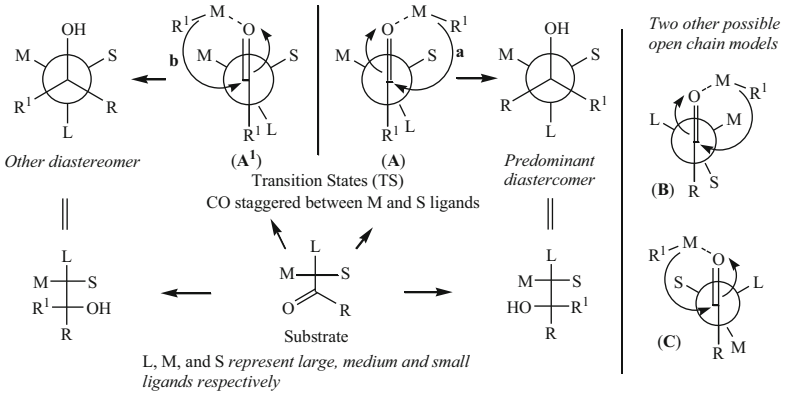


Fig. 2.81 Cram's open chain model

Thus, 1,2-asymmetric induction takes place by the chiral center already present. Reagents R^1M: EtLi, MeLi R^1Li, etc.; $LiAlH_4$, $NaBH_4$; R^1MgX: R^1 = Me, Et, Ph, iPr, $iPrCH_2$, etc., X = Br, I.

One other possible open chain TS model (**B**) (Fig. 2.81) with carbonyl group staggered between L and M and the nucleophile a attacking the carbonyl carbon from the side of M (smaller than L) correctly predicts the stereochemical course to give the predominant product, but it involves stronger steric interactions of the bulkiest complexed O (of CO) with gauche L and M groups making the TS energy much higher and hence is not tenable. The third possible TS model (**C**) (Fig. 2.81) with CO staggered between S and L involving the preferential nucleophilic attack on the left diastereotopic face (the side of S) leads to the less predominant product, and hence is eliminated.

Cram's rule is equally applicable to racemic substrate when the products are also racemic.

Limitations of Cram's Open Chain Model

(i) It applies only to kinetically controlled reactions; for Meerwein–Pondorf–Varley (MPV) reaction Cram's model may hold good for a short reaction time.
(ii) It does not apply to catalytic reduction. These two limitations hold good for other models also, to be discussed later.
(iii) It does not apply when the small ligand S is OH, OR, NH_2—capable of complexing with the reagent R^1M or R^1MgX. In such cases cyclic model will be applicable.

Some examples of the applications of Cram's rule are shown in Fig. 2.82.

Stereoselectivity through the open chain nonrigid model (Fig. 2.82) is not usually high except for a case in which the difference of bulkiness of the ligands M and S is high.

2.11.3.2 Cram's Chelate or Cyclic Model

If the substrate chiral ketone contains at the α-position an OH, NH_2, or OMe group which is capable of coordinating with the reagent, Cram's rule based on a rigid *chelate or cyclic model* predicts the stereochemistry of the predominant product, as delineated in Fig. 2.83. The metallic part of the reagent is doubly coordinated, as shown, to form a 5-membered ring. The nucleophile preferentially approaches the electrophilic carbon from the side of the ligand S. If the chelating group is M (usual case), the cyclic model predicts the same stereochemistry as the open chain model (case 1). If the chelating group is the ligand S or L, the cyclic model predicts the correct stereochemistry of the predominant product, while the open chain model predicts the opposite stereochemistry (case 2). For such substrates the chelate model should always be applied, irrespective of the bulkiness of the chelating ligand.

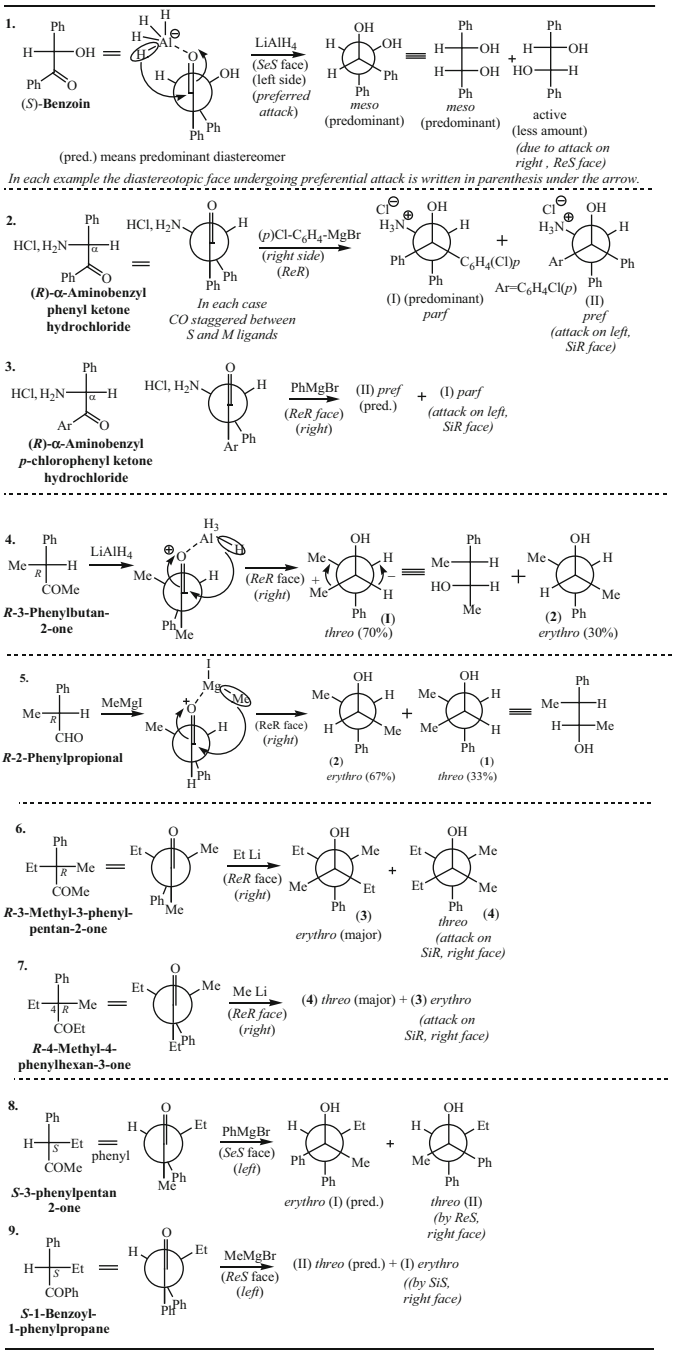


Fig. 2.82 Cram's rule. Open chain model, nine examples

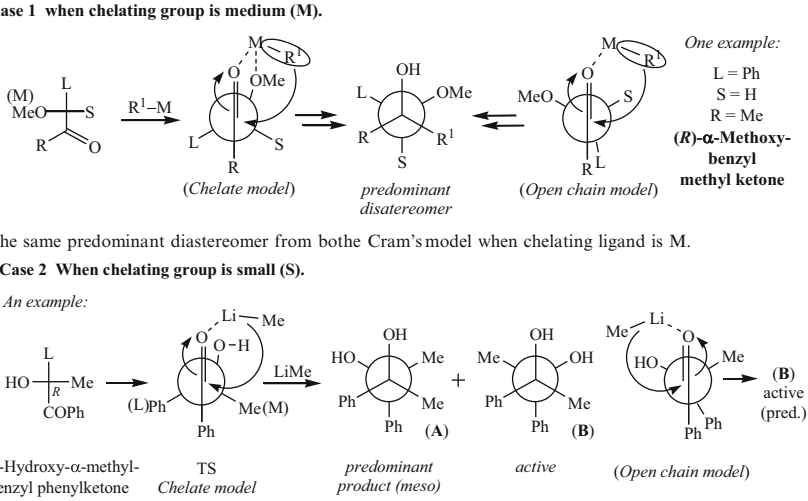


Fig. 2.83 Cram's chelate (cyclic) model. Examples

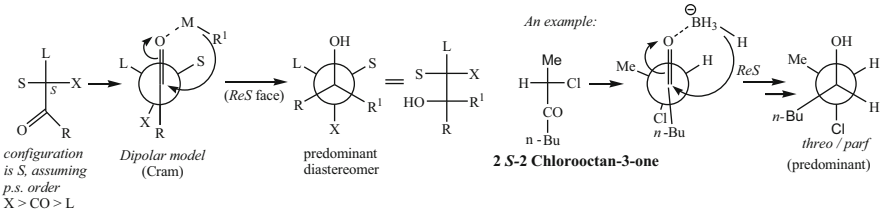


Fig. 2.84 Cram's dipolar model. An example

2.11.3.3 Cram's Dipolar Model

Cram suggested a *dipolar model* for prediction of stereochemistry when the substrate carries a strongly electronegative group, *e.g.,* a halogen atom at C_α position [32, 34]. To minimize the dipole repulsion and to increase the electrophilic character of the carbonyl carbon, the C=O bond and the C–X bond (having opposite dipoles) are placed anti in the dipolar model (Fig. 2.84). The nucleophile adds from the side of the small ligand S, giving the predominant product, as shown. Cram's model correctly predicts the stereochemical course of the reactions but does not always succeed to give a quantitative assessment of the asymmetric induction based on steric interactions. A few alternative models have been suggested [33] of which the Felkin–Anh model [35, 36] has been widely accepted.

2.11.4 Felkin–Anh Models [35, 36]

2.11.4.1 Felkin–Anh Open Chain Model

In this model three reactive transition state (TS) conformations (**A**), (**B**), and (**C**) (Fig. 2.85) are considered in terms of the orbital and the nonbonded steric interactions. Because of the destabilizing four-electron interactions between the HOMO (highest occupied molecular orbital) of the nucleophile and the HOMO of the carbonyl group [(**a**) in Fig. 2.86], the incoming nucleophile in each case approaches the carbonyl at an angle of about 109° with the carbonyl plane, little away from the orthogonal approach, corresponding to the Bürgi–Dunitz trajectory [37–39]. The TS (**A**) encounters minimum steric interaction in addition to the orbital destabilizations (discussed later) and involves mainly the two-electron stabilizing interactions between the HOMO of the nucleophile and the lowest unoccupied molecular orbital (LUMO) of the carbonyl group. Hence, it leads to the predominant diastereomer ($\mathbf{D}^1$) by nucleophilic attack on the right side. The TS conformation (**C**) might also lead to the same predominant diastereomer ($\mathbf{D}^1$), but its contribution should be *very little* because of its high TS energy due to nonbonded interactions R↔L and L↔R^1 (approaching nucleophile). The TS conformation (**B**) involves the

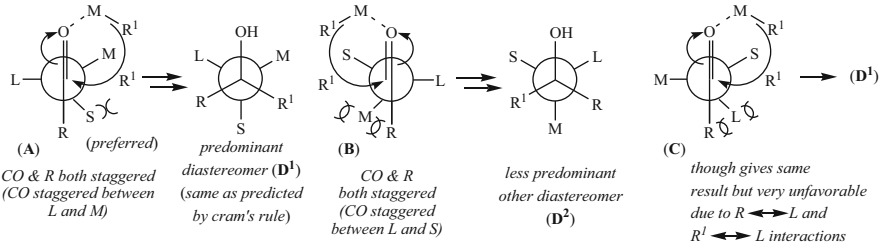


Fig. 2.85 The Felkin–Anh model for the nucleophilic addition to the carbonyl group.

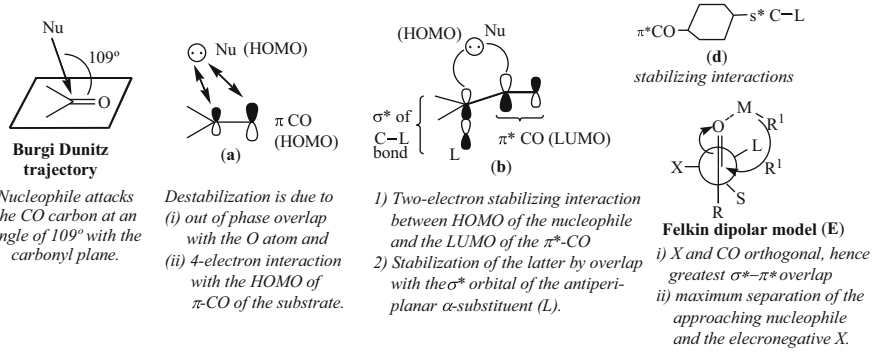


Fig. 2.86 Felkin dipolar model. Orbital interactions in the Felkin–Anh model

nonbonded interactions M↔R and R^1↔M; hence, it possesses higher free energy and leads to the less predominant diastereomer (**D^2**).

Moreover, the antibonding π* orbital is stabilized by its overlap with the σ*orbital.

Thus, consideration of the *molecular orbitals involved* reveal that the *orthogonal approach* of the nucleophile leads to its destabilization due to (i) out of phase overlap with the oxygen atom and (ii) from the four-electron interaction with the HOMO of the π-CO of the substrate [(**a**) in Fig. 2.86], and hence the approach of the nucleophile invokes the *Bürgi–Dunitz trajectory* to minimize these destabilizing factors. The approach of the nucleophile involves further stabilization if the largest ligand L is placed orthogonal to the C = O double bond, thus providing greatest overlap between the antibonding π* orbital (lowest unoccupied molecular orbital, LUMO) of the carbonyl group and the antibonding orbital σ* of the antiperiplanar *α*-substituent, in addition to the stabilizing two electron $n-\sigma^*$ interaction with the electron pair of the nucleophile as shown in (**b**).

In fact, any example of the Cram's open chain model (see Fig. 2.83) can be explained in a more quantitative manner by Felkin–Anh's model (**A**) (Fig. 2.85).

2.11.4.2 Felkin–Anh Dipolar Model

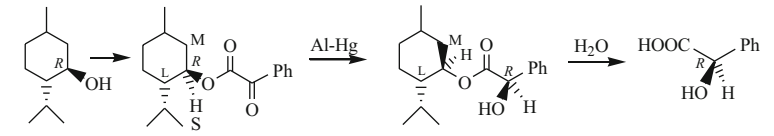
Cram's dipolar model for the prediction of the predominant diastereomeric product may also be replaced by a different Felkin–Ahn model (**E**) (Fig. 2.86), based on the tenets already mentioned, with the additional proviso that in the TS the halogen or any strongly electronegative ligand is placed orthogonal to the C=O bond, thus providing greatest overlap between σ* orbital of the C–X bond and the π* orbital of the C=O, as well as allowing maximum separation of the electronegative *α*-substituent (X) and the negatively charged nucleophilic reagent (Fig. 2.86). Of course, Felkin–Anh dipolar model also leads to the same predominant diastereomer as the Cram's dipolar model (Fig. 2.84).

2.11.5 Prelog's Rule

Prelog's rule, though empirical, allows the prediction of the steric course of an asymmetric synthesis carried out with a chiral α-ketoester (usually a glyoxylate or pyruvate) of a chiral secondary or tertiary alcohol, SMLC-OH (S, M, and L stand for small, medium sized, and large substituents, respectively). The prediction has been confirmed in the majority of the cases.

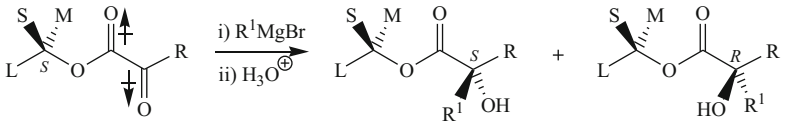
It is the outcome of generalization of the results of the asymmetric synthesis carried out by McKenzic group in early twentieth century. In McKenzie's first example [40] the phenylglyoxalate ester of (−)-menthol was reduced with aluminum amalgam to give the (−)-mandalate ester of (−)-menthol as the predominant product over the corresponding (+)-mandalate ester (Fig. 2.87).

For application of Prelog's rule [41, 42], by convention, in the transition state the α-ketoester (**1A**) (Fig. 2.88) is so oriented that the two carbonyl groups are



Note: *This example may be rationalized by Prelog's rule, as indicated.*

Fig. 2.87 Asymmetric synthesis of (−)-mandelic acid

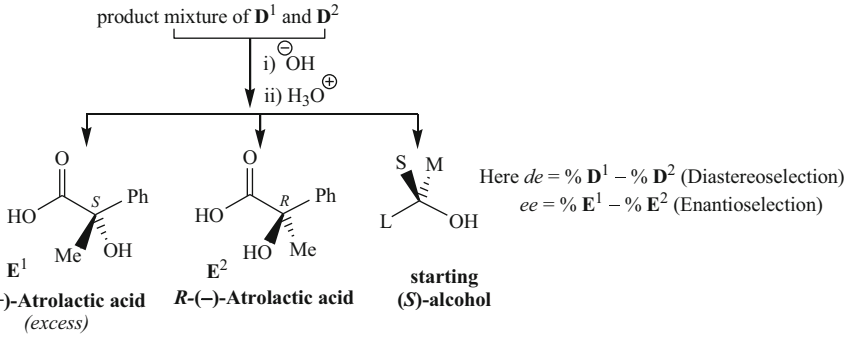


If the priority sequence order is same as the bulkiness order i.e. L>M>S, and provided the priority sequence of R>R^1, the chiral center generated preferentially will be of the same descriptor as the starting alcohol, (here S)

Diastereoselectivity: [$\mathbf{D}^1$] > [$\mathbf{D}^2$]

$\mathbf{D}^1$ *and* $\mathbf{D}^2$ *may also be separated.*

An example: In the above Grignard reaction, when R=Ph *and* R^1=Me, *i.e., the reaction of a phenyl glyoxalate with mathyl magnesium bromide*



So, the separated mixture of the hydroxyacids displays positive rotation, assigning (S) configuration to the starting chiral alcohol

Fig. 2.88 Prelog's rule. Diastereoselection. Enantioselection

antiperiplanar (to minimize the dipolar repulsion), and the L group occupies the same plane as the two carbonyl groups and the alkyl oxygen bond; thus, the bulky ester carbonyl group (complexed with the reagent) becomes staggered between two smaller groups S and M (Fig. 2.88). In this TS conformation the nucleophile (usually the Grignard reagent R^1MgX) will predominantly approach the ketocarbonyl function from the side of the small group S. This rule thus correlates

the configuration of the starting chiral alcohol SMLC-OH with the predominant α-hydroxyester formed, and therefore, of the α-hydroxy acid obtained in excess by hydrolysis along with the starting alcohol. Prelog's rule holds good since such nucleophilic addition is kinetically controlled.

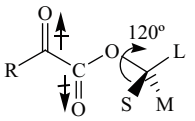
2.11.5.1 Attempted Rationalization of Prelog's Model

The generalization for predicting the stereochemistry of the products from the α-keto-ester asymmetric synthesis was put forward as an empirical model; yet its reasonable success implies some correlation of the model with the conformation of the TS (Fig. 2.89).

It is pertinent to note that trans-coplanar conformation does not represent the most stable ground state; it may represent the most stable TS, because of maximum separation of charge between negative centers is possible in this form. Thus, it represents better distribution of charge density under the influence of the reagent in the TS. X-ray crystal studies revealed that the ϕ between two CO's of (**2**) (Fig. 2.89) is 104°. This conformation may exist in solution also.

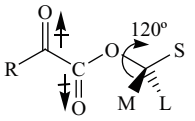
2.11.5.2 More Examples of The Application of Prelog's Rule

The atrolactic acid synthesis and the Prelog's rule have been widely used for the determination (or confirmation) of the absolute configuration of secondary alcohols, *e.g.*, alicyclic alcohols, monoterpene alcohols, sesquiterpene alcohols, triterpene alcohols, and steroid alcohols [43]. Some examples of Prelog's rule are shown in Fig. 2.90. For the asymmetric atrolactic acid synthesis, the chiral secondary alcohol is converted to its phenylglyoxylate ester. The latter is then reacted with MeMgBr to give the α-hydroxy ester in excess predicted by Prelog's rule.



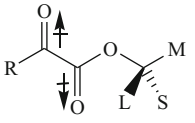
(**1A**) (*cf.* Figure **2.88**)

Choosing this TS conformation (**1A**) *was largely intuitive, but there is a rationale as follows: The ester CO being complexed with the reagent is staggered between the smaller groups* M *and* S.



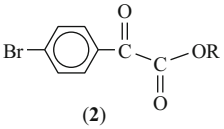
(**1B**)

Although this TS (**1B**) gives the *right diastereomer* $\boldsymbol{D^1}$*, in excess, the complexed ester C=O is staggered between* **M** *and* **L**, *so* (**1B**) *becomes of higher energy and may have minor contribution.*



(**1C**)

This TS conformer gives the wrong diastereomer as the predominant one, hence is discarded.



(**2**)

Fig. 2.89 Rationalization of Prelog's rule

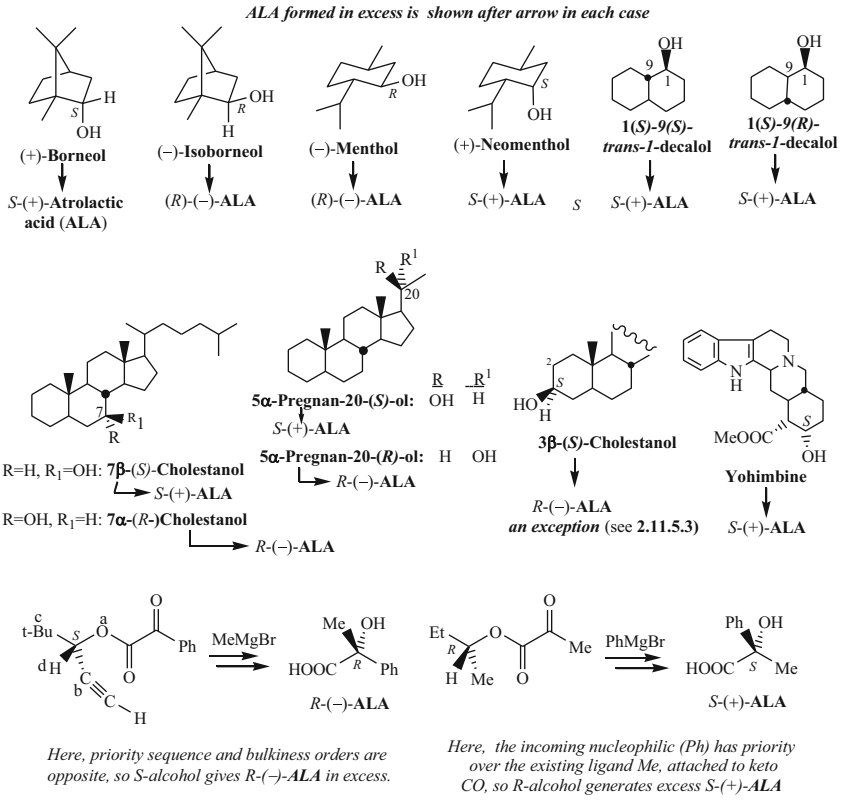


Fig. 2.90 More examples of application of Prelog's rule. Atrolactic acid synthesis

The product mixture upon hydrolysis gives the predicted atrolactic acid in excess (as shown in Fig. 2.88). In case the *priority sequence order is the same as the bulkiness order and (R) (here Ph) has higher priority than the incoming nucleophile (R)*[1] (here Me), *(R)-alcohol gives the (R)-hydroxy acid (here (R)-atrolactic acid) in excess* and the *(S)-alcohol gives (S)-hydroxy acid* (here (*S*)-atrolactic acid). The other two possibilities of getting (*R*)-hydroxy acid from (*S*)-alcohol and *vice versa* are illustrated in the last row of Fig. 2.90.

2.11.5.3 Exception to and Anomalies of Prelog's Rule

In atrolactic acid synthesis with (*S*)-3β-cholestanol (see Fig. 2.90), *R*-(−)-atrolactic acid is obtained in only 1.7 % enantiomeric excess [43]. Thus, *S*-alcohol is providing the *R*-atrolactic acid. This may be taken as an exception to Prelog's rule. Perhaps, here bulkiness order is not the same as the CIP priority sequence. Examination of models reveals that the steric crowding at C2 (with its environment)

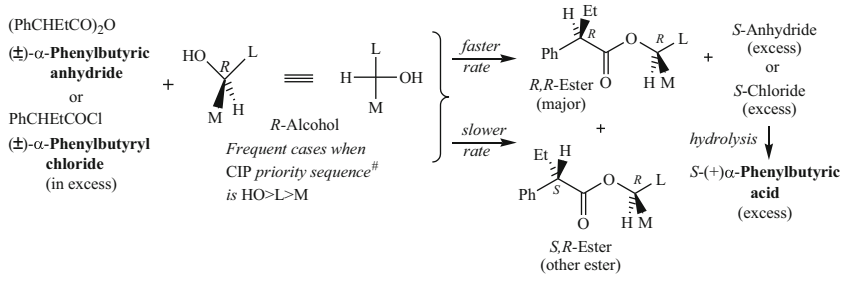
is greater than that at C4. However, asymmetric induction being so low, no definite conclusion can be drawn from this result.

Atrolactic acid synthesis of the tetramethyl ethers of both (−)-epicatechin (*R*-3-ol) and (+)-catechin (*S*-3-ol) leads to the preferential formation of *R*-(−)-atrolactic acid. These results must be considered as an anomaly rather than an exception to the rule [43] (vide Sect. 14.5.3, Fig. 14.25).

2.11.6 Horeau's Rule

Horeau developed an empirical method [44, 45] for the correlation of configuration of secondary alcohols based on the principle of *kinetic resolution*. During the esterification of an optically active secondary alcohol, say, (−)-RR^1CHOH with *an excess of racemic* (±)-R^2COOH the transition states, [(+)-RR^1CHOH.(+)-R^2COOH]$^{\#}$ and [(+)-RR^1CHOH.(−)-R^2COOH]$^{\#}$ are diastereomeric and thus are unequal in enthalpy (*cf.* Fig. 2.77). Hence, the activation energies and the reaction rates of esterification with the enantiomeric acids will be unequal. Thus, the enantiomeric acid involving lower activation energy will be esterified at a faster rate, leaving the other enantiomeric acid in excess being unreacted (kinetic resolution) (Fig. 2.91).

An enantiomerically pure (or enriched) secondary alcohol is treated with an excess of (±)-α-phenylbutyric anhydride (Fig. 2.91) (or sometimes the corresponding chloride). The residual anhydride is hydrolyzed and the optical rotation of the resultant α-phenylbutyric acid is measured. The Horeau's rule states that (provided, the CIP priority L > M holds good) an alcohol with *R*-configuration gives an excess of *S*-(+)-phenylbutyric acid and vice versa (Fig. 2.91). Brewster [46] and Horeau [47] have published detailed reviews on this method and its limitations.



Thus, excess of S-(+)-α-phenylbutyric acid implies R-configuration of the unknown alcohol.
Likewise, excess of R(−)-α-phenylbutyric acid implies S-configuration of the unknown alcohol.

#In rare cases when priority sequence is HO>M>L, the conclusion will be reverse, i.e. excess S-acid implies S-alcohol and excess R-acid implies R-alcohol. Examples: M= −C≡CH and L= t-Bu; M= −CH=CH$_2$, L= −CHMe$_2$

Fig. 2.91 Horeau's rule illustrated

Horeau's method has the advantage that in case the optically active alcohol is not available, the racemic alcohol itself may be treated with optically active α-phenylbutyric anhydride taken in much excess. The rotation of the unreacted alcohol is measured. Based on kinetic resolution now it may be concluded that if *S*-(+)-phenylbutyric anhydride is used, the unreacted alcohol would have *R* configuration (provided L gets priority over M) and *vice verse* (Fig. 2.91). The sign of rotation of the remaining unreacted alcohol needs to be determined. The rule is strictly empirical, and no rationalization has yet appeared.

The Horeau's procedure can be used [48, 49] for microscale determination if a highly sensitive method (GC, HPLC, MS, or CD) is available to determine the composition of the diastereomeric esters formed.

2.11.7 Sharpless Enantioselective Epoxidation

Sharpless in 1980 discovered a new metal-catalyzed asymmetric epoxidation process [50] which is by far more selective than any previously described methods for such asymmetric transformation. The strained epoxide ring serves as a valuable synthon for many natural products including insect pheromones. One of the most attractive aspects of this new process is the use of its readily available simple components.

In this method a prochiral primary allyl alcohol (mono-, di-, or tri-substituted, R^1, $R^{2,}$ and R^3 are achiral) is treated with *t*-butylhydroperoxide (TBHP) in the presence of titanium (IV) isopropoxide and optically active diethyl tartrate (DET) (or di-isopropyl tartrate, DIPT) to produce the corresponding epoxide in a highly enantioselective manner, as shown in Fig. 2.92. The system provides consistent enantioselection.

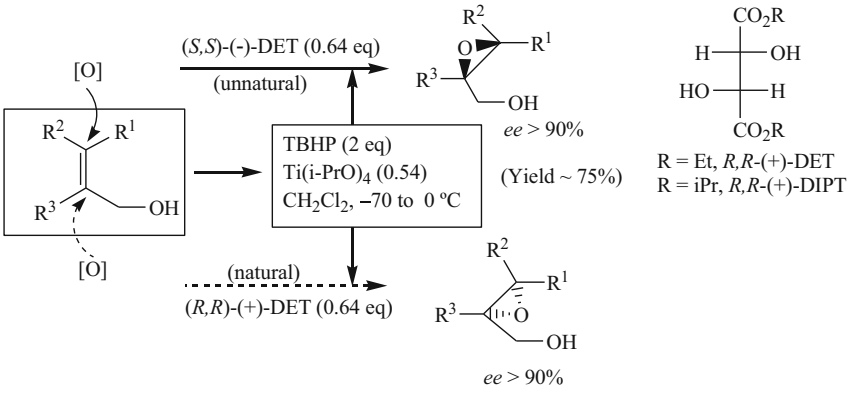


Fig. 2.92 Prediction of the chiral epoxides formed enantioselectively from prochiral allylic alcohols by Sharpless rule

With Sharpless reagent using (+)-DET geraniol undergoes regiospecific epoxidation of the allylic 2,3-double bond to form 2*S*,3*S*-epoxygeraniol in 90 % *ee* in accordance with the Sharpless rule [50]; here attack takes place almost entirely at the 2*Re*-,3*Si* (α-face) [*cf.* ex. (V) of Sect. 6.2.6]. Extensive application of the Sharpless epoxidation method to numerous multistep syntheses demonstrates its utility and reliability [51, 52].

The catalyst is sensitive to preexisting chirality in the substrate. Thus, the epoxidation of racemic secondary allylic alcohols with Sharpless reagent proceeds rapidly with only one enantiomeric alcohol, leaving behind the other slower reacting enantiomer.

2.11.7.1 Kinetic Resolution of Racemate Allyl Alcohols

Resolution of racemate allyl alcohols may be accomplished *by treating with half equivalent of TBHP in the presence of the chiral catalyst like Ti-(i-Pro)$_4$ complexed with an active DET (or DIPT).*

The epoxidation pattern of *E*-cyclohexylpropenylcarbinol using (+)-Diisopropyltartrate is shown in Fig. 2.93 [53].

Interestingly, for steric reasons in the transition states, the *S*-enantiomer (in Fig. 2.93) reacts much faster than the *R*-enantiomer, thus under the reaction condition permitting the isolation of the unreacted *R*-isomer.

Kinetic resolution of the racemate allylic alcohols (*cf.* Fig. 2.93) has been achieved by Sharpless epoxidation method [54].

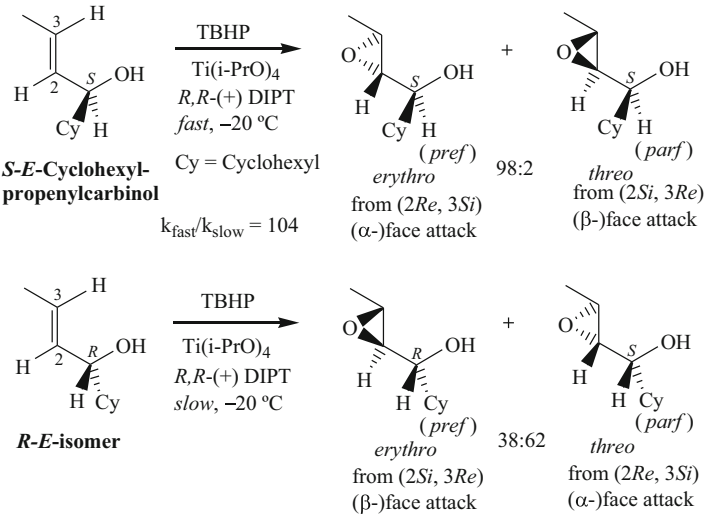


Fig. 2.93 Kinetic resolution of racemate allyl alcohol by Sharpless epoxidation method

2.11.7.2 Mechanism of the Sharpless Reaction

Nature of the titanium alkoxide system has the following properties crucial to the success of the reaction:

(i) Exchange of monodentate alkoxide ligands is rapid in solution.
(ii) Of the four covalent bonds of Ti^{IV} two participate for the divalent chiral auxiliary (tartrate) and one each for TBHP and the allyl alcohol.
(iii) The coordination chemistry of Ti^{IV} (d°) alkoxide system is flexible since this system displays a range of coordination numbers and geometries. This property may be responsible for the catalysts' ability to accommodate sterically widely differing substrates.
(iv) Ti^{IV} alkoxides are weak Lewis acids and thus activate a coordinated alkylperoxo ligand toward nucleophilic attack by olefin of a bound allylic alcohol.

The mechanism of the reaction has been extensively studied by NMR, and numerous crystal structures for the complexes involved have been determined. The active catalyst was initially believed to be the 10-membered structure [55]. Comprehensive analyses [56, 57] favor a mechanism (Fig. 2.94) based on the structure (**I**) to be converted to (**II**), by use of *R*,*R*-(+)-DET, epoxidizing much predominantly the enantiotopic α-face of the allylic segment (when written as in Fig. 2.92).

Most importantly, the species containing equimolar amount of titanium tetraalkoxide and tartrate is shown to be the most active catalyst in the reaction mixture, mediating the reaction at a much faster rate than titanium tetraalkoxide alone [56].

In (**I**) due to weak coordination the carbonyl oxygen atoms readily dissociate and recoordinate to the metal centers, providing a means of exchanging the alkoxide ligands for the substrate molecules [*R*,*R*,-(+)-DET], TBHP, and allylic alcohol as shown in (**II**). The lower energy conformation (here oxygen is delivered from the α-face of the double bond of the β-substituted allylic alcohol) of the allylic segment dictates which enantiotopic face is epoxidized. Many allylic alcohols with different substituents have been highly enantioselectively epoxidized following the Sharpless rule.

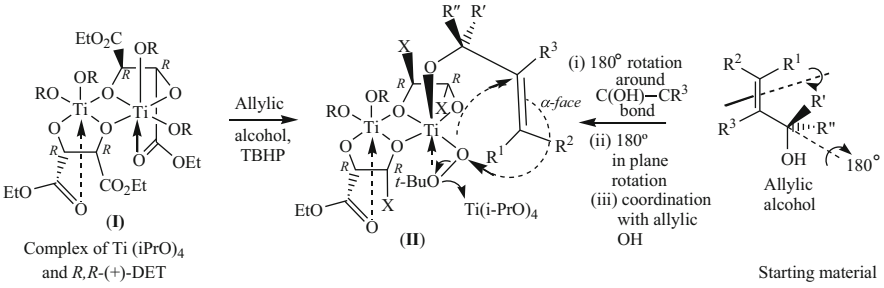


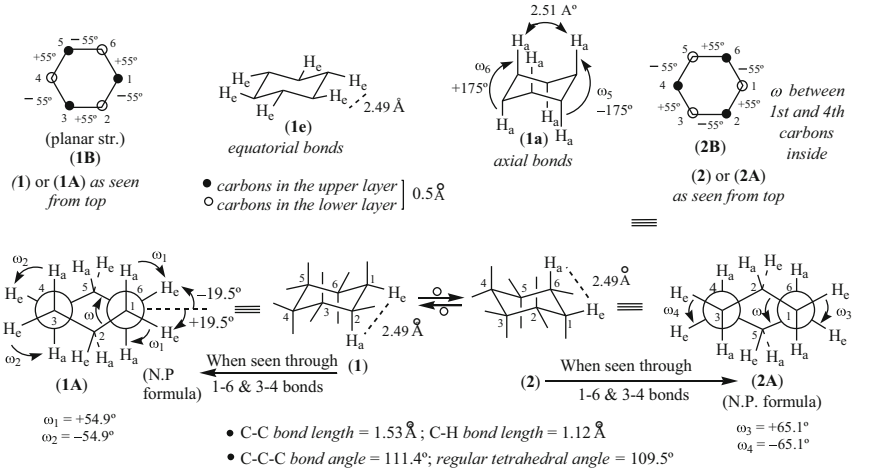
Fig. 2.94 Mechanism of the Sharpless epoxidation of an allylic alcohol by the active catalyst (**I**) from *R*,*R*-(+)-DET

2.12 Conformation of Saturated Six-Membered Ring Compounds

2.12.1 Conformational Aspects of Cyclohexane

2.12.1.1 Geometry of Cyclohexane Chair. Bond Lengths. Bond Angles. Torsion Angles

Baeyer considered that cyclohexane is a strained planar molecule. In 1890 Sachse first pointed out that cyclohexane might be nonplanar puckered chair or boat shaped and unstrained (see D. H. R. Barton, reference 2 of Chapter 11). As evident from X-ray and electron diffraction experiments by Hassel in 1943, cyclohexane exists almost exclusively in its chair conformation. The electron diffraction experiments reveal the C–C and C–H bond lengths, bond angles, the intraannular (C–C–C–C), and the external H–C–C–H torsion angles, which are shown in (**1A**) and (**2A**) (Fig. 2.95). The C–C–C– bond angle (111.4°) is more than the regular tetrahedral angle (109°) and little less than the C–C–C bond angle (112.4°) in *n*-propane. The (C–C–C–C) torsion angle (55°) deviates from the optimum of 60° in n-propane, because of the constraint in the ring. Such a decrease in the torsion angle is resisted due to an increase in torsional strain involved. A compromise is reached to



- C-C-C *bond angle in propane* = 112.4°
- *Torsion angle*, ω, of C-C-C-C, or ω_1 or ω_2 H_a-C-C-H_e = +54.9° or –54.9°. *Torsion angle* ω_3 or ω_4, H_e-C-C-H_e = +65.1° or –65.1°
- *For determining the sign of ω from chair orboat form (without drawing the N.P formulas) see* ***Section 2.15.1.2.***
- *The distance between hydrogen atoms at positions (i) 1a & 2e = 1e & 2a = 1e &2e = 2.49 Å (diverse directions) (ii) 1a & 3a = 2.51 Å (parallel directions). The distance between substituent(s) in case (i) increases (more in 1e, 2e), but in case (ii) remains unaltered if the substituents are same, but increases slightly when the substituents are different.*

Fig. 2.95 Salient features of the geometry of cyclohexane chair and its flipped conformer

minimize the total strain when the bond angle slightly increases and the torsion angle becomes little smaller than that of an open chain molecule like n-propane. Thus, cyclohexane is not an entirely strain-free molecule; some angle strain and torsional strain remain.

2.12.1.2 Equatorial and Axial Bonds

Cyclohexane has two geometrically different sets of H atoms, six are approximately parallel with the vertical C_3 axis, alternatively up and down, as shown in structure (**1a**) (Fig. 2.95) called axial (a) hydrogen atoms; the remaining six H atoms are distributed around the periphery of the ring making alternately +19.5° and −19.5° angles with the equatorial (horizontal) plane of the molecule and are called equatorial (e) as shown in the structure (**1e**) (Fig. 2.95) (see D. H. R. Barton, reference 2 of Chapter 11).

The decrease in the intraannular torsion angle to 54.9° brings with it a decrease in the external H–C–C–H torsion angle of *cis* (*ae or ea*) located H atoms from usual 60° (in ethane) to 54.9° [structure (**1A**)] and that of *trans* (*aa*) located H atoms from usual 180° to 174.9°, and an increase in the corresponding *trans* (*ee*) located H atoms from 60° to 65.1°. In ^{1}H NMR spectrum the vicinal coupling constants for such *cis* and *trans* protons vary according to Karplus relationship (see Sect. 4.2.6).

Since the distances between different pairs of adjacent hydrogens and between 1,3-diaxial hydrogens are 2.49 Å and 2.51 Å, respectively (Fig. 2.95), which are more than twice the van der Waals radius (1.20 Å) of hydrogen, there is no nonbonded interaction in the chair form of cyclohexane.

2.12.1.3 Symmetry of Cyclohexane Conformations

a. ***Chair*** **(1)**. The principal axis of symmetry is the C_3 axis passing vertically through the center of the chair; this is also an S_6 axis. Additionally, the chair conformation possesses three C_2 axes bisecting pairs of opposite sides (str. **3** and **4**) (Fig. 2.96), a center of symmetry, and also three vertical σ planes intersecting at the C_3 axis and passing through the diagonal (opposite) carbon atoms. Each σ_v plane also bisects the angle between two C_2 axes; hence, the σ_v planes are also known as σ_d. Thus, the cyclohexane chair belongs to the point group $\mathbf{D_{3d}}$ (Fig. 2.96) and cyclohexane molecule is achiral.

b. ***The flexible forms—boat and twist–boat. Symmetry***. The two extremes of the flexible forms of cyclohexane are boat (**5**) and twist–boat (**8**) (Fig. 2.96). These are interconverted by *pseudorotations*, which are low-energy processes, and involve only changes in torsional strain and other nonbonded interactions, but do not involve bond angle variation. This can be verified by molecular model (Dreiding or Fischer) study. In the true boat (**5**) the bowsprit and flagpole hydrogens are only about 1.8 Å apart, whereas the sum of the van der Waals

radii of two hydrogen atoms are about 2.4 Å. Thus, there is a significant *bowsprit–flagpole* (*bs–fp*) interaction.

To minimize the *bs–fp* interaction, the *bs* and *fp* H's are pulled a little apart resulting in the twist–boat in which the eclipsing of the adjacent H's at C2 and C3 and at C5 and C6 are also somewhat alleviated. The important stereochemical features including the symmetry point group of the boat and twist–boat forms are delineated in Fig. 2.96. The symmetry point groups of boat and skew–boat forms of cyclohexane are $\mathbf{C_{2v}}$ [$C_2 + 2\sigma_v$ orthogonal)] and $\mathbf{D_2}$ [$C_2 + 2C_2$ (orthogonal)], respectively (Fig. 2.96).

2.12.1.4 Enthalpy (*H*) or Potential Energy (*E*) Difference

Here b-ec stands for butane–eclipsing interactions and b–g stands for butane–gauche interactions (see Fig. 2.96).

H_{boat} = two *b-ec* (1,2,3,4 carbons + 4,5,6,1) interactions + 4 *b*-g (The other four successive 4 carbons) = 2 × 4.4 to 6.1) + (4 × 0.9) = (8.8 to 12.2) + 3.6 = 12.4 to 15.8 kcal/mol

H_{chair} = 6 b–g interactions (6 combinations of consecutive 4 carbons) = 0.9 × 6 = 5.4 kcal/mol

H_{boat}–H_{chair} = ΔH (or ΔE) = 12.4–15.8) – 5.4 = 7.0 to 10.4 kcal/mol

Since potential energy of skew–boat is 1.6 kcal/mol less than that of boat, the energy difference between skew–boat and chair form = 7.9 to 10.4—1.6 = 5.4 to 8.8 kcal/mol.

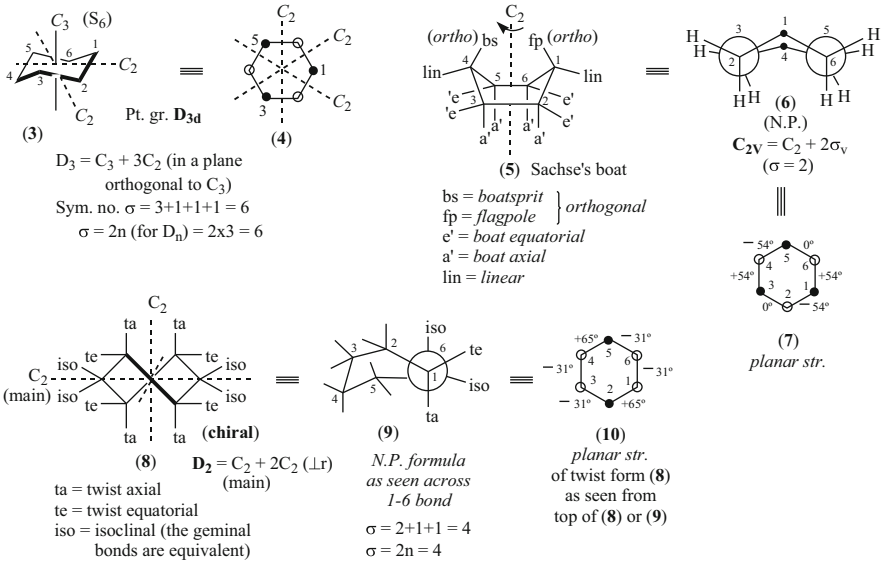


Fig. 2.96 Salient features of the geometry of cyclohexane boat and twist form

The enthalpy difference between flexible (skew–boat) form and chair form is estimated to be 5–6 kcal/mol based on heat of combustions and equilibrium determinations.

So the free energy difference $\Delta G = \Delta H - T\Delta S$ (entropy of flexible form is 5 e.u. greater than that of chair form at 298°K) = 5,560−5 × 298 = 4,000 cals = 4 kcal/mol.

Now from the expression $\Delta G = 4{,}000$ cals = RTlnK, one can calculate the equilibrium constant $K = 1{,}000$ (approx.) which means that only one molecule in a thousand will be in the skew–boat form.

2.12.1.5 Cyclohexane Ring Inversion

Cyclohexane chair form undergoes at ambient temperature *ring inversion* or *ring reversal* to another chair form of same energy (degenerate interconversion). Hassel called it *flipping*. Since the diastereomeric six equatorial hydrogens are exchanging with six axial ones in this transformation or *topomerization*, it is a case of *diastereotopomerization*.

Flipping or ring inversion or Hassel interconversion (Fig. 2.97) is accompanied by the following:

(i) Interchange of planes occur (distance between the two planes is ~0.5 Å).
(ii) The axial bonds are converted to equatorial bonds and *vice versa*.
(iii) α-Bonds and β-bonds remain unaltered.

The multistep inversion of cyclohexane chair (**1**) into the equienergetic chair (**1′**) through sequentially the transition state (**TS1**), skew–boat forms, and the transition state (**TS1′**) [enantiomeric with (**TS1**), is depicted in Fig. 2.98]. The interconversion of the skew–boat forms takes place through the intermediate Sachse's boat form, as evident from manipulation of molecular models. A pathway involving chair → envelop/half chair-like TS → intermediate boat form → inverted envelop/half chair TS → inverted chair is also possible. This is called σ-pathway since both the equivalent transition states and the intermediate boat form retain a symmetry plane of the ground state chair form. *But force field calculations make the existence of the boat form of cyclohexane as an intermediate in the cyclohexane inversion itinerary highly unlikely* [58], and hence the σ-pathway is ruled out. The skew–boat (or twist) form is obtained from the so-called *boat form* in the potential energy

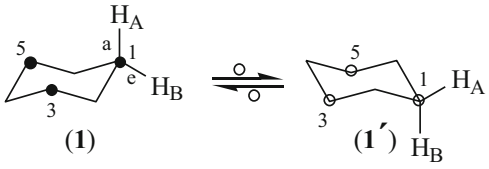
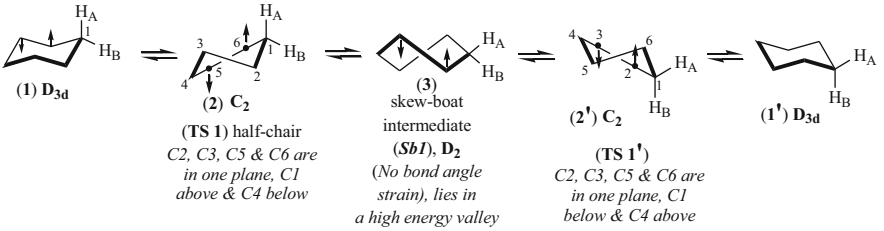


Fig. 2.97 Ring inversion of cyclohexane



- *Arrows show the direction of suitable twisting involving extensive bond angle deformation with increased torsional strain*
- *The enantiomeric (**TS1**) and (**TS1′**) have a C_2 axis (point group **C_2**), but no σ-plane.*
- *(**Sb1**) may be reversibly converted to two other skew-boat forms, one identical and the other enantiomeric, by pseudorotation without change of bond angles, but changing only torsion angles.*

Fig. 2.98 Conformational itinerary involved in cyclohexane ring inversion (C_2 pathway)

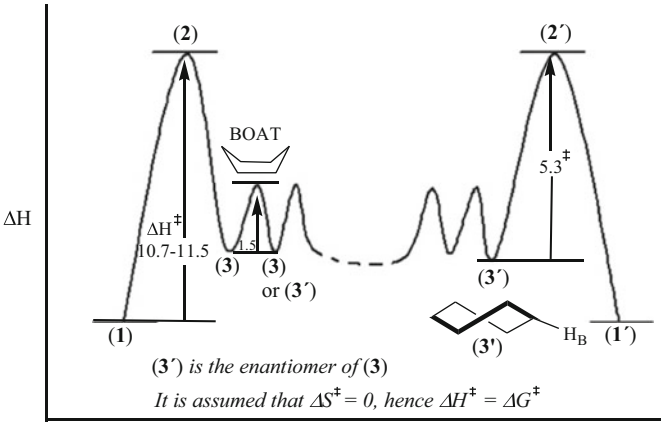


Fig. 2.99 Energy profile ($\Delta H^{\neq}$ in kcal/mol) of ring inversion of cyclohexane

diagram by slight deformation to alleviate the *fp* ↔ *bs* interaction and is less stable than the chair conformer by 4.7–6.2 kcal/mol according to various indirect experiments and also by force field calculations. The calculations also suggest that the true boat form is of about 1–1.5 kcal/mol higher energy than that of the twist form, and that the (**TS1**) or (**TS1′**) is of 10.7–11.5 kcal/mol higher energy than the chair form. The boat form is apparently at the energy maximum in the interconversion of the twist (or skew–boat) conformers of which two are mirror images. This pathway (Fig. 2.98) is called C_2 pathway, since the C_2 axis of the ground state chair form is retained along this pathway.

The energy profile diagram of ring inversion of cyclohexane following the C_2 pathway is depicted in Fig. 2.99.

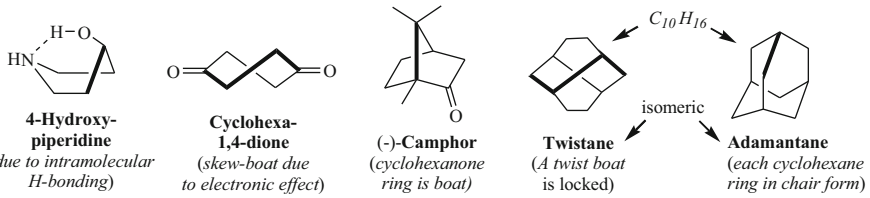


Fig. 2.100 Some compounds having fixed boat or skew–boat conformation of the cyclohexane ring

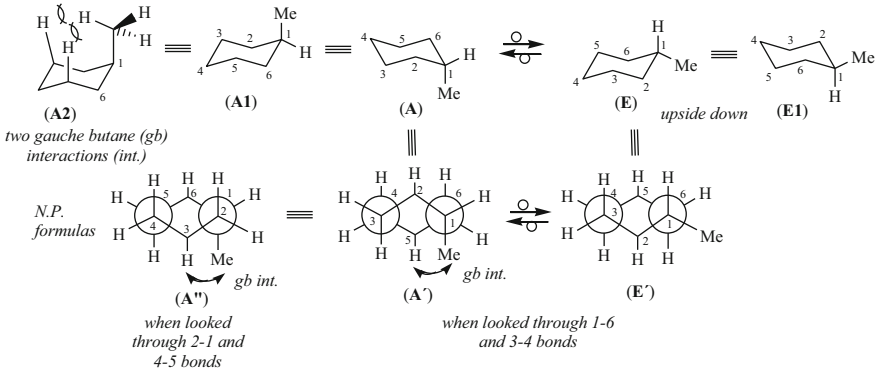
2.12.1.6 Stable Boat or Skew–boat Conformers

The chair form of cyclohexane is usually the most stable conformer. However, under certain conditions of structures the boat or the skew–boat forms can be stabilized in preference to the chair form. In case of certain molecules, as shown in Fig. 2.100, the configurational requirement does not allow the chair form of a particular ring. This is also true in cases of *trans-cisoid-trans*-perhydrophenanthrene (Fig. 2.136), *trans-transoid-trans*-perhydroanthracene (Fig. 2.140), the D/E rings in multiflorenol and friedelin, and the E ring in bauerenol and eapacannol (see Fig. 10.31, also see eupacannol in Fig. 2.111).

2.12.2 Monosubstituted Cyclohexanes. Conformational Energy

Monosubstituted cyclohexanes with the more stable equatorial substituent (E) flips into its nonequivalent diastereomeric less stable chair conformations with the substituent axial, as shown for methylcyclohexane in Fig. 2.101. Corresponding Newman projection formulas are also shown. The energy barrier of ring inversion in substituted cyclohexanes remains practically unaffected. The energy profile diagram is similar to that of cyclohexane ring inversion (Fig. 2.99); of course, the two chair conformers have different enthalpies and the rates of forward and backward interconversions are different. For example, in case of methylcyclohexane the equatorial conformer (E) will have two gauche–butane interactions (~1.8 kcal/mol) less enthalpy than the corresponding axial conformer (A), as shown in Fig. 2.101, both in chair formulas and Newman projection (N.P.) formulas.

Conformational Energy The energy (enthalpy or potential energy or free energy) of a given conformational isomer over and above that of the conformational isomer of minimum energy is called conformational energy.



Note: In (**A**′) or (**A**′′) (like A2) the distance between axial methyl protons and *syn*axial protons is ~ 1.83 A (~ van der Waals radii of two H's)

Fig. 2.101 Conformers of methylcyclohexane

As already mentioned before, the two diastereomeric chair forms (**A**) and (**E**) (Fig. 2.101) are of unequal free energy and hence their populations are different. Their equilibrium constant K is given by the equation:

$$\Delta G^{\circ} = -\mathrm{RTlnK} \ldots (\text{equation } \mathbf{a}), \text{where} K = [(\mathbf{E})]/[(\mathbf{A})].$$

The difference of free energy between equatorial (**E**) and axial (**A**) conformers, ΔG°, is negative (the more stable conformer to be written on the right side of the equilibrium). *Thus, $-\Delta G^{\circ}$, which is positive, is known as the conformational free energy of the substituent* (also known as A-value). Usually steric grounds (as explained in Fig. 2.101 in case of methylcyclohexane), in some cases electronic factors may dictate the more stable conformer. In the Newman projection (**E**′) for the equatorial conformer no extra gauche–butane interaction is present, whereas in Newman projections (**A**′) and (**A**″) of the axial conformer one extra *gb* is seen in each; the chair form (**A2**) shown both *gb* interactions. Thus, the enthalpy difference, ΔH, between the two diastereomeric conformers $= 0.8 \times 2 = 1.6$ kcal/mol (approx.). If $\Delta S = 0$, $\Delta G = \Delta H$ (from the equation $\Delta G = \Delta H - \mathrm{TAS}$) (equation **b**). This value corresponds to over 90 % population of the e conformer (**E**) (but NMR studies indicate it to be 95 %).

The conformational free energies ($-\Delta G^{\circ}$ values) of a number of common substituents are listed in Table 2.5.

The Table 2.5 reveals the following interesting facts

(i) In the halogen series the effective bulk of F being least, $-\Delta G^{\circ}$ value is least, $-\Delta G^{\circ}$ values for Cl, Br, and I are almost equal due to the increase in their bond lengths resulting in increase in the distance from the *syn*axial hydrogens. Moreover, larger atomic volume causes the increased polarizability of electrons and the ease of deformation to reduce the energy of the axial conformer. In general, the elements of the first two rows in the Periodic Table with relatively short C–X bonds and low polarizability show larger $-\Delta G^{\circ}$ values

Table 2.5 Conformational free energies ($-\Delta G^\circ$) in kcal/mol (approx.)[a]

Substituent $-\Delta G^\circ$ (~t, °C)	Substituent $-\Delta G^\circ$ (~t, °C)	Substituent $-\Delta G^\circ$ (~t, °C)	Substituent $-\Delta G^\circ$ (~t, °C)
D 0.006 (25)	CN 0.2 (−79)	OH[b] 0.60 (25)	Me 1.74 (27)
T 0.011 (−88)	NH_2[c] 1.23 (−80)	OH[d] 1.04 (−83)	Et 1.79 (27)
F 0.25 (−86)	NH_2[e] 1.7 (20)	OMe 0.63 (−93)	$CH(Me)_2$ 2.21 (27)
Cl 0.53 (−80)	NO_2 1.1 (−80)	$OC(Me)_3$ 0.75 (36)	$C(Me)_3$ 4.8 (−120)
Br 0.48 (25)	COOMe 1.2 (25)	OPh 0.65 (−93)	Ph 2.8 (−100)
I 0.47 (−78)	COOH 1.4 (25)	OCOMe 0.68 (25)	CH_2Ph 1.68 (−71)

[a]More detailed tabulations have been compiled in the textbook by Eliel, Wilen, and Doyle [59]
[b]in cyclohexane
[c]in toluene-d_6
[d]in CS_2
[e]in $MeOCH_2CH_2CH_2OH$

than the heavier elements with longer bonds to carbon and higher polarizability.

(ii) Groups like >NH, -NHMe, OH, etc. which may form H-bonds have different $-\Delta G^\circ$ values in protic and aprotic solvents (for OH see ref. [60]).

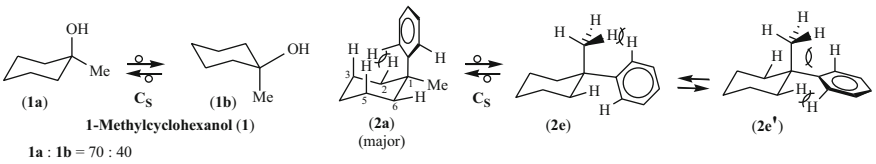
(iii) Substituents at the second atom (in italics), for example, *O*Me, *O*Et, *O*Ts, *O*COMe do not significantly affect the effective bulk. The ΔG° values of Me, Et, *i*Pr do not differ as expected, but it increases sharply for *t*-Bu group having greatest effective bulk. The *t*-butyl substituted cyclohexanes have almost exclusively equatorial conformation and have been called ***anancomeric***, meaning *fixed in one conformation* (derived from Gk *unankein* meaning to fix by some fate or law).

(iv) The ΔG° values are approximately additive, and they may be used for di- and polysubstituted cyclohexanes.

(v) The additivity of ΔG° values does not hold good in geminally substituted cyclohexanes [61].

2.12.3 1,1-Disubstituted Cyclohexanes

1,1-Disubstituted cyclohexanes having a plane of symmetry ($\mathbf{C_S}$) passing through C1 and carrying achiral substituents do not show any configurational isomerism even if the substituents are different. They exist in two interconvertible conformers when the substituents are different. The energy barrier is usually of the similar order as that of cyclohexane. For example, in 1-methylcyclohexanol (**1**) the ratio of the two diastereomeric conformers (**1a**) and (**1e**) (Fig. 2.102) should correspond to the difference in conformational free energies of the two substituents (1.74 − 1.04 = 0.70 kcal/mol). But actually (**1**) exists as a 70.30 mixture of (**1a**) and (**1e**) conformers in Me_2SO at 35 °C, corresponding to a free energy difference of 0.48 kcal/mol. Here the bulkier group Me in (**1a**) is predominating but to a much less amount due to some leveling effect.



Note: The compound (**2a**) flips into a β-equatorial phenyl and an α-axial methyl conformer which when rotated 180º around a horizontal axis gives the conformer (**2e**) with α-e-Ph and β-a-Me substituents.

Fig. 2.102 Conformations of two geminally substituted cyclohexanes

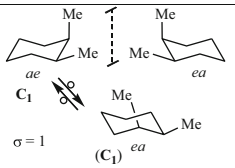
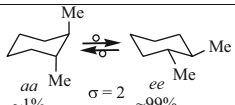
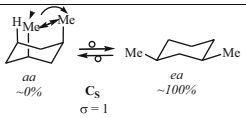
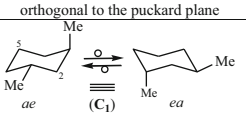
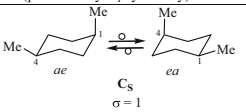
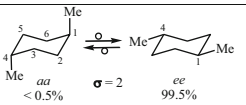
In case of 1-methyl-1-phenylcyclohexane (**2**) (Fig. 2.102), contrary to expectation, the conformer (**2a**) with axial phenyl and equatorial methyl is preferred over the other conformer (**2e**) with equatorial phenyl and axial methyl by 0.32 kcal/mol in spite of the fact that the difference of their conformational free energy (2.8−1.74 = 1.06 kcal/mol) would lead to the opposite conclusion. This anomaly may be explained as follows (Fig. 2.102). In the conformer (**2a**) the phenyl ring is so oriented that the ortho H's are away from axial H3 and axial H5 and so the *synaxial* interactions of the phenyl with these H's becomes minimal. However, there may be some interactions between the ortho H's and the adjacent e-H2 and e-H6. On the contrary, the phenyl group in the flipped conformer (**2e**) (see **Note** in the Figure) would interact strongly with the Me H's. Hence, the phenyl group must rotate by 90° (*cw*) about its pivotal bond to give the conformer (**2e′**), in which also strong interactions between the ortho H's and the adjacent *e*-H's exist. Thus, the additivity of ΔG° values does not hold good in geminally substituted cyclohexanes [59].

2.12.4 Non-geminal Disubstituted Cyclohexanes

Disubstituted cyclohexanes, other than the germinal ones (Sect. 2.12.3), exist in three sets of positional isomers, *viz*., 1,2-, 1,3-, and 1,4-isomers. Each set has a *cis*–*trans* pair of diastereomers. Each diastereomer can flip into interconvertible chair conformers. The relative enthalpy of each conformer depends upon the steric interactions present. Depending upon its symmetry property a particular isomer may exhibit enantiomerism. These features in case of all dimethylcyclohexanes are illustrated in Fig. 2.103. Calculation of relative free energy and entropy for each isomer are also shown as **Notes** in the Figure. Some important stereochemical features of dimethylcyclohexanes having *different substituents* are stated below.

cis-1,2-Isomers. The *cis*-1,2-isomer having *different substituents* is resolvable since the flipped conformers are different entities and not enantiomers. The bulkier group exists predominantly in the equatorial orientation.

1,3-Isomers. Both *cis*- and *trans*-isomers with *different substituents* are resolvable, since each of them has C_1 pt gr. and each flips into a different chiral variety.

Planar Structure (Optical activity)	**Conformation** (symmetry pt. gr.)	**Inter-actions**	**Relative enthalpy** ΔH kcal/mole	Kcal/mole **ΔH exptl. (ΔG exptl.)**	**Flips into**	**Regarding chirality**
 *cis-1,2* σ-plane (*meso*)	 ae ea **C₁** σ = 1 (**C₁**)	2 (axial Me) + (1e ↔ 2a Me's) = 3 *gb*.	2.7 ΔH (calc) 1.8 0.9 3.6	1.87 (1.66)	A non-superposable mirror image (energy barrier much less than 10 kcal/mole	Unresolvable (±)-pair
 *trans-1,2* **C₂** pt.gr. (active)	 *aa* ~1% σ = 2 (**C₂**) *ee* ~99% (The C_2 passes through the midpoints of 1-2 & 4-5 bonds)	*ee* 1 *gb* *aa* 4 *gb*			another chiral variety of the same enantiomer (lacks an σ-plane or i)	Resolvable (±)-pair
 *cis-1,3* (σ-plane) (meso)	 *aa* ~0% **C_S** σ = 1 *ee* ~100% Each variety has a vertical σ-plane passing through C2 and C5 and orthogonal to the puckard plane	*aa syn axial* MeMe, + 2 *gb*	5.4 0 1.8 −1.8	−1.96 (−1.56)*	another achiral variety	True *meso*
 *trans-1,3* **C₂** (active)	 ae (**C₁**) ea σ = 1 (possess only C_1 symmetry)	2 *gb* for one a-Me			a superposable (homotopic form)	Resolvable (±)-pair
 *cis-1,4* (*meso*) σ-plane through C1 & C4	 **C_S** σ = 1 (A vertical σ-plane passess through **C1** and **C4**)	2 *gb* for one a-Me	1.8 1.8 0	1.90 (1.55)*	a superposable (homotopic form)	True *meso*
 *trans-1,4* (meso) σ-plane through C1 & C4	 *aa* < 0.5% σ = 2 *ee* 99.5% (**C_2h** for each): C_2 axis bisect 2-3 and 5-6 bonds; σ_h orthogonal to C2, passing through C1 and C4)	No *gb* for *ee* form 4 *gb* for *aa* form	3.6		another achiral variety	True *meso*

Notes: • Symmetry consideration of the planar structures gives the correct inference regarding resolvability.

- *G* = Free energy; *H* = Enthalpy (or Potential Energy *E*); *S* = Entropy
- ΔG (Relative free energy) = $G_{cis} - G_{trans}$; $\Delta H = H_{cis} - H_{trans}$; $\Delta S = S_{cis} - S_{trans}$
- $\Delta G = \Delta H - T\Delta S$ (ΔG*exptl. is calculated from experimental ΔH and ΔS data)
- *Total relative entropy* = S_{calcd}

S_{calcd} (for *trans*-1,2) = –Rln σ (σ, symmetry no. is 2) + Rln (entropy of mixing) (due to *dl*) + S (due to ee, aa equilibrium)
= (–1.38) + 1.38 + 0.11

S_{calcd} (for *cis*-1,2) = 0 + 1.38 + 0 = 1.38

*Entropy of mixing = $-R (x_1 \ln x_1 + x_2 \ln x_2)$ where x_1 & x_2 are mole fractions of each component in the mixture
= –R (½ln½ + ½ln½) = Rln2 = 1.38 cal/deg. mole

Fig. 2.103 Conformations energies, symmetries, and optical activity of the non-geminal dimethylcyclohexanes

The *cis*-isomer exists in a preferred *ee* conformation and the *trans*-isomer in an *ea* conformation with the bulkier group predominantly in the *e* orientation.

Though the 1,3-*cis* isomer exist almost 100 % in the diequatorial (*ee*) conformation, if necessary it can adopt the diaxial (*aa*) conformation by ring inversion to bring the substituents within reacting distance, *e.g.*, formation of an anhydride from *cis*-1,3-cyclohexanedicarboxylic acid or formation of intramolecular H-bond in *cis*-1,3-cyclohexanediol.

1,4-Isomers. The *cis*- and *trans*-1,4-isomers are always meso because they possess a vertical σ-plane passing through C1 and C4, even if the substituents are different. The *cis*-1,4-isomer exists preferentially in the conformation having the bulkier group in the *e* conformation, whereas the *trans*-1,4-isomer exists almost exclusively in the *ee* conformation.

2.12.4.1 Some Typical Disubstituted Cyclohexanes (Fig. 2.104)

(a) In *trans*-1,2-dihalocyclohexanes the diaxial (*aa*) conformer (which does not exist in dimethyl series) is substantially populated, sometimes as the major conformer (more than the *ee* conformer (**2**)). The percentage of *aa* conformer (**1**) having no dipole repulsion between the halogen atoms increases in the series: C1 < Br < I, since the increased bond length decreases the synaxial interactions. The *ee* conformer (**2**) is also stabilized by solvation with polar solvents. The effects of dipole–dipole repulsion in (**3**) and synaxial interactions in (**4**) result in the 50 % of each isomer in the equilibrium mixture of these two diastereomers of 4-t-butyl-1,2-dibromocyclohexane.

(b) The *cis*- and *trans*-isomers of cyclohexane-1,2-diol show intramolecular H-bonding (little stronger in *cis*). The *ee* conformer (**5e**) of the *trans*-2-halocyclohexanol is stabilized by H-bonding, and the diaxial conformer (**5a**) is free from dipole–dipole repulsion resulting in almost equal population of the two conformers.

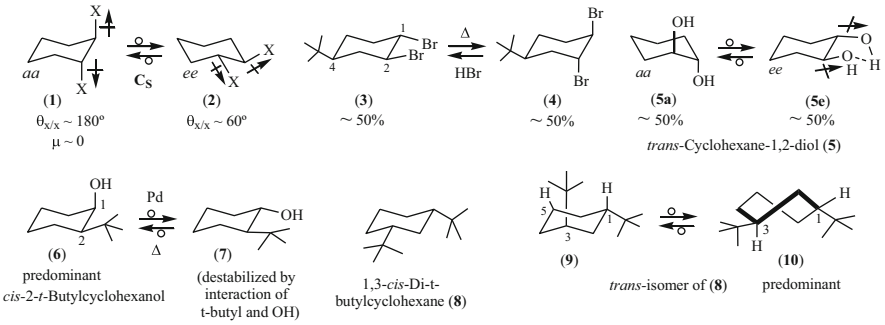


Fig. 2.104 Effect of dipole moment, H-bonding and bulky substituents on conformation in cyclohexane derivatives

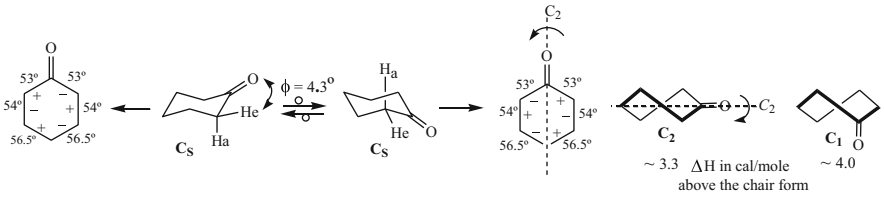
(c) Equilibration of *cis*-2-*t*-butylcyclohexanol (*anancomeric* system) with Raney Ni or with Pd upon heating gives a predominant amount of the *cis*-isomer (**6**) over the *trans*-isomer (**7**). The interaction between a methyl group of the t-Bu and OH destabilizes the *trans* isomer. The *cis*-isomer of 1,3-di-*t*-bulylcyclohexane (**8**) exists in the chair conformation with both bulky substituents in equatorial orientation. However, the corresponding *trans* isomer almost exclusively exists in the skew–boat conformation (**10**), since in the chair conformation (**9**) one *t*-butyl group have to occupy axial orientation involving severe interaction with H1 and H5.

2.13 Cyclohexanone

Cyclohexanone exists almost exclusively (99 % at 25 °C) in the chair form and only a small amount (~1 %) in skew–boat forms (Fig. 2.105). The conformational features of cyclohexanone are shown briefly in the Figure.

2.13.1 Torsion Angles, Stability

Torsion angles between pairs of adjacent C–C bonds (Fig. 2.105) show flattening of the ring at the region of the carbonyl group. The chair conformation has only a vertical σ-plane passing through C1 and C4 and belongs to the point group $\mathbf{C_S}$. The skew–boat forms belong to chiral point groups $\mathbf{C_1}$ and $\mathbf{C_2}$. Due to flattening at the site of the CO group, the *e*-H's at C2 and C6 are partially eclipsed with carbonyl oxygen ($\theta = 4.3°$), whereas the corresponding a-H's lean slightly outwards. Cyclohexanone is *slightly destabilized* relative to cyclohexane due to the angle strain and torsional strain. The equilibrium of cyclohexanone cyanohydrin (sp^2 sp^3 equilibrium) lies more toward cyanohydrins side than that of di-*n*-octyl ketone indicating the lower thermodynamic stability of cyclohexanone. Again, cyclohexanone is reduced with $NaBH_4$ at a rate 355 times as fast as di-*n*-hexylketone manifesting the lower kinetic stability of cyclohexanone. The twist–boat forms with $\mathbf{C_2}$ and $\mathbf{C_1}$



CH_2-CH_2 bond length = 1.545 Å; CH_2-CO bond length = 1.51 Å; C-CO-C bond angle = 116.5°

Fig. 2.105 Conformations, geometry, and torsion angles of cyclohexanone

point groups are having enthalpies (calcd.) 3.3 and 4.0 kcal/mol (approx.), respectively, above that of the chair form (Fig. 2.105).

2.13.2 Ring Inversion

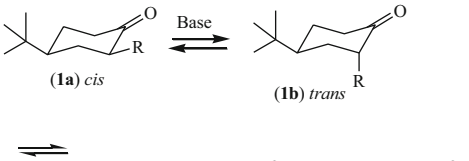
The free energy of activation $\Delta G^{\neq}$ (exptl.) [62] for the ring inversion in cyclohexanone at $-170°$ is ~ 5.0 kcal/mol, much lower than in cyclohexane. This is because of lower torsional barrier around an sp^3–sp^2 C–C bond than an sp^3–sp^3 one. Pseudorotation among the flexible forms is more facile in cyclohexanone than in cyclohexane, and the transition states for $\mathbf{C_2}$ and $\mathbf{C_1}$ skew–boats are not equivalent.

2.13.3 Alkylketone Effects

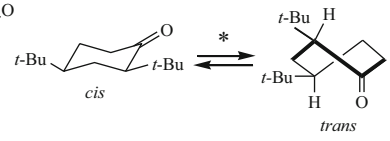
The alkylketone effect of the substituent $R = (-\Delta G_R)$ (in alkylcyclohexane)—$(-\Delta G'_R)$ (in 2, 3, or 4-alkylcyclohexanone) = the decrease in the conformational free energy of a substituent R in the alkylcyclohexanone with respect to the conformational free energy of the alkyl substituent in the alkyl cyclohexane.

2.13.3.1 2-Alkylketone Effect (Fig. 2.106)

The equatorial (e) substituents at C2 and C6 are nearly eclipsed with respect to CO oxygen and may destabilize the e conformer due to additional steric repulsion—thus decreasing the conformational free energy (ΔG between axial (a) and e) in comparison to that in alkylcyclohexane. Thus, in free energy, the term 2-alkylketone effect was first proposed by Walker in 1955 and Klyne in 1956. The 2-methyl ketone effect was estimated to be 1 kcal/mol, reducing the normal



R	kcal/mole		
	$-\Delta G_R{}^{\Psi}$	$-\Delta G'_R$	2-Alkyl-Ketone effect
Me	1.74	1.84	-0.10
Et	1.79	1.10	0.69
iPr	2.21	0.60	1.61



*cis-2,4-Di-t-butylcyclohexanone undergoes base catalyzed equilibration to give trans-2,4-di-t-butylcyclohexanone in skew-boat form (and not in chair form, and hence 2-t-butylketone effect cannot be measured).

Fig. 2.106 Measurement of 2-alkylketone effects

difference (ΔG_{Me} ~1.7 kcal/mol) by this amount making the $\Delta G'_{Me}$ (in 2-methyl cyclohexanone) as 0.7 kcal/mol.

But Allinger (1961) demonstrated that 2-methylketone effect is absent. The reason for absence of 2-methylketone effect is that the *e*-Me is too far (due to greater C–CH_3 bond length) from CO oxygen to have any van der Waals repulsion (steric interaction) between them; on the other hand, eclipsing of C–Me and C=O is electronically slightly favorable (*cf.* preferred conformation of propanal is having C=O and C–Me eclipsed). Thus, the 2-methylketone effect appears to be slightly negative, −0.10 kcal/mol. 2-Alkylketone effect can be estimated from the equilibrium data of 2-alkyl-4-*t*-butylcyclohexanones or 2,6-dialkylcyclohexanones (Fig. 2.106). A few alkylketone effects thus obtained are given in the Figure.

2.13.3.2 3-Alkylketone Effect

In 3-alkylcyclohexanone the axial conformer has only one alkyl-hydrogen synaxial interaction (gauche) instead of two such interactions in the axial conformer of alkyl cyclohexane. Thus, there is a decrease in $-\Delta G^{\circ}$ value [of the alkyl (R) group], which is known as 3-alkylketone effect. In case of 3-methylcyclohexanone (R=Me), this effect is equivalent to one gauche–butane interaction (0.89 kcal or 3.75 kJ/mol). However, in view of the van der Waals repulsion between axial Me and CO oxygen in the axial conformer this value is reduced to a calculated value of 0.6 kcal/mol. Experimental study of the equilibrium between *cis* and *trans* isomers of 3,5-dimethylcyclohexanone (**a** in Fig. 2.107) suggest a value of 0.37 kcal/mol (1.73–1.36 kcal/mol) (taking ΔS = O) for the decrease (3-methylketone effect) in $-\Delta G_{Me}$. The equilibrium data of 2,5-dimethylcyclohexanone (between the *trans*

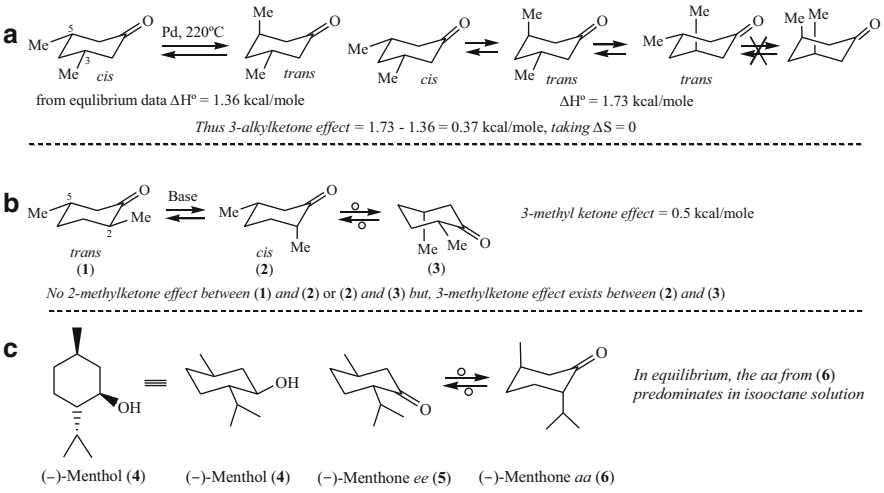


Fig. 2.107 3-Alkylketone effect. Few examples

(**1**) and *cis* (**2**) isomers) (**b** in Fig. 2.107) gives a value of 0.5 kcal/mol [Eliel 1965] which is explained in the Figure.

In (−)-menthone [(**5**) in Fig. 2.107] 2-isopropylketone effect (1.8 kcal/mol) and 3-methylketone effect (0.5 kcal/mol) cooperate with each other in the *aa* conformer (**6**) with respect to the *ee* conformer (**5**)—so much so that in a solvent of low polarity, *e.g.*, isooctane, the diaxial conformer (**6**) predominates, as evident from its CD spectrum. In the polar solvent the *ee* form (**5**) is also stabilized by dipole–dipole interaction with the solvent.

2.13.3.3 4-Alkylketone Effect

4-Alkylketone effect depends upon the relief of strain due to synaxial interactions between an axial 4-alkyl group and the axial H or substituent at C2 and C4, when C1 is converted from the tetrahedral (>CHOH) to the trigonal (>C=0) configuration, since this conversion leads to an outward motion (leaning) of the axial H's or substituents at C2 and C4.

This effect has been invoked to explain (Fig. 2.108).

(a) The greater rate of CrO_3 oxidation of 4,4-dimethylcyclohexanol as compared to that of cyclohexanol (*cf.* triterpene 3β-ol *versus* steroidal 3β-ol)
(b) The larger dissociation constant (K) of 4,4-dimethylcyclohexanone cyanohydrin as compared to that of cyclohexanone cyanohydrin.

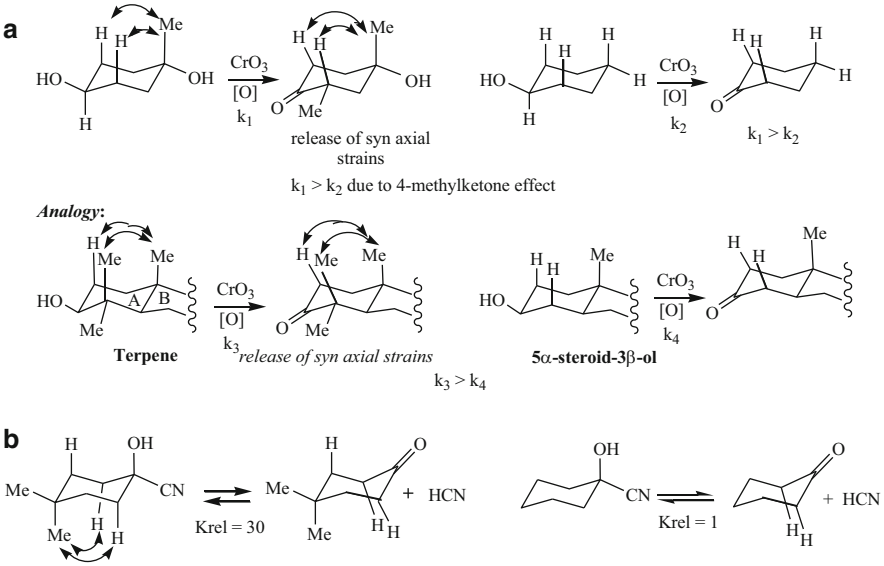


Fig. 2.108 Invocation of 4-alkylketone effect to explain some oxidation rates and relative equilibrium constants

2.13.4 Addition of Nucleophiles to Cyclohexanones. Stereochemical Aspects

The nucleophile can approach the carbonyl carbon of a cyclohexanone derivative from the axial side producing the equatorial alcohol and from the equatorial side producing the axial alcohol.

2.13.4.1 PDC (PSC) and SAC (SSC)

Dauben et al. in 1956 [63] first introduced the concept that in case of unhindered cyclohexanones having no substituent at C3 or C5 position, axial approach of the nucleophile does not encounter any steric hindrance and hence is preferred, producing the more stable equatorial alcohol in major amount (~90 %) (Fig. 2.109), as a result of *product development control* (PDC). This is illustrated with an example of the sodium borohydride reduction of an anancomeric cyclohexanone like 4-*t*-butylcyclohexanone [64] through the transition state (**A**) (Fig. 208a) which energetically resembles the product. If the attack takes place from the equatorial side, it involves the TS (**B**) of higher energy due to synaxial interactions with the developing axial OH and hence is formed in minor amount (~10 %).

On the other hand for hindered anancomeric cyclohexanones like *cis*-3-methyl-4-*t*-butylcyclohexanone, the axial approach becomes more hindered due to synaxial interaction with the 3-methyl group in the TS (**D**), compared to the equatorial approach through TS (**C**) having much less nonbonded interaction and hence

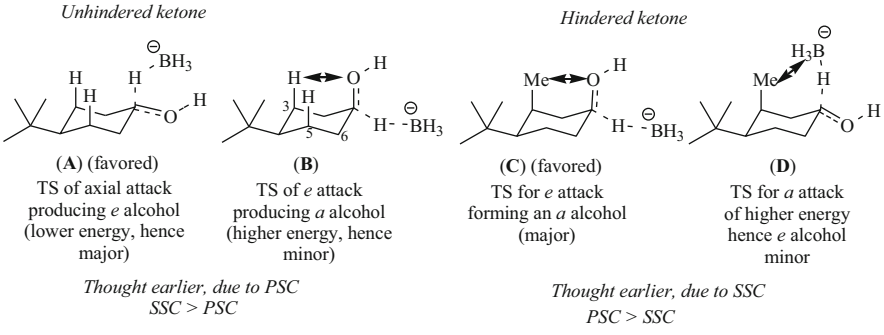


Fig. 2.109 Product strain control versus steric strain control

gives rise to the axial alcohol as the predominant product. The predominant formation of the axial alcohol as a result of *steric approach control* (SAC) was first conceptualized by Dauben [63]. The two terms, PDC and SAC, have been replaced later one by Brown [65] by *product stability control* (PSC) and *steric*

strain control (SSC) to give more emphasis on the transition state rather than on events prior to that the term PSC refers to a product-like or a late TS along the reaction coordinate, while the term SSC refers to a reactant-like or an early transition state.

2.13.4.2 Observations Against PSC

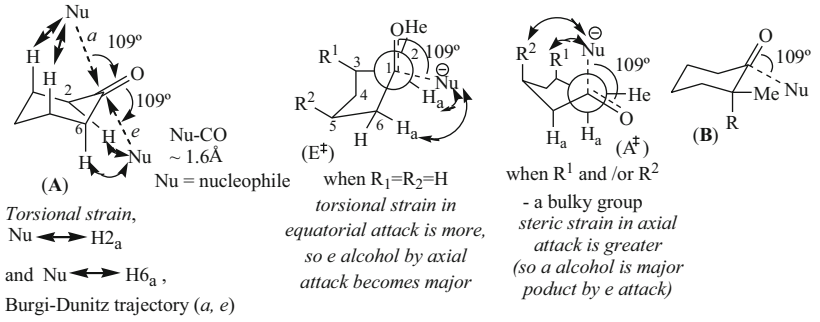
The role of SSC on the stereochemical course of addition of nucleophiles to cyclohexanones has never been in doubt, but the role of PSC has always been controversial. Some observations against the validity of PSC are as follows:

(i) Usually a higher proportion of an equatorial alcohol is obtained in lithium aluminum hydride reduction than that corresponding to thermodynamic equilibrium, *e.g.*, 90 % versus 80 % in the reduction of 4-*t*-butylcyclohexanone.
(ii) The rate constants of a number of sterically hindered ketones, relative to that of 4-*t*-butylcyclohexanone, support the concept of SSC, but show little effect of PSC [66].
(iii) Reduction of ketones with hydrides are highly exothermic; hence, according to Hammond's postulate the transition states are expected to be reactant-like (*cf.* SSC) and not product-like.
(iv) The deuterium isotope effect (k_H/k_D) in borohydride/borodeuteride reduction of cyclohexanones with varying degrees of steric hindrance [67] is almost constant in spite of widely varying ratios of the equatorial and axial alcohols indicating that the extent of B–H bond breaking at the TS is similar for cyclohexanones having different degrees of steric hindrance—a fact inconsistent with the PSC concept.

While teaching it occurred to the present authors long ago that PSC involves product strain which itself cannot have any role in the TS and activation energy in any reaction of kinetic control, and hence the specific contribution of PSC is unexpected, as is substantiated by the above observations. The same is true for reduction of acyclic carbonyl groups regulated only by steric factors such as Cram's rule and Prelog's rule (Sect. 2.11).

2.13.4.3 Torsional Strain. Role of C2 and C6 Axial Hydrogens

In 1965 it was suggested by Richer [68] that the axial H's at C2 and C6 offer steric resistance to the equatorial approach of a nucleophile (torsional strain) which competes with that offered by the axial H's at C3 and C5 to axial approach (Fig. 2.110). The relative strength of the resistances would, however, depend upon the bulk of the hydride reagent and the exact position of the TS in the reaction coordinate. If C. . . Nu is shorter than 1.6 Å the equatorial approach would be more hindered in case of reduction with hydride reagent.



Note: *When* $GE^{\ddagger} > GA^{\ddagger} \rightarrow$ *e Alcohol is major. When* $GA^{\ddagger} > GE^{\ddagger} \rightarrow$ *a Alcohol is major*

Fig. 2.110 Felkin transition states for addition of a nucleophile to a cyclohexanone, torsional strain versus steric strain. Formation of equatorial alcohol versus axial alcohol

The most satisfactory and widely accepted alternative to PSC is the concept of *torsional strain* introduced in 1968 by Cherest and Felkin [35]. Two competing factors, namely (1) the steric interaction of the incoming nucleophile with the 3,5-diaxial groups (SSC) in the *axial attack* and (2) the *torsional strain* which arises between the semiformed bond of the nucleophile and the two axial CH bonds at C2 and C6 during the *equatorial approach* (Fig. 2.110) arise, as already stated.

In the absence of any steric hindrance (factor 1) (in case of unhindered ketones), the effect of the torsional strain prevails leading to a preferential axial attack forming the equatorial alcohol. On the contrary, in the presence of one bulky axial substituent at C3 or C5 (or at both), the steric strain control (SSC) (2) prevails reversing the steric course and forming the axial alcohol predominantly. The bulk of the hydride reagent will increase the SSC, more so if C3 and C5 substituents are present and will definitely increase the proportion of axial alcohol. In Fig. 2.110 appropriate transition states are shown for both cases.

This torsional strain effect is not evident in the reduction of 2-methylcyclohexanone (**B**, R=H) and 2,2-dimethylcyclohexanone (**B**, R=Me) indicating that torsional strain is relatively insensitive to the bulk of the group involved (*cf.* rotational barrier of propane is barely larger than that of ethane, Sect. 2.3.4).

The application of Bürgi–Dunitz trajectory [37–39] proposed in 1973, based on molecular orbital interactions (Sect. 2.11.3.2) which states that a nucleophile attacks the carbonyl carbon at an angle of 109° with the carbonyl plane, makes the torsional strain stronger by making it almost like eclipsing strain (Fig. 2.110). Moreover, the torsional strain theory also successfully explains the stereochemistry of the reduction of acyclic ketones (see Sect. 2.11, Felkin models). Both steric strain and torsional strain can be minimized for acyclic substrates.

Several examples of sodium borohydride/lithium aluminum hydride reduction of hindered cyclohexanones are given in Fig. 2.111. The more hindered is the ketone

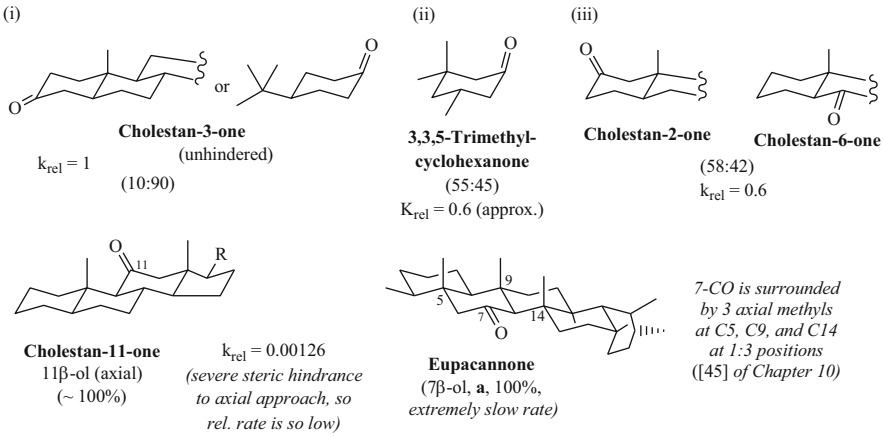


Fig. 2.111 Increased amount of axial alcohol by increased SSC in hindered cyclohexanones

the more equatorial attack leads to the formation of greater percentage of the axial alcohol, and the less is the rate of the reaction.

Hydride reductions of cyclohexanones.

The percentages of *a*:*e* alcohols are given in parenthesis.

Studies on π-facial selectivity in the addition of nucleophiles to double bonds, especially the carbonyl-based systems have generated more models and hypotheses (~10) (not necessarily mutually exclusive) than any other subject in the field of stereoselective synthesis [69], some of which, *e.g.*, Cram, Prelog, Felkin–Anh, have been discussed earlier.

2.13.5 Cieplak Hypothesis

Cieplak in 1981 suggested [69] that the stereochemistry of nucleophilic addition to cyclohexanones is determined by a combination of steric factor, which as usual favors the equatorial approach, and the stereoelectronic factor which involves electron donation from the cyclohexanone C–C and C–H σ bonds into the vacant σ* orbital of the incipient bond formation between the nucleophile and the carbonyl carbon (Fig. 2.112). Since axial C–H bonds (at C2 and C6) next to the carbonyl group are better electron donors than the 2–3 and 5–6 σ bonds, the transition state with axially oriented incipient bond (axial approach) is preferred over the one with the equatorially oriented incipient bond (Fig. 2.112). Thus, the hypothetical model proposed by Cieplak is based on the concept that the carbonyl group undergoes extensive pyramidialization, and that the outcome is primarily a consequence of the aforesaid interactions between the occupied vicinal σ-orbitals by electron donation into the vacant σ*-orbital leading to the stabilization of the transition state.

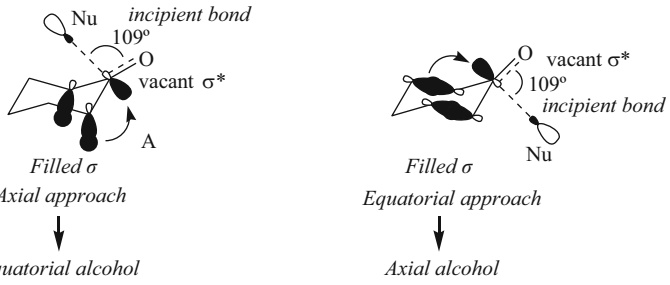
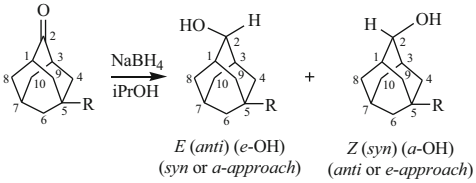


Fig. 2.112 Participating orbitals in the Cieplak model



	R	*E/Z*
EWG	Cl	59:41
	p-Cl-Ph	60:40
	Ph	58:42
EDG	*p*-HO-Ph	44:56
	p-H_2N-Ph	34:66

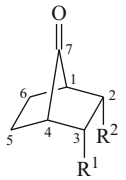
Fig. 2.113 Hydride reduction of 5-substituted 2-adamantanones

Cheung et al. [70] in 1986 reported that in the nucleophilic addition of $NaBH_4$ to the carbonyl groups of 5-substituted 2-adamantanones, depletion of electron density on the C1–C9 and C3–C4 bonds by halogen or phenyl groups possessing *para* electron-withdrawing substituents favored *syn* approach forming (*E*) (*anti*) alcohols. On the contrary, the electron donating groups like *p*-aminophenyl and *p*-hydroxyphenyl analogs favored the *anti* approach forming the (**Z**) (*syn*) alcohols as major products (Fig. 2.113).

The above observations [70] strongly supported Cieplak view of electronic effects in such asymmetric induction through preferential interaction with newly developing σ* orbital with electron-rich *anti*-periplanar bonds and vice versa.

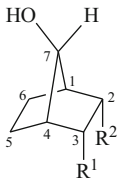
Cieplak in 1989 [71] observed that the electronegative substitution of the substrate by CF_3 at C3 of cyclohexanone or in the reagent, *e.g.*, sodium tri-isopropoxyborohydride reverses the π-facial diastereoselection by increasing the relative proportion of the axial attack leading to the favored formation of the equatorial alcohol. The same type of favored axial attack has been found in a number of widely different reactions including alkyllithium addition and also in cases of peracid epoxidations of methylenecyclohexenes. The findings appear inconsistent with the predictions of Felkin, Klein, Ashby, and Anh models of stereochemistry of reactions in cyclohexane-based systems, but are consistent with Cieplak model [70].

In 1990 Mehta [72] demonstrated that electronic induction by the 2,3-*endo, endo* substituents reverses the π-facial selection (resulting in the reversal of the *E:Z* ratio)

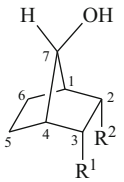


2,3-*endo-endo substituted*
7-Norbornadiones

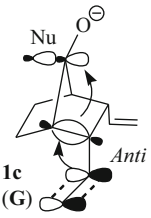
1a: R^1=R^2=COOMe
1b: R^1=R^2=CH_2OMe
1c: R^1=R^2=vinyl
1d: R^1=vinyl, R^2=ethyl
1e: R^1=R^2=ethyl



E
(from *syn face attack*)
EWG



Z
(from *anti face attack*)
EDG



Anti face selectivity in **1c** *may be stabilized through space donation to* s * *orbital in a perpendicular conformation*

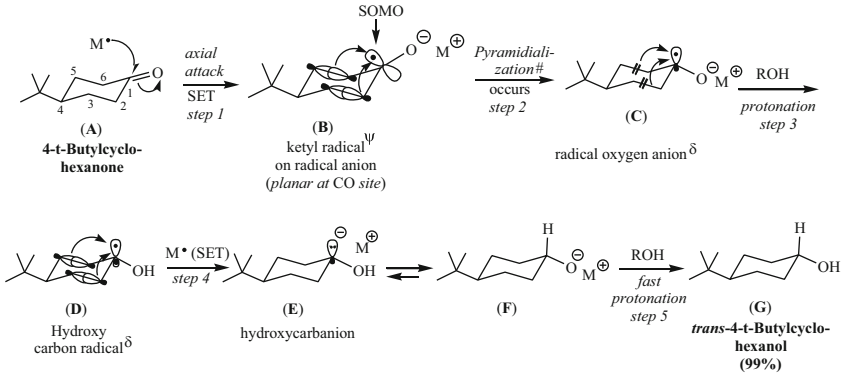
Product Ratios in the Metal Hydride Reductions and Methyllithium Addition

E:Z distribution

Substrate	$NaBH_4$	$LiAlH_4$	$Li(t\text{-}BuO)_3AlH$	MeLi
1a	84:16	87:13	77:23	>90:<10
1b	40:60			34:66
1c	36:64	35:65	34:66	27:23
1d	25:75			
1e	20:80	21:79	29:71	17:83

Fig. 2.114 Metal hydride reductions of and methyllithium additions to 2,3-*endo, endo* substituted 7-norbornadiones. *E:Z* ratios of the products support Cieplak hypothesis.

in the nucleophilic addition of hydrides and methyllithium to 7-norbornadione, as shown in a tabular form in Fig. 2.114. The predominant approach of nucleophiles to the *syn* face in **1a** and to the *anti* face in **1e** is fully consistent with the prediction based on the Cieplak's hyperconjugative model, according to which delocalization of σ electrons in the electron-rich antiperiplanar σ bond into the incipient σ* orbital lowers the transition state energy (*cf.* Fig. 2.114). The unexpected *anti*-face selectivity in the cases of **1b** and **1c** (with 2- and 3-substituents supposed to be electron withdrawing groups) (see the Table) may be attributed to *through space donation in a perpendicular conformation* as shown for **1c** in (**G**) in Fig. 2.114. For an authoritative background review and a comprehensive list of references, see [73].



ψ *70% unpaired electron density is located at C1; and 2-3 and 5-6 s -bonds (HOMO) are favorably disposed to interact with singly occupied p*-orbital (SOMO)*

\# *SOMO is extended in the axial direction*

δ *OM in (**C**) or OH group in (**D**) is oriented exclusively in the equatorial direction and is antiperiplanar with interacting 2-3 and 5-6 s -bonds*

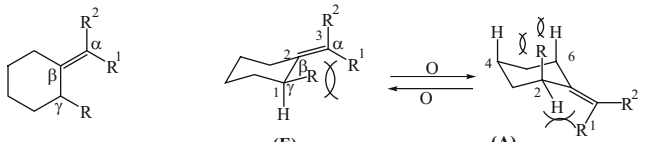
Fig. 2.115 Stepwise mechanism of Birch reduction of an anancomeric saturated cyclohexanone

2.13.6 Highly Stereoselective Reduction of Saturated Cyclohexanones by Dissolving Metals. Birch Reduction

Reduction of saturated anancomeric or rigid unhindered cyclohexanones by $LiAlH_4$ or $NaBH_4$ produces the equatorial alcohols to the extent of 90 % (*cf.* Sect. 2.13.4).

Reduction of aromatic systems and α,β-unsaturated ketones (1:4 reduction) and of saturated ketones (1:2 reduction) by use of alkali metals (Li, Na or K) dissolved in liquid ammonia in the presence of a proton donor-like alcohol (little amount) is known as **Birch reduction**, which is of versatile synthetic utility [74, 75]. Saturated anancomeric or rigid cyclohexanones are reduced to equatorial cyclohexanols (~99 % diastereoselectivity) by Birch reduction.

The mechanism of such reduction of 4-*t*-butylcyclohexanone (**A**) proposed by Pradhan [76] is delineated stepwise in Fig. 2.115, based on *Fukui effect* [77], rationalized by Fukui's Frontier Molecular Orbital (FMO) approach. The stereoelectronic effect provides a *kinetic preference of the axial attack of* an electron of the metal (M) on the carbonyl carbon, by a single electron transfer (SET) mechanism leading through a stabilized transition state (TS), to a resonance stabilized ketyl radical; this effect has been termed as Fukui effect. In the Birch reduction 4-t-butylcyclohexanone (**A**) carbonyl carbon, the resonance-stabilized ketyl radical (**B**) with a singly occupied molecular orbital (SOMO) is formed by axial approach of an electron transfer from the metal (SET mechanism). In steps 2–4 the species (**C**), (**D**), and (**E**) are formed successively and the stabilizations of species (**B**) to (**E**) have been indicated in the Figure. The species (**E**) equilibrates to the more stable species (**F**), and either species undergoes protonation very fast to



alkalidene cyclohexane with allylic substituent

(E) *involves $A^{1,3}$ strain between R and R^1; when $R = R^1$ = Me, the distance between them is* ~ 2.45 Å

(A) *involves $A^{1,3}$ strain between R^1 and H, and synaxial interaction between R & H4, and R & H6*

When $R = R^1$ = Me the distance between them = 2.45 Å; enthalpy difference between ***(E)*** *and* ***(A)*** *conformers = $A^{1,3}$ Me/Me interaction – [2(1,3 diaxial Me/H interactions + $A^{(1,3)}$ Me/H interaction.] ≈ 1(1,3-diaxial Me/Me interaction) –[2(1,3 dixial Me/H interaction. +$A^{(1,3)}$Me/H interaction] ≈ 3.7 – 3(.9) = 3.7−2.7 = 1 kcal/mole. The axial conformer* ***(A)*** *should, therefore, exist to the extent of ~ 85% and the equatorial conformer* ***(E)*** *~ 15% in the ground state (as obtained from the equation $\Delta G = -RT\ln K$, taking $\Delta S = 0$)*

Fig. 2.116 Alkylidenecyclohexane. $A^{1,3}$ Strain

form the final product *trans*-4-t-butylcyclohexanol (**G**) (99 %), giving no chance of the carbanion (**E**) to undergo inversion at C1.

2.13.7 Alkylidene Cyclohexanes. Allylic$^{(1,3)}$ Strain

The C=O group of cyclohexanone is replaced by $C{=}CR^1R^2$ to form alkylidenecyclohexanes. The same geometry as in cyclohexanone (Fig. 2.105) is assumed for alkylidenecyclohexanes (Fig. 2.116). Thus, in a cyclohexane ring containing an exocyclic double bond and an allylic substituent, the steric interference between the allylic equatorial substituent at C_γ position and the ethylenic *syn (Z)* substituent (at C_α position) (Fig. 2.116) is given the trivial designation allylic 1,3- or $A^{(1,3)}$ strain for the sake of semantic simplicity since the groups involved are at the 1 and 3 positions of the allylic system. This is one of the two general stereochemical theorems introduced by Johnson and Malhotra [78, 79] in 1965; the other one, $A^{(1,2)}$ strain, will be discussed in Sect. 2.14.

In the equilibrium between the equatorial **(E)** and the axial **(A)** conformers, R and R^1 in **(E)** are nearly eclipsed (the spatial arrangement of $R–C_\gamma–C_\beta{=}C_\alpha–R^1$ is almost planar); the dihedral angle θ between $R^1–C_\alpha$ and $C_\gamma–R$ is ~ 4°. *The allylic 1,3 strain ($A^{(1,3)}$strain) between two substituents/atoms is almost same as the synaxial interaction between them.* Thus, *in the equatorial conformer* **(E)**, *even when* R *and* R^1 are *only moderate in size (medium to large),* the axial conformer **(A)** predominates.

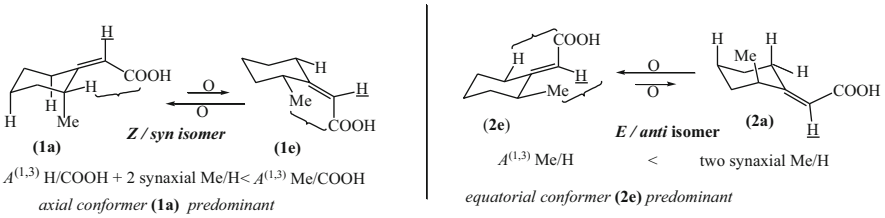


Fig. 2.117 Predominant conformers of the diastereomers of 2-methylcyclohexylidene acetic acid

Some examples illustrating $A^{(1,3)}$ strain follow.

2.13.7.1 Conformational Preference

Of the two geometrical isomers of *2-methylcyclohexylideneacetic acid*, the *Z*-diastereomer exists predominantly in the Me axial (**1a**) conformer, but the *E* diastereomer exists predominantly in the Me equatorial (2**e**) conformer, as evident from ^{1}H NMR study. These facts can be explained in terms of the interactions including $A^{(1,3)}$ strain present in the molecules (Fig. 2.117). Likewise, it may be predicted that in case of 3-*methylcyclohexylidenepropionic acid* (underlined H to be replaced by Me in all the structures of Fig. 2.117), both Z- and *E*-diastereomers exist predominantly in the axial conformer (due to severe $A^{(1,3)}$ Me/Me interaction present in the equatorial conformer of the *E* isomer).

2.13.7.2 Synthetic Utility [80] of $A^{(1,3)}$ Strain. Stereochemistry of Exoxyclic Enolate Anion Protonation

In the example shown in Fig. 2.116, if R and R′ are small, the equilibrium should lie to the left; if they are large or medium in size it should lie to the right, and the extent of conformation inversion to the axial conformer will depend on the free energy difference between the conformers. In 1955 Zimmerman and coworkers [81, 82] reported the addition of PhMgBr to benzoylcyclohexene to form the *less stable isomer*, *cis*-1-benzoyl-2-phenylcyclohexane (**4**). It was suggested that 1,4 addition of PhMgBr to benzoylcyclohexene takes place by its equatorial approach at C2, avoiding the steric hindrance in axial approach due to synaxial H4 and H6, to form the bromomagnesium enolate anion (**3**). They thought that C-protonation of the conformer (**3e**) with an acid takes place from the less hindered equatorial direction to give (**4**), since steric interference arising from axial H's at C3 and C5 seriously hindered axial protonation. However, according to $A^{(1,3)}$ strain, (**3**) should exist mainly as the conformer (**3a**) in the ground state, and the protonation at C1 should take place from the axial side in order to have continuous overlap with the π-orbital (stereoelectronic requirement, **SER**).

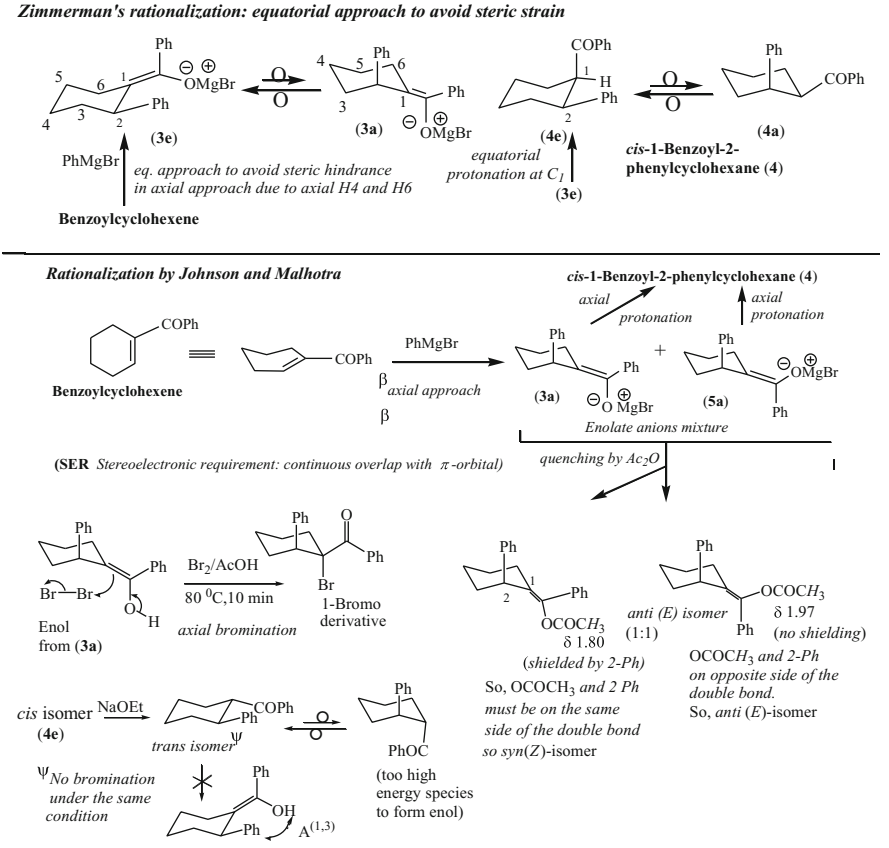


Fig. 2.118 Stereochemistry of protonation of exocyclic enolate anion (avoidance of $A^{(1,3)}$ strain and SER to be satisfied

Johnson and Malhotra's rationalization [80] has been delineated in Fig. 2.118, invoking the avoidance of $A^{(1,3)}$-strain and axial protonation of the exocyclic enolates to form the less stable *cis*-isomer (**4a**). The easy bromination of the *cis*-isomer and resistance to bromination of the *trans*-isomer under the same condition is also explained by invoking $A^{(1,3)}$ strain during formation of its enol.

2.13.7.3 Another Example of the Use of $A^{(1,3)}$ Strain Concept

Another Example of the Use of $A^{(1,3)}$ Strain Concept will be evident from the axial C-protonation of 1-*aci*-nitro-2-phenyhlcyclohexane using the more stable conformer avoiding severe $A^{(1,3)}$ strain, thus forming the less stable isomer

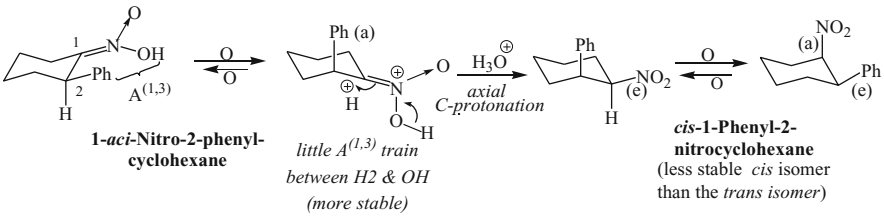


Fig. 2.119 Axial C-protonation of 1-*aci*-nitro-2-phenylcyclohexane. Use of $A^{(1,3)}$ concept

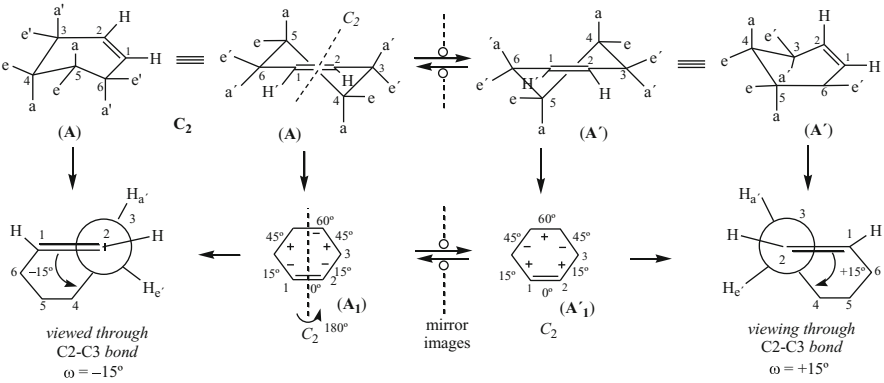


Fig. 2.120 Conformations and ring inversion of cyclohexene. Torsion angles

(Fig. 2.119) (*cf.* Fig. 2.118). Here, also equatorial protonation of the equatorial conformer was postulated by Zimmerman [83].

2.14 Cyclohexene. Conformation. $A^{1,2}$ Strain

2.14.1 Conformation of Cyclohexene. Torsion Angles (Fig. 2.120)

Cyclohexene with two sp^2 carbons possesses a half-chair conformation (**A**) or (**A′**) (Fig. 2.120) confirmed by X-ray crystallographic data of cyclohexene derivatives and also by electron diffraction studies of cyclohexene vapor. The geometry of cyclohexene possesses the following characteristic features:

(i) The C1, C2, C3, and C4 atoms and the two vinylic H atoms are almost in a plane. The C4 and C5 are alternately up and down (or down and up) of this plane.

(ii) Torsion angles shown in the planar projection formulas (**B**) or (**B′**) are supported by theoretical calculations and show considerable flattening of the ring near the double bond.

(iii) The homoallylic carbons C4 and C5 on the opposite sides of the π-plane have almost normal equatorial (e) and axial (a) bonds (perfectly staggered) as in cyclohexane—the axial bonds slightly leaning toward the center.

(iv) The conformation (**A**) or (**A′**) has a C_2 axis in the plane of the double bond, bisecting it and the C4–C5 bond, and belongs to the point group $\mathbf{C_2}$ (chiral).

(v) The conformation (**A**) is convertible to its enantiomer (**A′**) by ring inversion and vice versa, and so they form an inseparable (±)-pair. They are shown in a different perspective by structures ($\mathbf{A_1}$) and ($\mathbf{A_1}'$), respectively.

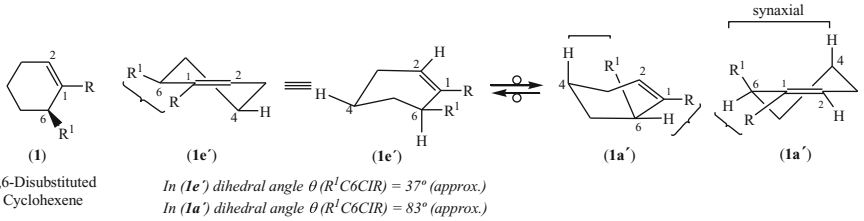
(vi) At the allylic carbons C3 and C6 the axial and equatorial character of the bonds is considerably changed. Vector analysis of cyclohexene by Corey [84] gives the dihedral angle 37° for $H_{e'}$–C6–C1–H (and $H_{e'}$–C3–C2–H) and 83° for $H_{a'}$–C6–C1–H and H_a + C3–C2–H. These are in excellent agreement with the more empirical measurements on Dreiding models. Thus, the substituents at C3 and C6 are imperfectly staggered and are said to occupy pseudoequatorial (e′) and pseudoaxial (a′) orientations, respectively.

(vii) The interconversion of the two half-chair forms probably goes through a TS with boat-like conformation. The energy barrier ($\Delta G^{\neq}$) determined by ^{1}H NMR from the coalescence of 1,2,3,3,6,6-d_6-cyclohexene at −164 °C is 5.3 kcal/mol.

(viii) Johnson and Malhotra has proposed allylic 1,2-strain ($A^{(1,2)}$-strain) in 1,6-disubstituted cyclohexene system, the discussion on which follows:

2.14.2 *Allylic 1,2-Strain ($A^{(1,2)}$-Strain)*

The strain or instability in 1,6-disubstituted 1,2-cyclohexenes (**1**) due to unfavorable interaction between ethylenic substituent and allylic pseudoequatorial substituent of the adjacent C6 is given the trivial designation $A^{(1,2)}$ strain [78, 79]



Notes: *The position and intensity of the pseudoallylic t-proton at C6 in NMR gives an estimate of the relative abundance of (1a′) and (1e′) conformers. The splitting patterns also help the assignment. $A^{(1,2)}$ Interference being weaker than synaxial interaction, will exert a controlling effect only in absence of synaxial interaction* [85, 86]

Fig. 2.121 Ring inverted conformers of 1,6-disubstituted cyclohexene

(Fig. 2.121). The dihedral angles between R^1 and R in the equatorial (**1e**) and axial (**1a**) conformers (Fig. 2.121) are 37° and 83°, respectively.

$A^{(1,2)}$ *strain is not a strong effect* and is manifested only when the groups are moderately large or bulky. However, in 6-methylcyclohexene, the equatorial conformer is more stable, since it does not possess any $A^{(1,2)}$ strain or synaxial strain. $A^{(1,2)}$ strain between two substituents is much weaker than the synaxial interaction between them.

$A^{(1,2)}$ strain operates best when R conjugates with the double bond (*e.g.*, phenyl group). The *ortho*-H of the phenyl group becomes planar with the double bond and comes still much closer to give stronger $A^{(1,2)}$ strain.

Some applications of $A^{(1,2)}$ *strain* are discussed in the sequel.

2.14.2.1 Conformational Preference

The outstanding examples of $A^{1,2}$ strain in simple 6-disubstituted 1-phenyl (and methyl) cyclohexenes (Fig. 2.122) have been provided by Garbisch [85, 86]. He determined the preferred conformations for C6 substituents in a number of such compounds (Table 2.6) from the widths at half height of the C6 proton magnetic resonance bands [85, 86]. Synaxial interaction between same groups or groups of similar bulk are stronger than the $A^{1,2}$ strain and between them and the former exerts a controlling effect. However, in the absence of synaxial interactions, presence of $A^{1,2}$-strain makes the particular conformer less predominant. The preferred conformer in each case can be rationalized comparing all the interferences involved in the C6-pseudo axial (a′) and C6-pseudo equatorial (e′) conformers. The preferred conformations of C_6 substituents of 1-phenylcyclohexenes are delineated in Table 2.6.

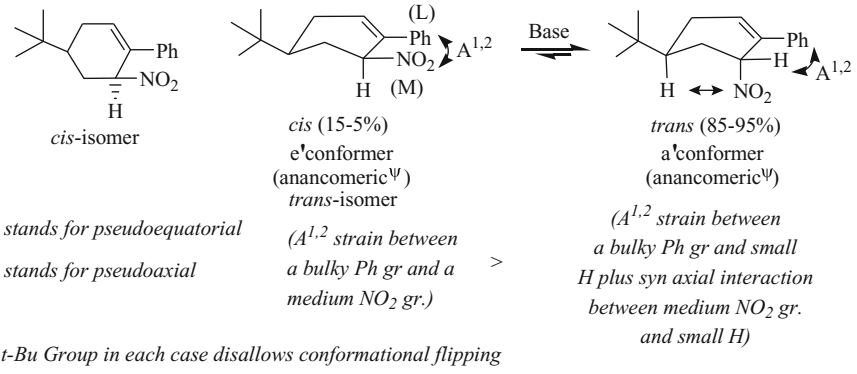
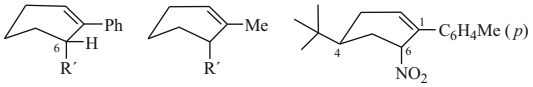
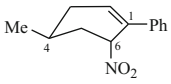
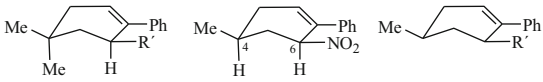
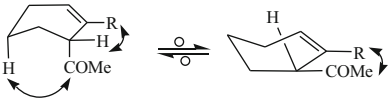


Fig. 2.122 Isomerizational preference in base catalyzed equilibration of *cis*- and *trans*-4-t-butyl-6-nitro-1-phenylcyclohexenes

Table 2.6 Preferred conformations of the C6 substituents of 1-Phenyl (or Methyl)-6-substituted or 4,6-substituted cyclohexenes

Preferred conformation of 6R′	1,6 or *trans*-1,4,6-substituted cyclohexenes
Pseudoaxial	 R′ = *t*-Bu, Ph, $Me_2C(OH)$ or NO_2 R′ = NO_2 or Br only conformer
Pseudoequatorial	  R′ = Me_2C (OH), COMe or, NO_2 *cis*-4-Me-6-NO_2- **Note**. Syn axial R′ ↔ Me interaction in the a′ conf. overrides $A^{1,2}$ interaction between NO_2 and Ph or R′ and Ph groups in the e′-conformer, leading to the preferred e′ conformer.
No Conformational Preference (NCP)	 synaxial R = Me or Ph

2.14.2.2 Isomerizational Preference

One of the best examples is the base catalyzed equilibration of *cis*- and *trans*-4-*t*-butyl-6-nitro-1-phenylcyclohexenes leading to an equilibrium composition of 85–95 % of the *trans* isomer (pseudoaxial nitro group) (Fig. 2.122).

2.14.2.3 Pseudoallylic 1,2-Strain in Enamines

The value of $A^{1,2}$ strain becomes apparent only from its generality. If we replace the ethylenic substituent of 1-alkyl- or 1-aryl-6-substituted cyclohexene by a nitrogen or an oxygen atom, we get an enamine (**2**) or an enol ether. Thus, cyclohexanone can be converted to 1-pyrrolidinyl-6-methylcyclohexene (**2**), which may be regarded as a pseudoallylic system (Fig. 2.123). This reaction is known as Stork reaction [87, 88]. Now, the pseudoallylic 1,2-strain, Me ↔ pyrrolidinyl α-H in (**2e′**), is greater than the sum of the pseudoallylic 1,2-strain, e′H ↔ pyrrolidinyl α-H, and the synaxial interaction between 6-Me and 4-H in (**2a′**). Thus, the pseudoaxial conformer (**2a′**) becomes predominant in the ring inversion of (**2**) (Fig. 2.123). The enamine upon hydrolysis forms pure 2-methylcyclohexanone

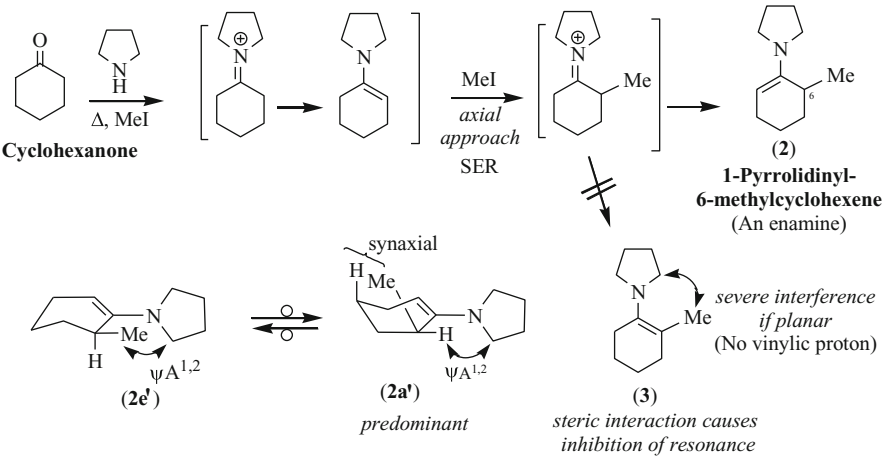
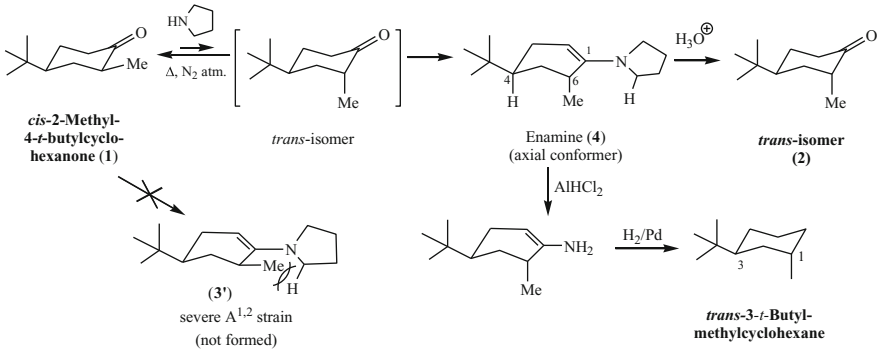


Fig. 2.123 Pseudoallylic 1,2-strain in a 6-methylenamine derivative



Note. *All compounds including the starting material are racemates, but, as usual, only one enantiomer is written in each case.*

Fig. 2.124 Complete conversion of the *cis*-2-methyl-4-t-butylcyclohexanone into the corresponding less stable *trans* isomer

without any further methylation at C2 or C6—this being the best method of its formation. The absence of any vinylic proton in the NMR establishes the non-formation of (**3**), which would involve severe interaction between the olefinic methyl and a planar pyrrolidinyl α-H, as shown in (3′) in Fig. 2.124.

Experimental verification of the pseudoaxial conformation of the 6-Me group in (**2**) is obtained by complete conversion of the more stable *cis* isomer (**1**) of 2-methyl-4-*t*-butylcyclohexanone to the less stable *trans* isomer (**2**) [89], which can be explained in terms of the non-formation of the enamine (**3′**) from the *cis* isomer due to severe $A^{1,2}$ strain (Fig. 2.124). The base (pyrrolidine) catalyzed

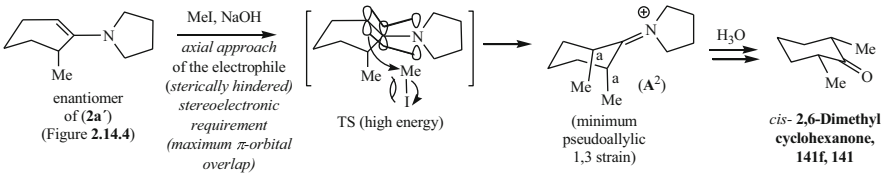


Fig. 2.125 Preparation of *cis*-2,6-Dimethylcyclohexanone (**A**)

equilibration produces the intermediate *trans* isomer (~5 %), which with axial Me is converted to the stable enamine (**4**) involving much weak $A^{1,2}$ strain between He and α-H of pyrrolidine, and the equilibrium is shifted till the whole amount of *cis* isomer is transformed to (**4**). The latter upon hydrolysis gives the *trans* isomer (**2**) quantitatively. The last two steps establish that the acid hydrolysis did not cause any change in orientation (here axial) of the Me group. Thus, the enamine (**4**) must have the Me group axial.

Another proof of pseudoaxial orientation of 6-substituent of an enamine:

The 6-methylenamine derivative enantiomer of (**2a′**) of Fig. 2.123 may undergo further methylation (or alkylation), but with difficulty, and the resulting Schiff base ($\mathbf{A^2}$) (Fig. 2.125) produces upon acid hydrolysis *cis*-2,6-dimethylcyclohexanone (**A**) (without any other accompanying polymethylated cyclohexanone). This is a convenient method for the preparation of (**A**). The sequence of reactions is illustrated in Fig. 2.125.

2.14.2.4 Synthesis of Solenopsin A. Application of $A^{1,2}$ Strain concept [90]

The concept of $A^{1,2}$ strain has been elegantly applied for the synthesis of solenopsin A(**X**), starting from the precursor imine (**B**), as delineated in Fig. 2.126.

2.15 Fused Ring Systems

2.15.1 Decalins

Of the fused carbocyclic systems with 6-membered rings like 6–3, 6–4, 6–5, 6–6, and 6–7 systems, the most important fused system is the 6–6 system, [4.4.0] bicyclodecane, trivially named as *decalin* (=decahydronaphthalene).

This system is very useful for consideration of conformation and reactivity, more quantitatively than other bicyclic systems. The decalin system occurs abundantly in natural products, like diterpenes, triterpenes, steroids, and alkaloids.

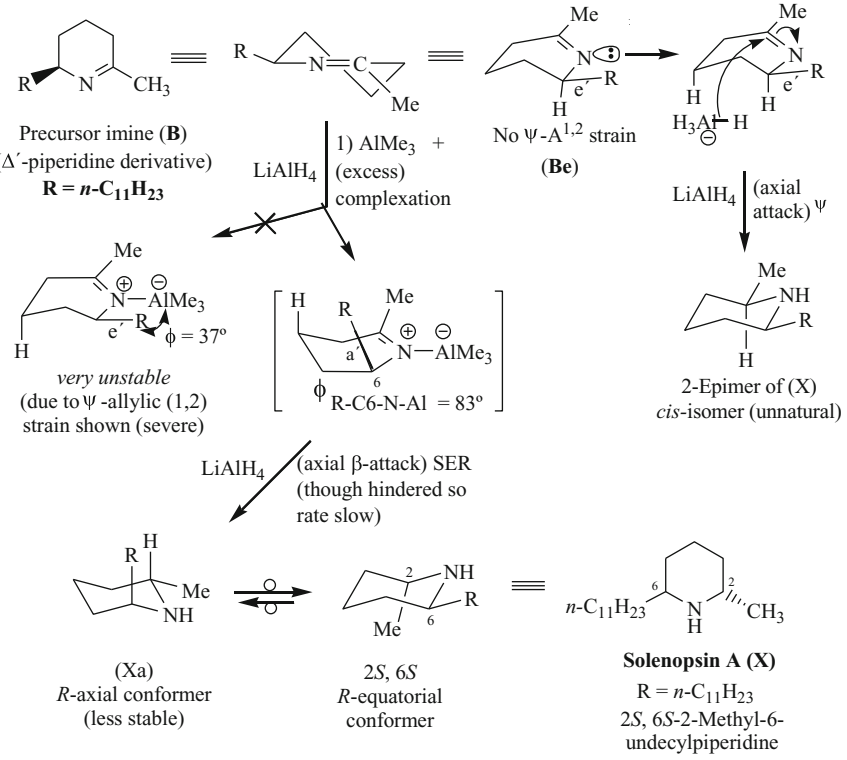
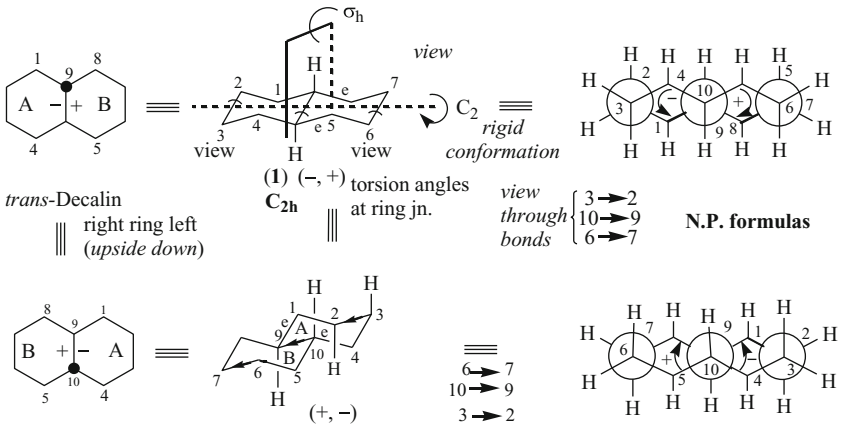
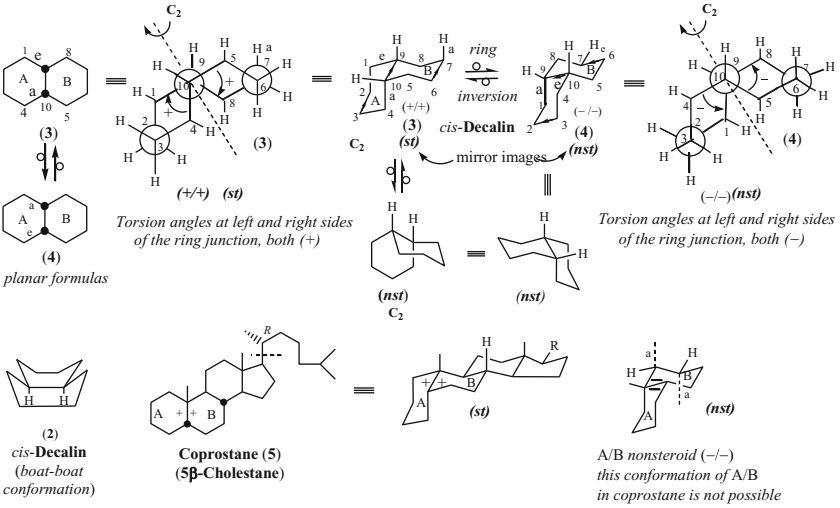


Fig. 2.126 Synthesis of solenopsin A by application of $A^{(1,2)}$ concept



Upon monosubstitution at any position C_2 *disappears, and* σ *also disappears, if the substitution is in a position other than C-9 and C10, and the substituted product is optically active.*

Fig. 2.127 Conformation and symmetry point group of *trans*-decalin



Note. *Monosubstitution product of cis-decelin at any position other than C9 and C10 gives a diastereomer upon inversion and hence is optically active.*

Fig. 2.128 Steroid (st) (+/+) and nonsteroid (nst) (−/−) conformations of *cis*-decalin

2.15.1.1 Brief History

- The existence of *cis* and *trans* decalins with two puckered six- membered rings was first predicted by Mohr in 1918. He also predicted chair/chair conformation (1) (Fig. 2.127) for *trans*-decalin and boat–boat conformation (2) (Fig. 2.128) for *cis*-decalin. On the contrary, two planar six-membered rings, as postulated by Baeyer, can only be fused as cis and not as trans.
- In 1925 Mohr's prediction was confirmed by isolation of *cis*- and *trans*-decalins by Hückel, and *trans* isomer was found to be more stable.
- In 1943 Hassel recognized that the chair form of cyclohexane is at least 5.6 kcal/mol more stable than its boat/skew–boat form, and for the first time predicted the chair–chair conformation for both *trans*-decalin (**1**) (Fig. 2.127) and *cis*-decalin (**3**) (Fig. 2.128).
- This was also substantiated by electron diffraction data in 1946.

The conformations of the two diastereomeric forms, *trans*-decalin and *cis*-decalin, are discussed in the sequel.

2.15.1.2 *trans*-Decalin. Conformation. Torsion Angles. Symmetry

In *trans*-decalin the ring junction H's at C9 and C10 are on the opposite sides. *The sign of any torsion angle involving three consecutive bonds is written against the*

central bond. Torsion angles around all C–C bonds in both *trans*-decalin and *cis*-decalin are ~55–56°, the same as in cyclohexane. In *trans*-decalin the two rings (chair) are fused through *e,e* bonds and cannot undergo ring inversion. From the Newman projection formula of *trans*-decalin, obtained by looking through the bonds mentioned in Fig. 2.127, it is quite clear that the torsion angles (ϕ or ω) in rings A and B on the left and right side of the common central bond are (−) and (+), respectively (see also Sect. 2.12.1.1). The signs are reversed if the B ring is on the left side (Figure). The sign of ϕ can be determined directly from the chair/chair conformation of the *trans*-decalin using the following rule. One does not have to draw the Newman projection formula for this purpose, as is shown on the right side of the Figure.

Rule of determining the sign of the torsion angle (ω or ϕ): While moving our eyes in a clockwise direction from the top of a ring (in a slanting manner) in the chair or boat or skew–boat form, the *β-axial* H or substituent in that ring *is preceded by* (+) sign of the torsion angle ω or ϕ and *is followed by* (−) sign.

Corollary: The α-axial *H* or substituent in that ring is preceded by (−) sign and is followed by (+) sign of the torsion angle ω or ϕ.

Symmetry. The chair–chair conformation of *trans*-decalin (Fig. 2.127) has a center of symmetry at the midpoint of C9–C10 bond and is achiral. Additionally, it has a C_2 axis passing through the midpoints of C2–C3, C9–C10, and C7–C6 bonds, and a σ_h plane passing through the ring junction, and perpendicular to the C_2 axis. Thus, its point group is $\mathbf{C_{2h}}$.

2.15.1.3 *cis*-Decalin. Conformations. Torsion Angles. Symmetry (Fig. 2.128)

In the *cis*-decalin the ring junction H's at C9 and C10 are on the same side of the puckered plane of the molecule, and the two rings are fused through *ea* or *ae* bonds. Thus, ring inversion takes place when the ring fusion bonds *ea* [to a ring, say(A)] (**3**) are converted to ae (**4**). From the Newman projection formulas of *cis*-decalin (Fig. **b**), it is clear that torsion angle ϕ at the left and right side of the common central C9-C10 bond in rings A and B are (+,+) for left side formula (**3**) and (−,−) for the right side formula (**4**), which are undergoing rapid interconversion. Such a concerted change in torsion angles of a *cis* fused junction is quite feasible. One can easily see this interconversion in its Dreiding or Fischer model.

The signs of the torsion angle ϕ at the ring junction in the rings A and B can be determined directly from either chair–chair conformations (**3**) (+/+) and (**4**) (−/−), by application of the rule, stated above.

The conformation (**3**) of *cis*-decalin is called *steroid* conformation since it resembles the A/B rings of 5β-steroids like coprostane (**5**), whereas the conformation (**4**) which cannot exist in 5β-steroids is called *nonsteroid*.

Symmetry. *cis*-Decalin chair–chair conformation (**3**) or (**4**) is chiral since either has no reflection symmetry (no σ plane or center of symmetry). Each conformer possesses a C_2-axis which passes through the midpoint of C9-C10 bond to which it is orthogonal, and it bisects the dihedral angle between 9-H and 10-H bonds (ϕ 9-H/10-H). So the point group is $\mathbf{C_2}$ (chiral). But it forms an *unresolvable* (±) *pair* because of the rapid interconversion of the two flipped forms: *steroid and nonsteroid* (*which are enantiomers*), *at even low temperatures*, the energy barrier between them being much lower than 15 kcal mol^{-1}. Although it is appreciably higher than that in cyclohexane, the barrier to ring inversion $\Delta G^{\neq}$ in *cis*-decalin is *12.3–12.6 kcal* mol^{-1} (51.5–52.7 $kJmol^{-1}$).

The inversion path involves the following transformations: chair–chair (CC) → chair–twist (CT) → twist–twist (TT) → alternate TT → CT → CC; the alternate TT is reconverted to the CT and CC by a reversal (mirror imaged) of the path by which it was formed.

2.15.1.4 Ring Inversion in *cis*-Decalin

In rigid *trans*-decalin the equatorial and axial protons are distinguishable which appear in ^{1}H NMR as two broadbands due to spin–spin coupling.

cis-Decalin and its derivatives undergo ring inversion (*cf.* cyclohexane)—already discussed. This can be studied by ^{1}H NMR. The equatorial and axial protons are averaged out due to ring inversion and appear as a narrow band at ambient temperature. However, at temperatures lower than the coalescence temperature, they can be split up into two broadbands. The *coalescence temperature* gives a value of 12.76 kcal mol^{-1} at –18° in CS_2 for the free energy barrier of ring inversion. 2,2-Difluoro-*cis*-decalin exhibits a barrier of 12.26 kcal mol^{-1} at –30 °C in ^{19}F NMR. The energy barrier of *cis-decalin* is thus considerably higher than that of cyclohexane and *cis*-1,2-dimethylcyclohexane ($\Delta G^{\ddagger} = 10$ kcal/mol). Ring inversion data of many cyclic compounds are available in a monograph by Oki [91]. Some coalescence temperatures and energy barriers are given in Fig. 2.129.

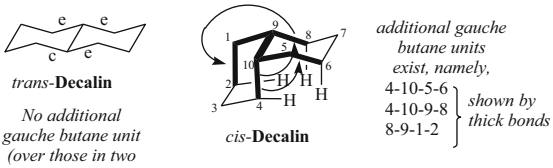
	~ *Coalescence temp.*	Energy barrier (kcal $mol^{-1)}$
cis-Decalin	≥ –30ºC	12.3 – 12.5
Cyclohexane[#]	– 66.7ºC	10.1
cis-Hydrindane	– 121ºC	6.5

[#]The rate of chair inversion is 105 sec^{-1}.

Fig. 2.129 Some coalescence temperatures

Isomer	Symmetry no. σ	Entropy cal. / deg. mole^{-1}				
		– R ln σ	R ln 2 due to ± forms	Total S	*Calcd.* ΔS $S_{cis} - S_{trans}$	*Exptl.* ΔS
cis-Decalin	2	– 1.38	1.38	0	+ 1.38	0.55
trans-Decalin	2	– 1.38	–	– 1.38		

Fig. 2.130 Entropy difference in decalins



$\therefore \Delta H_{calc.} = H_{cis} - H_{trans} = 3$ gauche butane interactions

$= 3 \times 0.8$ kcal mol$^{-1} = 2.4$ kcal mol^{-1} (= 10.05 k J mol^{-1})

Good agreement with $\Delta H_{exptl.}$ (2.10 to 2.72 kcalmol^{-1}) (≡ 8.8 to 11.4 k J mol^{-1}) determined by temperature dependence of *cis* ⇆ *trans* equilibrium and also from heat of combustion data.

According to ***Auwers-Skita rule or*** *conformational rule*, *cis*-decalin having higher enthalpy has higher b.p., density, refractive index than those for *trans*-decalin.

cis-Decalin : b.p. 193ºC.743 mm; d_4^{20} 0.895; n_D^{20} 1.4811

trans-Decalin 184ºC/747 mm; 0.870; 1.4697

Fig. 2.131 Enthalpy and physical constants difference of *cis* and *trans*-decalins

2.15.1.5 Entropy Difference in Decalins

Symmetry number of *cis*- and *trans*-decalin is 2. The difference in entropy of *cis*-decalin results from the fact that the *cis*-isomer is a (±)-pair (although non-resolvable). The total entropy difference is shown in Fig. 2.130.

Thus, ΔS experimental value (0.55 cal K^{-1} mol^{-1} ≡ 2.3 J K^{-1} mol^{-1}) is much less than the calculated value 1.38 cal K^{-1} mol^{-1} ≈ 5.8 J K^{-1} mol^{-1}.

This is contrary to the popular belief that *cis*-decalin is more flexible than *trans*-decalin; in that case ΔS would have been more rather than less. Here ring inversion ability possessed by *cis*-decalin should not be confused with the flexibility of the system as in case of *trans*-decalin.

2.15.1.6 Enthalpy and Physical Constants. Auwers-Skita Rule

Difference in enthalpies of *cis*-decalin and *trans*-decalin can be counted in terms of gauche interactions arising out of carbon atoms of two different rings. In *trans*-

decalin each ring is fused through *e* bonds, and hence no additional gauche unit is introduced. The calculated and experimental results of ΔH (enthalpy difference) are presented in Fig. 2.131.

2.15.1.7 Free Energy Difference in Decalins

The free energy difference has been determined experimentally from the equation $\Delta G = \Delta H - T\Delta S$ as 2.39 kcal mol^{-1}, which is in good agreement with the equilibrium data for 1-decalones [*trans* ⇆ *cis* (5–10 %) from $\Delta G = -$ RT ln *K*]. The comparison is, however, not strictly valid because of 2- and 3-alkylketone effects (*cf.,* Sect. 2.13.3) in 1-decalone.

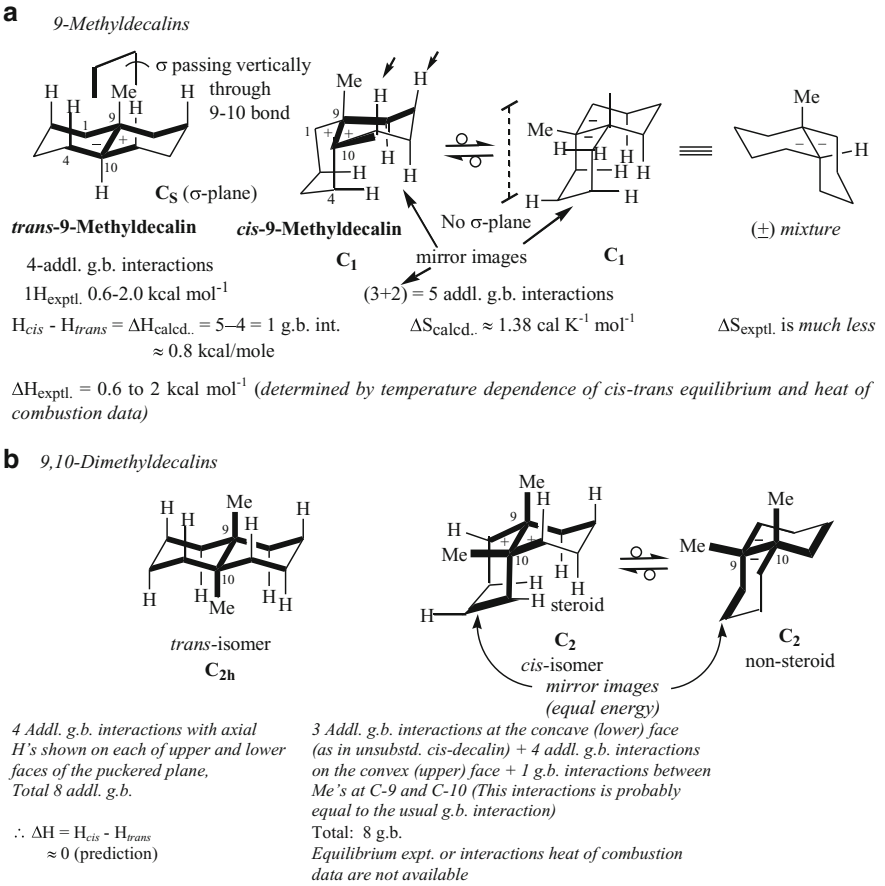


Fig. 2.132 Conformations and enthalpy differences of 9-methyldecalins and 9,10-dimethyldecalins

2.15.1.8 Effect of Introduction of Angular Methyl Group/s

The enthalpy difference between *cis*-decalin and *trans*-decalin after introduction of 9-methyl group and 9,10-dimethyl groups are delineated in Fig. 2.132a and b, respectively. The additional gauche–butane interactions between an axial methyl group and synaxial hydrogen atoms are shown in each case. The axial hydrogen atoms at the concave face of *cis*-decalin sustaining three additional gauche–butane interactions are also shown (*cf.* Fig. 2.131).

trans-Decalins unsubstituted at positions other than bridgeheads are always *achiral*, having a σ-plane vertical to the common 9–10 bond. *cis*-Decalins substituted only at C9 or C10 or at both carbons although chiral exist as unresolvable (±)- or racemic mixtures (*cf. cis*-decalin). However, a *cis*-decalin substituted at any position/s other than bridgeheads will exist as a resolvable (±)-pair, since flipping now converts it into a diastereomer (and not an enantiomer).

2.15.1.9 *cis*-Decalones and *trans*-Decalones

When a carbonyl group is introduced to give 1- or 2-decalones, the two bridgehead carbons, C9 and C10, become chiral, and both the *trans*- and *cis*-decalins exist as resolvable (±)-pair. Thus, *trans*-1-decalone can be resolved into two enantiomers (**1**) and (**1′**) (Fig. 2.133), having unequivocal conformations. However, each enantiomer of *cis*-1-decalone (**2**) or (**2′**) exists as two nonequivalent conformers (**2a**) and

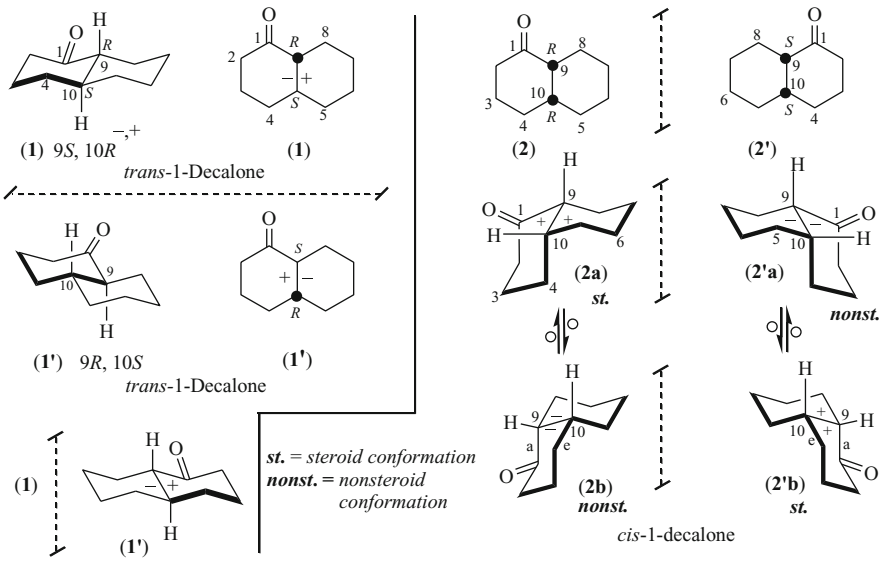


Fig. 2.133 Conformations of *trans*-1-decalones and *cis*-1-decalones

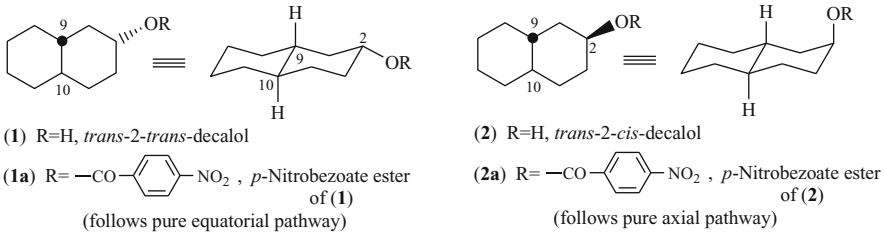


Fig. 2.134 Conformational analysis of *trans*-2-decalol diastereomers

(**2b**) or (**2′a**) and (**2′b**), respectively, by ring inversion. Conformer (**2a**) having steroid conformation flips into (**2b**) having nonsteroid conformation. Similarly, conformer (**2′a**), the enantiomer of (**2a**), having nonsteroid conformation, undergoes ring inversion to form a nonequivalent conformer (**2′b**) having steroid conformation (Fig. 2.133).

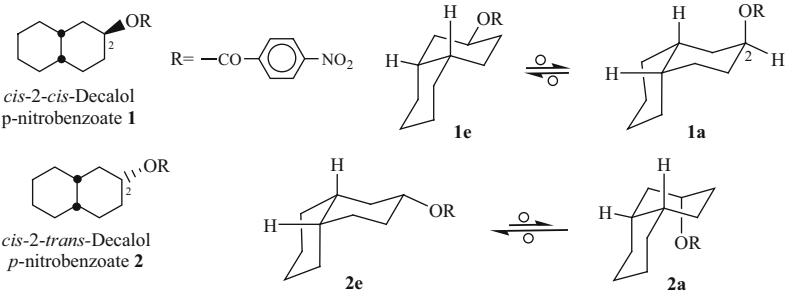
2.15.1.10 *trans*-2-Decalols. Conformational Analysis

Trans-2-Decalol exists as two diastereomers, *viz*., *trans*-2-*trans*-decalol (**1**) and *trans*-2-*cis*-decalol (**2**) (Fig. 2.134); they have the hydroxyl group *trans* and *cis*, respectively, to the 9-H atom. Since *trans* decalin possesses unique rigid conformation (ring inversion being not possible), the OH groups in the diastereomers (**1**) and (**2**) (Fig. g) must be equatorial and axial, respectively. Thus, the conformational analysis here is easy and gives definite conclusions. Compound (**1**) is converted to its *p*-nitrobenzoate ester (**1a**) at a faster rate than compound (**2**) under the same condition, *because of the steric hindrance* in the latter case. Likewise, the saponification of the ester (**1a**) follows pure equatorial pathway whereas the reactions of (**2**) or (**2a**) follow pure axial pathway involving steric hindrance.

2.15.1.11 *cis*-2-Decalols. Conformational Analysis

Let us now consider the relative reactivity of the *p*-nitrobenzoates of isomeric *cis*-2-dacalols [92]. The configurations of these epimeric decalols were established by Dauben et al. [93, 94]. Because of the flexibility (due to ring inversion) of *cis*-decalols, conformational analysis does not give definite conclusions regarding stereochemistry of the molecules. The relative rates of saponification of the diastereomers are delineated in Fig. 2.135 in terms of the equatorial **1e** and axial **1a** conformers arising due to ring inversion of **1** and in terms of the equatorial **2e** and axial **2a** conformers arising out of the ring inversion of **2** and by the application of Winstein–Holness equation (i) (Fig. 2.135); here k_e and k_a denote the specific rate

Relative rates of saponification of the diastereomers



Winstein-Holness equation: overall rate $k = k_e N_e + k_a N_a$

In case of compound **1**

$k_1 = k_{1e}N_{1e} + k_{1a} N_{1a}$

both greater, e pathway | *both smaller, but not negligible; a pathway*

$G_{1e} \approx G_{2e} < G_{1a} << G_{2a}$

$\therefore N_{1e} > N_{1a}$; $N_{2e} >> N_{2a}$

Again, $\Delta G^{\neq}_{1e} < \Delta G^{\neq}_{1a}$ $\therefore k_{1e} > k_{1a}$

and $\Delta G^{\neq}_{1e} < \Delta G^{\neq}_{2a}$

(*cases of steric hindrance*)

$\therefore k_{2e} >> k_{2a}$

$\therefore$ *The ester **1** undergoes saponification by both e and a pathways*

In case of compound **2**

$k_2 = k_{2e} N_{2e} + k_{2a} N_{2a} \approx k_{2e} k_{2a}$ (approx.)

both much greater | *both negligibly smaller, and the product is even more negligible*

Note: *The equatorial pathway for the saponification of an ester is faster than the axial pathway (case of steric hindrance).*

$\therefore$ *Compound 2 undergo saponification by equatorial pathway.*

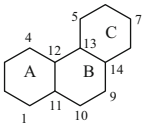
$\therefore$ *k_2 should be greater than k_1.*

Experimentally it has been found [94] that the ester **2** is saponified 1.5 times more rapidly than that of **1**.

Fig. 2.135 Conformational analysis of *cis*-2-decalol *p*-nitrobenzoate diastereomers

constants of the equatorial and axial conformers, and Ne and Na denote the mole fractions of the equatorial and axial conformers, respectively.

2.15.2 Perhydrophenanthrenes (PHP's). Stability. Point Groups. Optical Activity

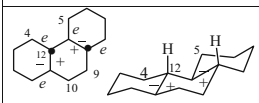
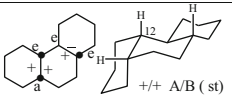
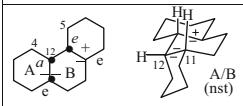


PHP

Three cyclohexane rings when fused successively to an angular arrangement gives rise to perhydrophenanthrene (PHP). It constitutes the ABC rings of terpenoids and steroids. It contains two equivalent pairs of chiral centers—the four

bridge-head carbons, C11 and C12 and C13 and C14. Thus, it constitutes an ***ABBA*** system and can have four pairs of enantiomers and two meso forms. (cf. CH_2OH-$(CHOH)_4$-CH_2OH); total *10 stereoisomers* and *six diastereomers* (Fig. 2.54).

In the nomenclature of the diastereomers, the prefixes *cis* and *trans*, here abbreviated as ***c*** and ***t***, define the stereochemistry of the ring junctions A/B or B/C, whereas the other terms *cisoid* and *transoid*, abbreviated as ***ci*** and ***tr***, define the steric relationship of the nearest bridgehead atoms (which also denotes the orientation of the terminal rings with respect to each other).

Planar structure **(A)** **(Point group)** (***chirality***) / *Conformation* **(B)** e equatorial a axial	No. of gauche butane interactions **(C)**	Relative $\Delta\Delta H^0$ calcd. kcal mol^{-1} [89] **(D)**	Relative $\Delta\Delta H^0$ calcd. Molecular mechanics [90] **(E)**	$\Delta\Delta G^0$ exptl. [91] **(F)**	σ plane in planar projection formula† **(G)**
 **(1) *t-tr-t*** ***four e ring fusion bonds (rfb)*** ***(chiral)*** (C_1) 11α, 12β, 13α, 14β *R S S R*	1 (C4-C12-C13-C5)	0	0	0	*Absent so resolvable*
Note: *A dot at a ring juncture indicates a forward or upward hydrogen, whereas the absence of a dot indicates a backword or downward hydrogen. In case of (±) pairs only one enantiomer with H12 β- is shown.*					
 +/+ A/B (st) **(2) *c-tr-t*** ***three e rfb*** ***(chiral)*** (C_1) *S S S R*	1+3 (for cis-decalin)	2.4	2.44	2.25	*Absent, so resolvable*
†*the chirality or otherwise can be judged by looking for a vertical σ-plane passing through the 9-10 and 12-13 bonds in the planar structure*					
 **(3) *c-ci-t*** ***three e r f b*** ***(chiral)*** (C_1)	1+3 (for cis-decalin)	2.4	2.57	2.66	*Absent so resolvable*

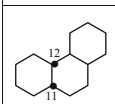
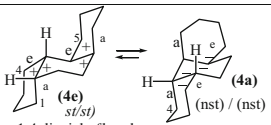
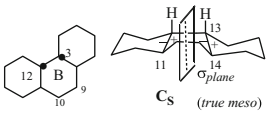
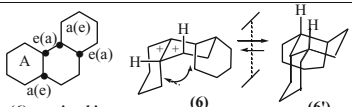
(A)	**(B)**	**(C)**	**(D)**	**(E)**	**(F)**	**(G)**
 **(4) c-tr-c** (C_1) ***chiral two e rfb)***	 **(4e)** *st/st)* 1,4,diaxial *rf* bonds. *A/B* +/+ *B/C* +/+ **(4a)** (nst) / (nst) 1,2-diaxial *rf* bonds –/–, –/–	1+6(two cis-decalins) ⇅ 6(two *cis*-decalins)	4.8 **(4e)** (4,5 di eq) ⇅ 4.0 **(4a)**	4.01	4.6	*Absent, so resolvable;* *flips into a diastereomer*

Fig. 2.136 (continued)

 C_S (*true meso*) **(5) *t-ci-t*** ***Achiral*** (**4*be rfb***) *In ring B the sign at 11-12 bond is +* *" " 13-14 " is –,* (*should have been + in chair form*) *Impossible in chair conformation; so B ring is boat.*	B-ring true boat [89]	7.2	7.03	8.98	*Present in planar as well as conformational structure.* *So true meso*
 **(6)** ***c-ci-c bi*** unresolvable ***(2e, 2a, rfb)*** **(6)** ***chiral (conformers)*** ***bi*** **(6')** *A/B B/C* +/+ +/+ *st st* *A/B B/C* –/– –/– *nst nst*	1+6+ *syn* axial $CH_2 \leftrightarrow CH_2$ ¥ interaction 	4.8+ *>3.6*¥ = >8.4 total (5.2-1.6 = 3.6) for Me ↔ Me	9.01	7.43	*present in planar str, so unresolvable;. actually unresolvable (±) pair formed by flipping.(cf. cis-decalin).* *So not true meso*

Notes : Approx. agreements between H^0(calcd.) and G^0(exptl.) energies are satisfactory in view of the differences, though small, in entropy of mixing and entropy of symmetry between isomers.

- *For the highly strained isomers **(5)**and **(6)** the calculated. energies are the reverse of the experimental.*
- ¥ *This is between 1, 3 synaxial ring fusion bonds; being rigid it would be more than synaxial Me Me interaction.*

Fig. 2.136 Perhydrophenanthrene (PHP)diastereomers: conformations, torsion angles, energies, resolvabilities

The six perhydrophenanthrene diastereomers (**1**), (**2**), (**3**), (**4**), (**5**), and (**6**) are included in Fig. 2.136 in order of increasing enthalpy, *i.e.*, decreasing stability. This basic ring system is of wide occurrence in nature, and the relative stabilities of the diastereomers are of interest. In this figure the calculated relative enthalpies ($\Delta\Delta H^0$) based on nonbonded interactions **(C)** [95], and on molecular mechanics [force field calculations **(D)**] [96], and the experimental relative free energies ($\Delta\Delta G^0$) **(E)** [97] are expressed in kcal/mol (approx.)

Conformations of the enantiomers, (marked with superscript 1) of the chiral perhydrophenanthrene diastereomers (**1**), (**2**), (**3**), and (**4**) are shown in Fig. 2.137.

It should be noted that the torsion angles of the central ring at 11–12 and 13–14 bonds at the ring junctions are the same in (**1**) to (**4**) and (**6**), as expected for chair conformation. But in the isomer ***t-ci-t*** (**5**) these signs are opposite which suggest a non-chair conformation for the central ring. The same conclusion is also drawn from the orientation (*a*,*e*) of the ring fusion bonds. If the ring B were a chair, the ring fusion bonds would be *e, e, a, a* with respect to B, and it is known that the *a, a* fusion in the *trans*-decalin moiety is sterically not possible. Because of the rigidity of the ***t-ci-t*** (**5**) diastereomer, the central ring assumes a *true boat* form (instead of a flexible boat).

The predicted order of stability of the PHP's has been confirmed by equilibration of the 9-ketones in the cases shown in Fig. 2.138.

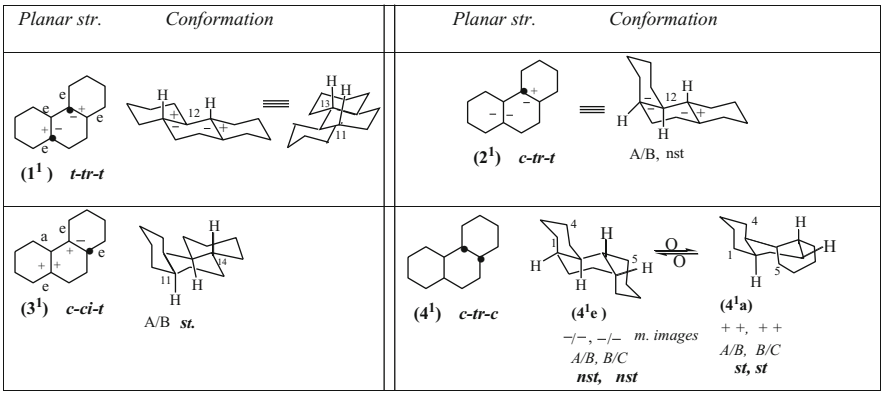
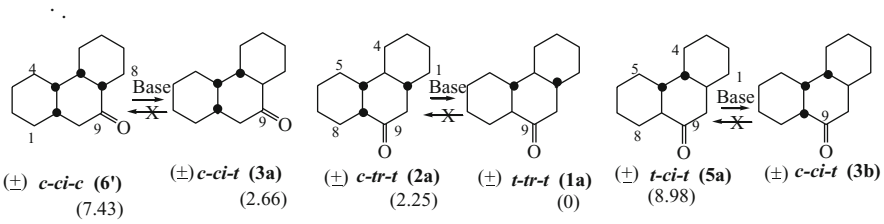


Fig. 2.137 Conformations of the enantiomers of the active perhydrophenanthrene diastereomers of Fig. 2.136



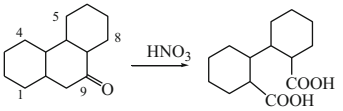
*Approx. rel. enthalpy (**H**) in kcal/mole (within parenthesis)*

Fig. 2.138 Confirmation of the predicted stability of perhydrophenanthrene-9-ones

2.15.2.1 Stereochemistry of Some Perhydrophenanthrones and All Perhydrodiphenic Acids (PHDPA's)

An elegant piece of work by Linstead and coworkers [98–104] presents a challenging exercise in stereochemical reasoning.

Several perhydrophenanthrones were correlated with PHDPA's in the following way:



Epimerization in this reaction must be guarded if same configuration is to be assumed

Syntheses of PHDPA's and the process of their configurational assignments from the study of catalytic hydrogenation of diphenic acid giving *cis* isomers (**A**), (**B**), and (**C**) of PHDP's are summarized in Fig. 2.139. The hydrogenated diastereomers were subjected to preferential epimerization of the monomethyl esters and

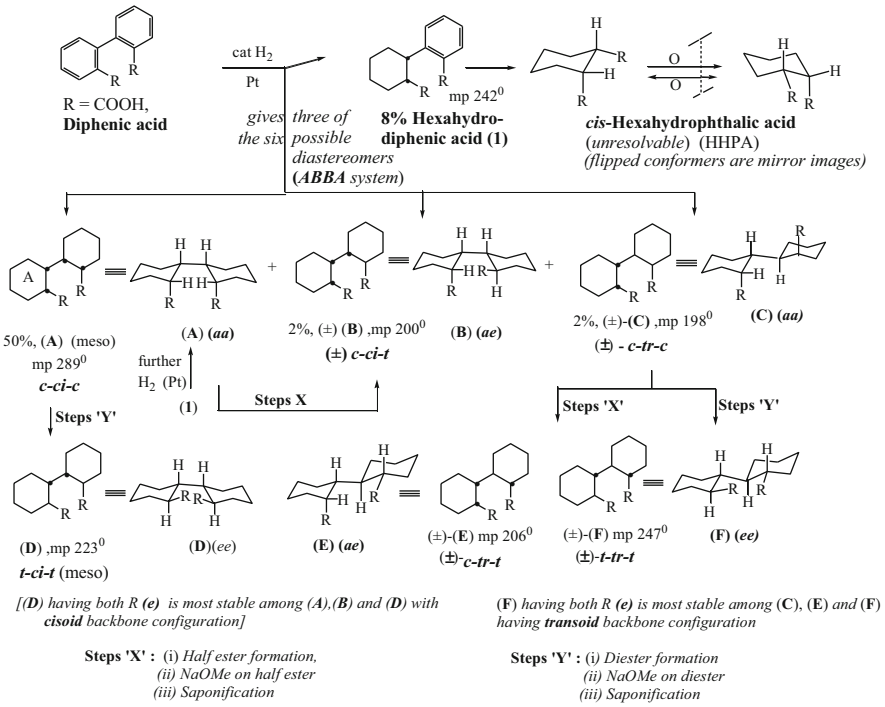


Fig. 2.139 Synthesis and configurational assignments of the perhydrodiphenic acids

dimethyl esters with sodium methoxide giving ultimately the most stable diastereomer (**D**) among the *cisoid* isomers, and the most stable diastereomer (**F**) among the *transoid* isomers (Fig. 2.139). The following important points should be noted:

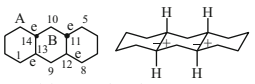
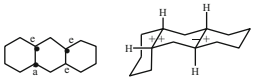
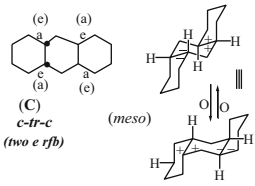
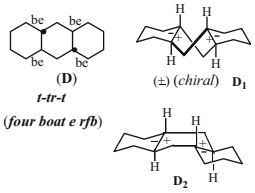
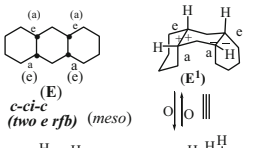
- Formation of *cis*-hexahydrophthalic acid from hexahydrodiphenic acid (**9**) and the conversion of the latter to (**A**) by further hydrogenation indicates that ring A in (**A**) is *cis* 1,2- disubstituted.
- Epimerization takes place at the α-carbon to the ester and not at the backbone carbons. Thus, (**A**), (**B**), and (**D**) belong to the same backbone configuration; this is also valid for (**C**), (**E**), and (**F**).
- Stoermer–Steinbach principle was applied to unresolvable (**A**) and (**D**) as follows: Both of them were converted to the monomethyl ester (dissymmetry introduced), which could be resolved. An active monoester was converted to the inactive diester (plane of symmetry again introduced).
- Thus, **PHDPA's**, (**A**) and (**D**), were established to be meso/unresolvable. Hence, (**A**), (**B**), and (**D**) must belong to cisoid backbone configuration.
- The PHDPA's (**C**) and (**F**) were resolved, so (**C**), (**E**), and (**F**) must belong to the *transoid* backbone configuration.

- Stability order in each series easily determines the relative configuration of the compounds in both *cisoid* and *transoid* series.
- For designation of PHDPAs the two COOH groups must be on the same side of the backbone C–C bond. (*cf*. the nomenclature of the perhydrophenanthrenes).

2.15.3 Perhydroanthracenes: Relative Stability. Torsion Angles. Point Group. Optical Activity

In perhydroanthracene (**PHA)** diastereomers (Fig. 2.140) the rings are fused in a linear arrangement. In the nomenclature of the diastereomers, the prefixes *c* and *t* define the stereochemistry of the ring junctions A/B and B/C, whereas the other terms *cisoid* and *transoid*, abbreviated as *ci* and *tr*, define the steric relationship of the nearest bridgehead atoms. Here, all the four chiral centers are equivalent, corresponding to **AAAA** system and there are five diastereomers—two of them are enantiomeric pairs and the other three are *meso*. The diastereomers (A), (B), (C), (D), and (E) in Fig. 2.140 are in order of increasing enthalpy and hence decreasing stability.

The relative stability of the isomers was originally predicted by Johnson [95]. Their planar structures, conformations, the relative enthalpies (calculated by molecular mechanics and determined experimentally [105]), and resolvability are outlined in Fig. 2.140. In contrast to the least stable PHP diastereomer, here the ***t-ci-t*** isomer (**A**) is the most stable isomer having no extra gauche interaction among the atoms of different rings. It possesses a plane of symmetry and hence is meso. The ***c-ci-t*** isomer (**B**) may also be called ***c-tr-t*** isomer, if one looks at the bridgehead atoms in the clockwise or anticlockwise direction, respectively. The ***c-tr-c*** isomer (**C**) flips into an identical conformer and possesses a center of symmetry and hence is *meso*. The instability of the isomer (**D**) is due to the twist–boat conformation of the central ring. The ***c-ci-c*** isomer (**E**) possesses 1,3-*syn*diaxial methylene interaction and two *cis*-decalin interactions and is the least stable one. Interestingly, an alternate conformer of the isomer (**E**) with the central ring as a twist–boat ($\mathbf{E^2}$) is of only slightly higher energy than the chair ($\mathbf{E^1}$) and contributes to the extent of 13 % at 271°C. The signs of torsion angles of the two junctions at 11–12 and 13–14 bonds in the central ring are consistent with the chair form in each isomer excepting (**D**). In case of the isomer (**D**) the same sign at the ring junctions 11–12 and 13–14 in the central ring in ($\mathbf{D_2}$) and in ($\mathbf{D_1}$) also indicates its boat or better twist–boat conformation.

Planar str. *Conformation*	No. of gauche int (s) (kcal/mole), relative to **A**[a]	Δ H^0 kcal/mole		Sym. pt. gr. (Sym.no σ)	*Flipping*	*Resolvability (opt. active)*
		Calcd.[b]	Exptl.[c]			
 **t-ci-t (A)** *four e rfb* *(meso)* *rfb : ring fusion bonds*	0 (0)	0	0	C_{2h} (2)	*not possible*	*no (no) meso*
 **(B)** ***c-ci-t or c-tr-t*** *(three e rfb)* *(±)(chiral)*	*one cis-decalin,* 3 (2.7)	2.62	2.76	C_1 (1)	*not possible*	*yes (yes)*
 **(C)** ***c-tr-c*** ***(two e rfb)*** (*meso*)	*two cis-decalins,* 6 (5.4)	5.56	5.58	C_i (both forms) (1)	*gives an identical form*	*no (no) meso*
 **(D)** ***t-tr-t*** ***(four boat e rfb)*** (±) (*chiral*) **D_1** **D_2**	*skew boat* (5.6)	5.86	4.15	C_2 (2)	*not possible*	*yes (yes)*
 **(E)** ***c-ci-c*** ***(two e rfb)*** (*meso*) **(E^2)** (13% at 271 °C) **(E^1)** −/−+/+	*two cis-decalins + syn-diaxial int.* (5.4+3.6 = 9.0) **(E_2)** *is of slightly higher energy than* **(E^1)**	8.13	8.74	C_S (1)	*gives an identical form*	*no (no) meso*

Notes: [a]*ΔH^0 calculated based on the number of gauche interactions [89]*
[b]*ΔH^0 calculated using molecular mechanics (force field method) [98]*
[c]*ΔH^0 determined experimentally from the composition of an equilibrium mixture of the hydrocarbons as a function of temperature [98].*
[d]*Both **c-ci-c** isomers of PHP (**5**) and PHA (**E**) are the least stable diastereomers of PHP and PHA respectively.*

Fig. 2.140 Conformation, stability, and optical activity of perhydroanthracenes

2.16 Stereoisomerism: Axial Chirality, (*R*,*S*) Notations

Different aspects of stereoisomerism in organic compounds, having chiral centers and pseudoasymmetric centers acting as stereogenic units, have been discussed in the preceding sections. Other elements of chirality, *viz.,* axis, plane, and helicity, also act as stereogenic units. Appropriately substituted allenes, spiranes, alkylidenecycloalkanes, biaryls, and adamantoids behave as stereogenic units, due to the presence of chiral axis. Likewise, appropriately substituted *trans*-cycloalkenes, cyclophanes, and their analogs display stereoisomerism due to the presence of a chiral plane. A helix is non-superposable on its mirror image; it possesses an inherent chirality, a stereogenic unit known as helicity.

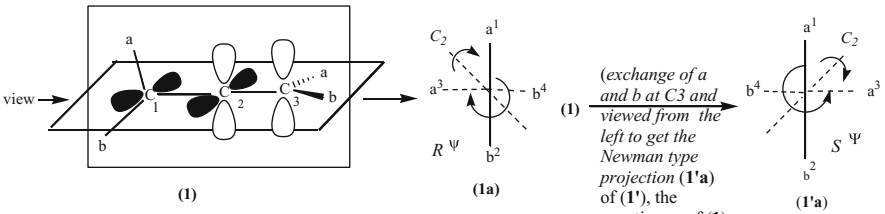
The configurational nomenclature of these stereogenic units will be illustrated briefly with examples in this and the following sections.

2.16.1 Stereochemistry of Allenes. Configurational Nomenclature

Of the three carbons of allene C1 and C3 are sp^2 hybridized and C2 is sp hybridized. The orbital picture of allene (**1**) is shown in Fig. 2.141. The shaded *p* orbitals as well as the unshaded p orbitals overlap with each other separately to form orthogonal π-bonds placing the ligands at C1 in a plane orthogonal to that of the ligands at C3. The structure (**1**) can be projected to a Newman-like projection formula (**1a**) to *R*-configuration, as shown, viewed from the left side with the front ligands in a vertical plane and the rear ligands in a horizontal plane (Fig. 2.141). The (*R*,*S*) nomenclature is independent of the direction of viewing, and the same specification will follow when viewed from the right side. The allenes of the general formula $C_{ab} = C = C_{ab}$ possess a C_2 axis, but no σ-plane, and belong to the point group $\mathbf{C_2}$. If the three or four of the ligands are different as in $C_{ab} = C = C_{ac}$ or $C_{ab} = C = C_{cd}$, the C_2 axis disappears, and the molecules are totally asymmetric and possess $\mathbf{C_1}$ point group. This is true for all axially chiral molecules.

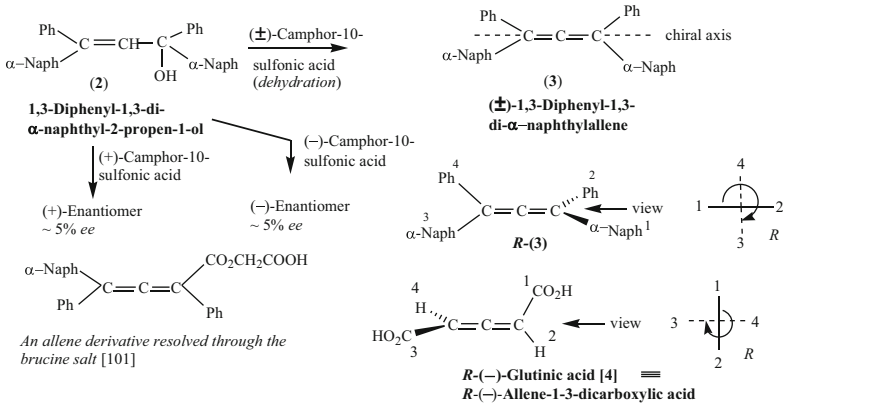
For configurational nomenclature of allenes and other axially chiral molecules, the standard subrule 0 (Sect. 2.6.2), which states that "the near groups precede the far groups," is considered first, ahead of other subrules. *R*,*S* nomenclature in cases of some specific examples of a few optically active allenes and their enantiomers are illustrated in Fig. 2.142.

The molecule is viewed from any end of the chiral axis and Newman-like projections are drawn; the groups near the viewer are numbered 1 and 2, whereas the groups at the far end are numbered 3 and 4, following the priority sequence rule. The order 1 → 2 → 3, clockwise or anticlockwise, gives the configuration as *R* or *S,* respectively. This type of nomenclature is applicable for other types of compounds with axial chirality, to be discussed in the sequel. Interchange of the two geminal groups at any end in these molecules leads to the enantiomer.

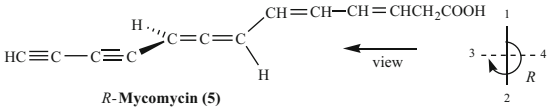


Ψ *By priority sequence rule if a precedes b,.*
Note: *Exchange of a and b at C1 or C 3 gives the enantiomer* **(1')**
Here, CIP ranking of substituents are indicated by supersripts 1,2,3,& 4.

Fig. 2.141 Orbital picture of an allene, its Newman projection, its enantiomer, and their (*R*,*S*) nomenclatures



Mycomycin **(5)** *is an α-substituted acetic acid. Its molecule contains two alkynes, one allene, and a butadiene moieties - all in conjugation. The allene part is responsible for enantiomerism. It is a natural antibiotic.)*



Nomenclature: *One has to look along the chiral axis of the biaryl (or any axially chiral) molecule and project on a plane orthogonal to the chiral axis; now, after assigning the priority sequence 1 (or a) and 2 (or b) to the near ligands shown by a solid line, and 3 (or c) and 4 (or d) to the far ligands shown by a dotted line the (R,S) specification can be made as shown in the examples given above.*

Fig. 2.142 A few optically active allenes and their (*R*,*S*) specification

Maitland and Mills [106, 107] prepared the first optically active allene (**3**), 60 years after Van't Hoff's prediction. 1,3-Diphenyl-1,3-di-α-naphthyl-2-propen-1-ol (Fig. 2.142) was separately dehydrated with (±)-, (+)-, and (−)-camphor-sulfonic acid to give (±)-, (+)-(~5 % *ee*), (−)-(~5 % *ee*), respectively. The absolute configuration of the (+)- or (−)-enantiomer was, however, not determined.

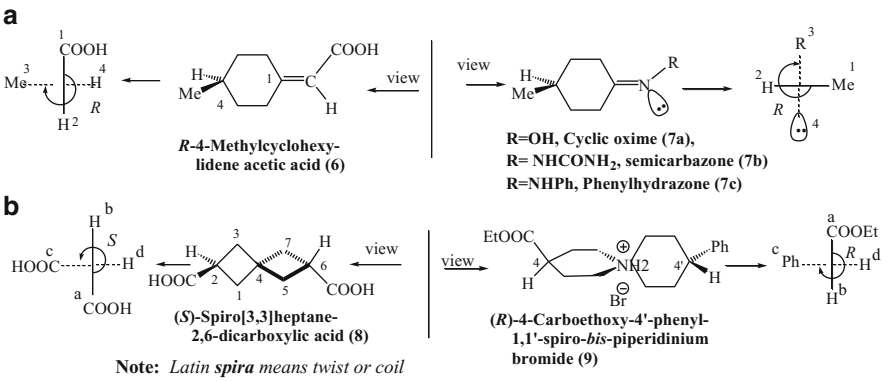


Fig. 2.143 Configurational nomenclatures of some known resolved axially chiral compounds (**a**) derivatives of an alkalydenecyclohexane and 4-carboxycyclohexylidene imine, (**b**) two spiranes

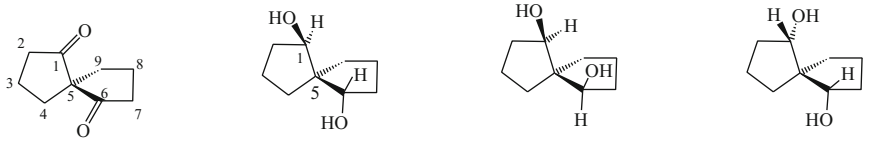
Naturally occurring (−)-glutinic acid and the antibiotic mycomycin [108] are also examples of optically active allenes (Fig. 2.142) [109].

For the (R,S) specification of other types of axially chiral molecules the same procedure [#] is followed (see Figs. 2.143, 2.145, 2.146, 2.147, 2.151, and 2.152).

2.16.2 Chiral Spiranes and Analogs. Configurational Nomenclature

Spiranes, alkylidenecycloalkanes, adamantanes, and catenanes with appropriate substituents can be dissymmetric like allenes. The name "*spirane*," derived from the Latin *spira* meaning twist or coil, implies that spiranes are nonplanar. One of the double bonds of an allene upon replacement by a ring, such as (**6**) (Fig. 2.143), gives rise to alkyledenecycloalkanes, which are also called hemispiranes. When both the double bonds of an allene are replaced by two rings, a *spiran*e, *e.g.*, (8), is generated. Here, also the two terminal disymmetrically substituted methylene planes are orthogonal to each other. The compounds having pairs of nonequivalent geminal substituents at both ends will exhibit enantiomerism, and (*R*,*S*) specification is achieved in such compounds as in case of allenes (Fig. 2.143). In all such compounds if one terminal methylene is substituted by same ligands, and the other terminal methylene carries different ligands, the molecule becomes prochiral—carrying a prochiral axis. When one of the same ligands at one terminal is replaced by a different ligand, the molecule becomes chiral, and the prochiral axis in the precursor molecule becomes chiral axis in the generated chiral molecule.

Spiro[4, 4]nonane-1,6-dione (**10**) [110], *a spirane having a chiral axis as well as a chiral center,* is shown in Fig. 2.144. Configurational nomenclature of (**10**) and its



***R*-Spiro[4,4]nonane-1,6-dione (10)**
C_5 is the chiral center
Priority sequence;
C1> C6> C4> C9
So, R

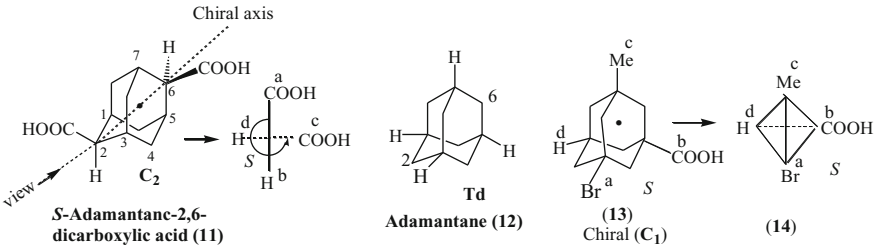
1*R*,5*R*, 6*S*
(10a)

1*R*, 5*R*, 6*R*
(10b)

1*S*, 5*R*, 6*S*
(10c)

In all the reduction products the spiro-carbon C5 has R configuration

Fig. 2.144 Spiranes with one chiral center (**10**) and three chiral centers (**10a, 10b, 10c**).



***S*-Adamantanc-2,6-dicarboxylic acid (11)**

Adamantane (12)

(13)
Chiral ($\mathbf{C_1}$)

(14)

Fig. 2.145 Adamantane, chiral adamantanoids, and specification of chirality

three distereomeric reduction products (**10a**), (**10b**), and (**10c**) are shown in Fig. 2.144. These compounds exhibit both central chirality and axial chirality; in such cases *central chirality has precedence for configurational nomenclature*.

2.16.3 Chiral Adamantoids. Configurational Nomenclature

Adamantane-2,6-dicarboxylic acid (**11**) (Fig. 2.145) is an axially chiral compound. In it C2 and C6 methylenes are dissymmetrically substituted and exist in orthogonal planes. The adamantoid (**11**) is shown to have *S* configuration. The imaginary chiral axis in adamantoids passes through the substituted terminal carbon atoms C2 and C6 and the geometrical center of the ring system.

It may be mentioned here that if the four bridgehead carbon atoms of adamantane (**12**) bear four different ligands as in (**13**), the molecule becomes chiral. Adamantane [111] is a highly symmetrical molecule having $\mathbf{T_d}$ symmetry like CH_4. On the other hand, the adamantane derivative (**13**), carrying four different chiral centers at the four bridgeheads, becomes completely asymmetric with the point group $\mathbf{C_1}$, and exists only as a (±)-racemate pair. The four different ligands in (**13**) form a tetrahedral arrangement like (**14**), and the chirality of the molecule may be

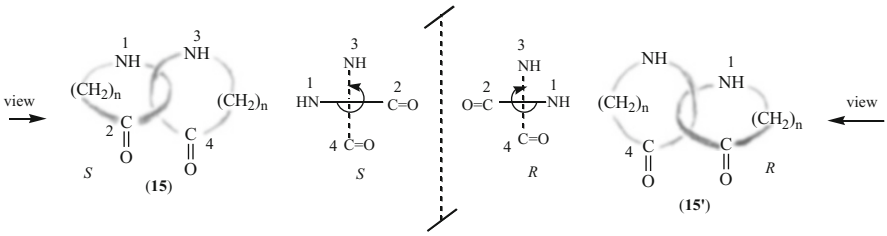


Fig. 2.146 Configurational nomenclatures of a chiral catenane and its enantiomer

associated with a center, represented by a dot, in the unoccupied space of the adamantane skeleton (Fig. 2.145). Like a centrodissymmetric compound exchange of any pair of ligands abcd in (**13**), or of the pair ab at C2 or C6 in (**11**), will lead to reversal of chirality and will give the enantiomer.

2.16.4 Chiral Catenanes. Configurational Nomenclature

The name catenane (from Latin *catena* meaning chain) was coined by Wasserman [112] to molecules of a type of unusual topology containing two or more *dissimilar* intertwined (or knotted) rings. The two rings are *to be held* with their planes perpendicular to each other as shown in (**15**) (Fig. 2.146) with regard to the arrangement of the four distinguishable groups in the chains. Configurational nomenclature may be given from similar projection formulas. Thus, the catenane (**15**) and its enantiomer (**15′**) are determined to possess *S* and *R* configurations, respectively.

2.16.5 Biphenyl Derivatives and Atropisomerism

2.16.5.1 Introduction

In biphenyls an sp^2–sp^2 pivotal single bond joins the two phenyl rings. The distance between two same side ortho Hs in unsubstituted biphenyl (**1**) is 2.90 Å (approx.) $>$ van der Waals radii of two H's, 2×1.2 Å $= 2.24$ Å. Hence, the rotation about the pivotal bond is not hindered by steric factor (Fig. 2.147). A dissymmetrically substituted (at C2 and C6) phenyl group of such a biphenyl lacking a vertical plane of symmetry is two-dimensionally chiral. A planar combination of two such groups in opposite ways would lead to cisoid [(**2**), $\mathbf{C_{2v}}$] and *transoid* [(4), $\mathbf{C_4}$, $\mathbf{C_{2h}}$] conformers.

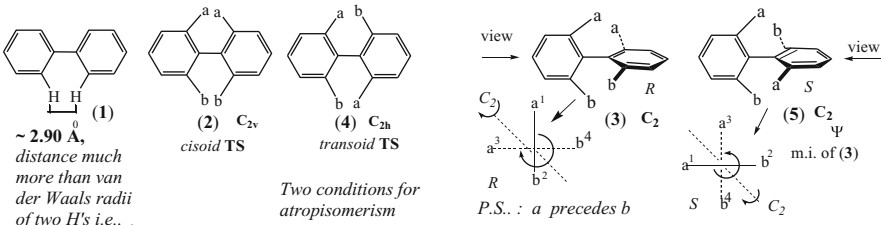


Fig. 2.147 Principle of atropisomerism. *R*,*S* nomenclature

On the contrary, a nonplanar combination of such groups would lead to two enantiomers (**3**) and (**5**), each belonging to $\mathbf{C_2}$ point group. When the energy barrier exceeds 19–20 kcal/mol (80–85 kJ mol^{-1}), the enantiomers are separable at room temperature. This type of enantiomerism was first discovered by Christie and Kenner in 1922 in the case of 6,6′-dinitro-2,2′-diphenic acid [113] (Fig. 2.149). Richard Kuhn later in 1933 called it *atropisomerism* and such enantiomers as *atropisomers* (from Greek *a* meaning "not" and *tropos* meaning "turn"), since such molecules do not turn around the molecular axis (due to steric hindrance).

2.16.5.2 Energy Profile Diagram

Energy Profile Diagram (approx.) is shown in Fig. 2.148 for a 360° displacement of torsion angle (ω) about the pivotal bond or showing the interplanar angle in *ortho* substituted biphenyls. The following points are to be noted:

(i) Nonplanarity of the two aromatic planes is caused by the steric demands of the *ortho* substituents. This is opposed by π electron overlap of the two rings; maximum stabilization occurs when the rings are coplanar. Even the biphenyl itself is nonplanar in the ground state (the inter-ring torsion angle being 44° in the vapor phase, but in the crystal phase the rings are coplanar because of packing forces [114].

(ii) The planar diastereomeric conformations (**2**) and (**4**) represent the energy maxima due to severe steric interference of the ortho substituents on either side; the former [*cisoid* **TS**, (**2**)] having similar groups on the same side has higher energy than the *transoid* **TS** (**4**) having dissimilar groups on the same side.

(iii) Recemization of the two enantiomers (**3**) and (**5**) takes place with greater ease through the transoid conformation (**4**) involving less activation energy than that involving the cisoid conformation (**2**).

(iv) The bulkier the *ortho* substituents are, the higher is the energy barrier between the enantiomers.

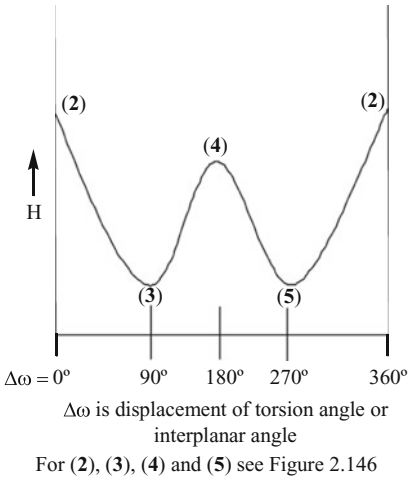


Fig. 2.148 Energy profile diagram of *ortho* substituted biphenyl

(v) Due to complete absence of resonance stabilization at $\omega = 90^\circ$ or 270°, the decrease of resonance stabilization may be more than the decrease in steric repulsion (which may be even absent) at these positions and a situation may be that of a double minima when ω is around 90° and 270°, and of a small maximum at ω 90° and 270°.

(vi) Excess resonance energy of a biphenyl over two benzene rings is a function of the angle of twist (ω) between the phenyl groups. At even as large twists as 45°~ there is still 50 % resonance energy.

2.16.5.3 Examples of Atropisomerism

Sufficient bulky groups must be present in *ortho* positions of biphenyls to destabilize the planar conformations due to steric repulsion and to generate atropisomers. They may be *ortho ortho* disubstituted, trisubstituted, tetrasubstituted, and even monosubstituted. Examples are given in Fig. 2.149.

2.16.5.4 Orders of Steric Hindrance and of Buttressing Effect

These are depicted in Fig. 2.150.

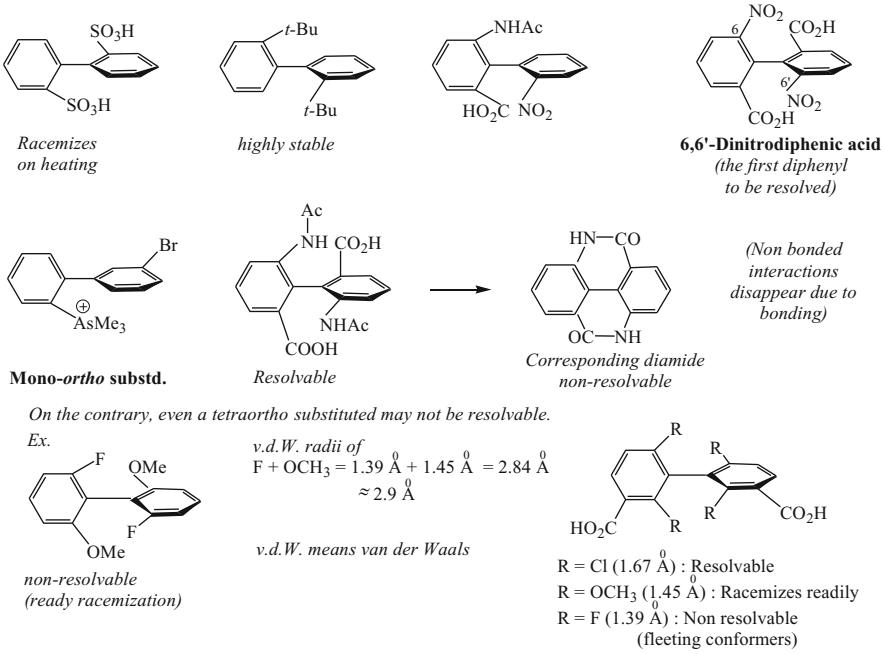
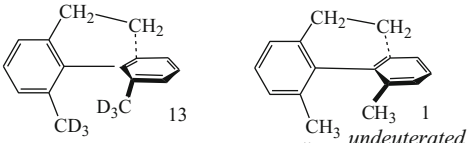


Fig. 2.149 Resolvable (stable) atropisomers

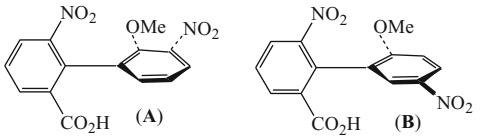
Order of Steric hindrance : Br > Me > Cl > NO_2 > COOH > OMe > F > H > D
roughly corresponds to v. d. W. radii of atoms or groups



Rel. Rate of racemization: 13.1#

since D has smaller van der Waals radius than that of H, because of the lower zero point vibration frequency of D

* ***Buttressing Effect:***



*Rate of racemization of (**A**) is much lower than that of (**B**) due to the buttressing effect of the bulky NO_2 group in (**A**) adjacent to the OMe group.*

* *Order:* NO_2 > Br > Cl > Me (different from bulk order)

Fig. 2.150 Order of steric hindrance and buttressing effect on configurational stability of biphenyls

2.16.5.5 Configurational Nomenclature of Chiral Biphenyls (*R,S* or *aR*, *aS*)

Selection rules (new convention*, 1966 [5]) for axial chirality has been introduced in case of atropisomers.

Subrule 0: Proximity rule: Groups about the near end of the chiral (molecular) axis precede over groups about the far end (same as in other axially chiral molecules).

a*Ordering of groups: Only the four atoms C2, C6, C2′, and C6′ which correspond to the four vertices of the *elongated tetrahedron* and which contribute more to the chirality properties of the molecule are considered for sequencing as shown in Fig. 2.151.

c. ***P, M-Nomenclature.*** Alternatively, molecules with chiral axes may be viewed as helicenes (since they resemble the helicenes to be discussed in the sequel). Their configuration may be designated as *P* or *M*. If the turn from the higher priority front ligand "a" to the higher priority rear ligand "c' is clockwise, the configuration is *P*, and if anticlockwise it is *M* (see Fig. 2.151). In general, *aR* corresponds with *M* and *aS* with *P*. The compound **A** possesses *aS* or *P* configuration and **B** possesses *aR* or *M* configuration according to the new convention introduced in 1966 [5]. According to the old convention published in 1956 [8], the nomenclature is the opposite in these cases.

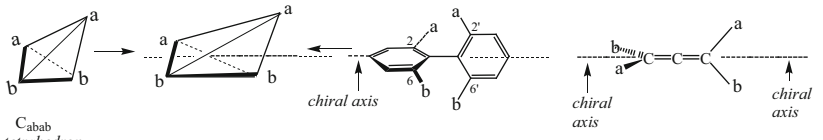
2.16.5.6 Some Interesting Examples of Axially Chiral Molecules Exhibiting Atropisomerism

A few examples are illustrated in Fig. 2.152. Molecules of the type in which one planar ring is replaced by a dissymmetrically substituted trigonal atom (two-dimensionally chiral) may exhibit atropisomerism if sufficient steric hindrance exists around the pivotal bond. A substituted stilbene (**A**) has been resolved. In the substituted naphthylamine (**B**), the peri nitro group gives rise to restricted rotation around the pivotal bond (chiral axis). Their (*R,S*) nomenclatures are also illustrated (Fig. 2.152).

Again, of the two oximes of 1-acetyl-2-hydroxynaphthalene-3-carboxylic acid, the *E*-isomer (**C**) (Fig. 2.152) is not resolvable (since the restriction around the pivotal aryl-carbon bond is not sufficient), whereas the *Z*-isomer (**D**) is resolvable because of restricted rotation around the pivotal bond.

Natural products possessing axial chirality have been reported, *e.g.*, natural dimeric coumarins, desertorin A, B, and C and triumbelletin (Fig. 13.71) and colchicine alkaloids (Fig. 24.1). Additionally, many asymmetric catalysts also possess axial chirality. Configurational nomenclature has been specified for each such compound (*vide* Figs. 6.14, 6.37, 6.38, 7.66, 7.67, 7.69, 13.56, 13.71, 17.10, 18.9, and 24.1).

a Ordering of groups

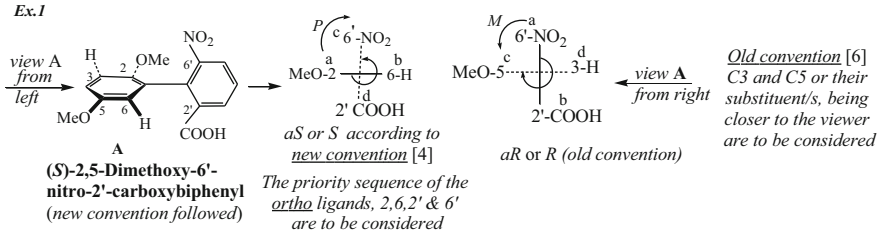


C_{abab} *tetrahedron, chiral center* — *Elongated tetrahedron* — *chiral axis in chiral biphenyl and chiral allene*

Ordering starts from the first off axis chiral biphenyl atoms (near the observer) i.e., 2nd and 6th atoms of the front ring.

- The priority sequence is fixed by the four ortho carbons after due complementation for quadriligancy proceeding along the branch of higher priority.
- molecule viewed from either end leads to the same configurational descriptor. *R* or *S*.

b. Nomenclature : *One has to look along the chiral axis of the biaryl molecule and project on a plane orthogonal to the chiral axis; now, after assigning the priority sequence 'a' and 'b' to the near ligands shown by a solid line, and 'c' and 'd' to the far ligands shown by a dotted line the (R,S) specification can be made as shown in the following examples. In many cases the nomenclature remains same according to new and old conventions. Here we are citing two examples where nomenclature changes according to the convention. Viewing from left or right gives the same specification.*



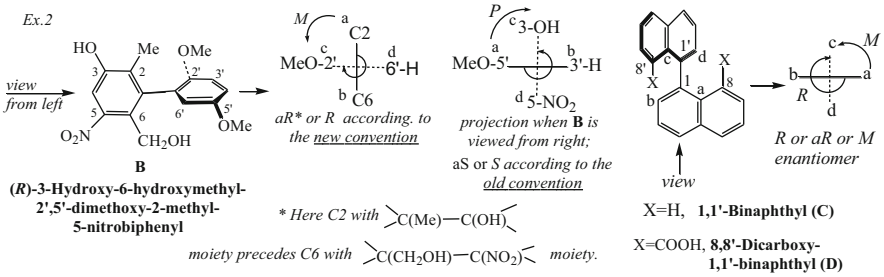


Fig. 2.151 Configurational nomenclatures (new and old conventions) of some chiral biphenyls and binaphthyls

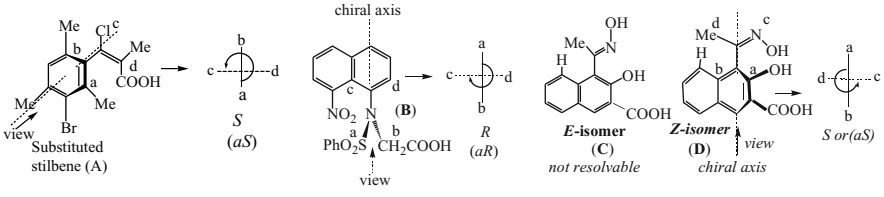


Fig. 2.152 Atropisomerism in acyclic analogs of biphenyl and in oximes

2.17 Planar Chirality

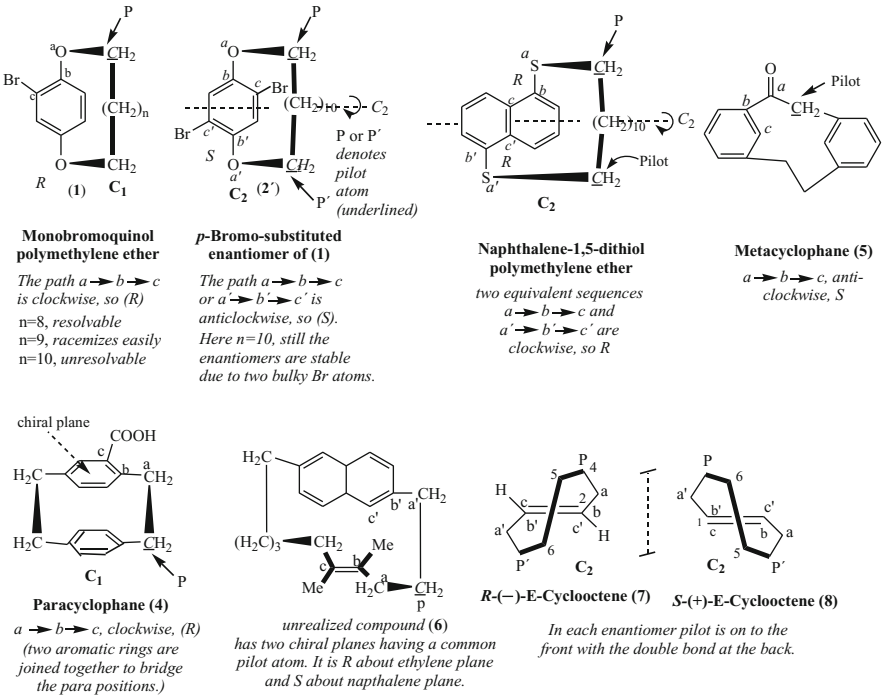
2.17.1 Introduction

A molecule possessing a chiral plane exhibits planar chirality. A chiral plane is not definable as easily as a chiral center or axis. A chiral plane contains as many of the atoms of the molecule as possible. Chirality is due to the fact that at least one ligand (usually more) is not in the chiral plane.

Moelcules with planar chirality include *ansa* compounds, *paracyclophanes, metacyclophanes* (Fig. 2.153), and a few *trans-cycloalkenes*. In the enantiomers the methylene chain is on either side of the aryl ring or C=C bond. The interconversion between the enantiomers is prevented by the inability of the alicyclic ring, being too small, to swing from one side to the other of the aryl or olefinic plane.

2.17.2 The (R,S) Specification of Planar Chirality

The specification of these compounds (Fig. 2.153) is done by application of the following *selection rule*.



Note: (**1**), (**2**) and (**3**) are *ansa* compounds; in Latin *ansa* means handle.

Fig. 2.153 Some compounds of planar chirality and their (*R,S)* specification

Selection rule [8]: The most preferred atom directly bound to atoms in the chiral plane is selected as the *pilot atom* (spectator point). It is the first out-of-plane atom linked to the sequence-preferred end of the chiral plane. The *sequencing starts with the first in-plane atom directly bound to the pilot atom* (the underlined *C*) and going along the in-plane sequence (marked as a, b, and c) involving the more preferred atom at each branch. The order in which a, b, and c appear when seen from the pilot atom, specifies the absolute configuration, *i.e.*, *R* for clockwise and *S* for anticlockwise order (see Fig. 2.153).

The compounds of planar chirality: (**1**), (**2**), and (**3**) are *ansa* compounds, (**4**) and (**5**) are cyclophanes, (**6**) is an imaginary compound containing two chiral planes having a common pilot atom. Cyclooctene is the smallest ring which can accommodate a *trans* double bond. Two enantiomeric configurations (**7**) and (**8**) are possible.

The interconversion of the two enantiomers (**7**) and (**8**) of *trans* or *E*-cyclooctene requires the swinging of the tetramethylene chain over and below the plane of the double bond (chiral plane) which is opposed by angle strain (ring strain). The two enantiomers have been resolved by Cope and coworkers [115, 116] who determined the absolute configuration of the enantiomers [117]; the (−)-enantiomer has been shown to be *R* and the (+)-enantiomer *S*. The molecule possesses $\mathbf{C_2}$ point group, having only the C_2 axis passing through the center of the double bond and bisecting the 5–6 bond. In the higher homologues increase in the mobility of the polymethylene chain decreases the rotational barrier. Thus, *trans*-cyclononene exists in enantiomeric forms only at −80 °C, and *trans*-cyclodecene is an extremely mobile system.

2.18 Helicity and *P,M*-Designation

A helix is inherently chiral and may be considered as displaying axial chirality—its axis serving as the chiral axis. However, it is more convenient to discuss such type of chirality as helicity. A helix possessing a C_2 axis orthogonal to the chiral axis is called *palindromic*, and it looks same from either end. If a helix moves from one end to the other in a clockwise direction, it is designated *P* (plus) and if it moves in an anticlockwise direction, it is designated *M* (minus) (Fig. 2.154). It is known that a polypeptide chain derived from natural L-amino acids often coil to form an α-helix with *P* helicity, but it is not palindromic since its two ends are nonequivalent and hence it lacks a C_2 axis. Its point group is $\mathbf{C_1}$. The nucleic acids present in DNA or RNA possess the skeleton of a double *P*-helix, preserved by strong intramolecular H-bondings [118].

Molecular overcrowding [119] is exemplified by hexahelicine (**A**) [120] and 4,5-disubstituted phenanthrenes as in (**B**) [121], (**C**) [122], and (**D**) and (**E**) [123] (Fig. 2.154).

The ring structures, due to overcrowding, assume helicity since the two terminal rings, as in (**A**), or the substituents at 4 and 5 positions of phenanthrene, as in (**B**) to (**E**), are large enough to prevent their existence in the same plane as that of the aromatic rings, thus giving rise to this type of optical isomerism, due to molecular asymmetry [118] arising out of helicity. With respect to some parts of the central

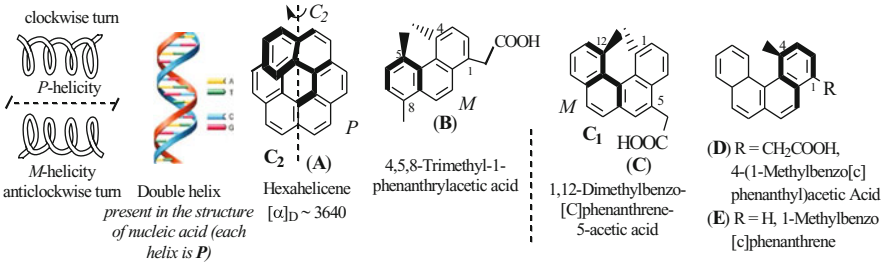


Fig. 2.154 Helical structures. Helicity due to molecular overcrowding

rings, the ring planes on the upper right side, as written in the Figure, are gradually bent up, and the ring planes on the upper left side are gradually bent down by small angles, (10°–14°) in case of *P* helicity, as have been evident from X-ray diffraction analysis [118]. In case of *M*-helicity the ring planes will bend just in opposite direction. Thus, in each case two helical enantiomers exist. The synthetic racemates (**A**) to (**E**) have been resolved. The *P, M* nomenclature and the point groups of these molecules are depicted in Fig. 2.154.

Hexahelicene having very high rotation, $[\alpha]_D$ ~3,640, racemizes slowly at its mp 266 °C. Higher helicenes readily racemize at their mps (about 200°) [124]. The absolute configuration, however, has not been assigned. As shown earlier in Sect. 2.16.5.5c, chiral biphenyls may also be viewed as helicenes and designated as *P* or *M* (see Fig. 2.151). For the relationship between the sign of the CE and absolute configuration of helicenes, see Sect. 2.19.6.2.

2.19 Chiroptical Properties. Optical Rotation. ORD, CD [125–127]

2.19.1 Origin of Optical Rotation. Circular Birefringence, Its Effect

Optical rotation and optical isomerism have been briefly discussed in Sect. 2.2.8. Now we would dwell on the origin of optical rotation. The electric field associated with the light wave in ordinary radiation oscillates in all directions perpendicular to the direction of propagation along the *z*-axis. Such radiation is isotropic or unpolarized. If the radiation is filtered to remove all oscillations other than in one direction, say in the *xz* plane, then the light becomes linearly polarized, since the projection in *xy* plane is linear, and the light becomes anisotropic.

When a plane-polarized (better called linearly polarized or LP) monochromatic light wave passes through a dissymmetric medium (nonracemic sample of a chiral substance), the plane of polarization rotates giving an optical rotation—a chiroptical property exhibited by nearly all chiral molecules. Two enantiomers exhibit optical rotations, equal in magnitude, but opposite in sign.

The electrical field vector (E) of an LP wave oscillates in the *xy* plane along the direction of propagation (*z*-axis). The LP wave is the resultant of two chiral components—a right circularly polarized (RCP) ray and a left circularly polarized (LCP) ray, whose projections on the *xy* plane are circles (Fig. 2.155).

In a symmetric or isotropic medium, the two components RCP and LCP travel at the same velocity. The resultant of the two rays in phase constitutes the linearly polarized light.

RCP ray + LCP ray = LP ray.

These rays make diastereomeric relationship with the two enantiomers and so interact differently. When the linearly polarized light is passed through a nonracemic sample of a chiral or dissymmetric compound (or medium), the velocities of LCP and RCP rays become different; the two circularly polarized rays will have different refractive indices, *viz*., n_L and n_R, being the refractive indices of left and right circularly polarized rays; this is known as *circular birefringence*. If $n_L > n_R$, $V_R > V_L$ since $n = V_O/V$, V_O and V are velocities in vacuum and the medium, respectively; thus, the plane of polarization or the LP wave will rotate toward right, i.e., the observed rotation α will be positive. The compound is said to be dextrorotatory. Thus, if $n_R > n_L$, $V_L > V_R$ and hence the observed rotation α will be levorotatory.

The observed rotation changes continuously as the LP proceeds (α varies directly with length l). Optical rotation due to circular birefringence for 1 cm path length is given by the expression $\alpha = (n_L - n_R)$. π/λ radian = Δn 180/λ degree. The specific rotation at a wavelength λ may be given by the expression $[\alpha]_\lambda$ in degrees = $\alpha \times 10/c$ for 1 dm path length, c = concentration in g/ml. The optical rotation of a chiral compound is usually reported as the specific rotation at sodium D line (589 Å), *i.e*., as $[\alpha]_D$. The molar rotation at a wavelength λ, $[M]_\lambda$, or $[\Phi]_\lambda = ([\alpha]_\lambda \cdot M)/100$ (cf. Sect. 2.2.9).

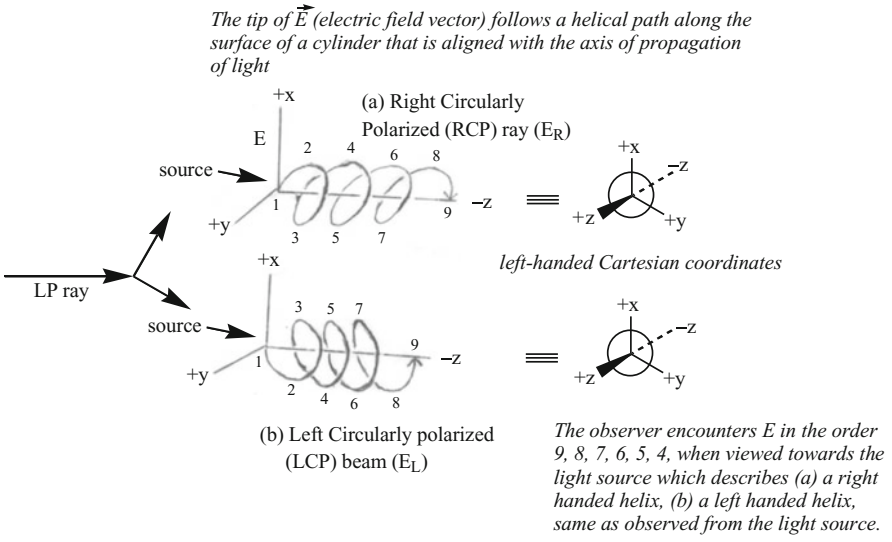


Fig. 2.155 LP wave is the resultant of RCP and LCP rays (*E* electric field vector)

2.19.2 *Optical Rotatory Dispersion. Plain Curve*

The specific rotation $[\alpha]$ of a chiral compound depends upon the wavelength of the monochromatic light wave. The measurement of specific rotation as a function of wavelength is called optical rotatory dispersion (ORD). Specific rotations of chiral compounds are generally reported for sodium D-line (589 nm wavelength), which is quite far from UV region. Consequently, the values of specific rotations are much lower than those recorded near the UV absorption maximum; if $[\alpha]_D$ is too low to be detected, and it is not possible to ascertain whether such a compound is dextrorotatory or levorotatory, the enantiomer may be mistaken as a racemic variety. In old literature the $[\alpha]_D = \pm 0$ has been assigned to such chiral compounds whose rotation at 589 nm could not be measured.

Specific rotations of such compounds having very low $[\alpha]_D$ values or any chiral compound having no UV absorption if measured at shorter wavelengths undergo many fold increment as one approaches the absorption maximum below 210 nm (due to $\sigma \rightarrow \sigma^*$ transition). Such an ORD curve is called a plain curve (Fig. 2.156). A plain curve results when measurement is done at wavelengths away from the absorption maximum (λ_{max}), and only circular birefringence is operative. By measuring a plain curve one can assay in a precise way the enantiomeric purity of a natural product or a bioorganic compound having no UV absorption. A chiral compound, if racemic, remains inactive throughout the measureable wavelength.

A plain curve for certain molecules may or may not cross the zero rotation axis. Thus, the *ortho* isomer of α-(iodophenoxy)-propionic acid shows levorotation at the D-line whereas the *meta* and *para* isomers show $[\alpha]_D$ positive, although all three isomers have the same absolute configuration (Fig. 2.156) [125]. Each of the three position isomers shows plain positive curve; the observed positive rotation increases with decrease of wavelength. In case of negative plain curve the observed negative rotation increases with decrease of wavelength.

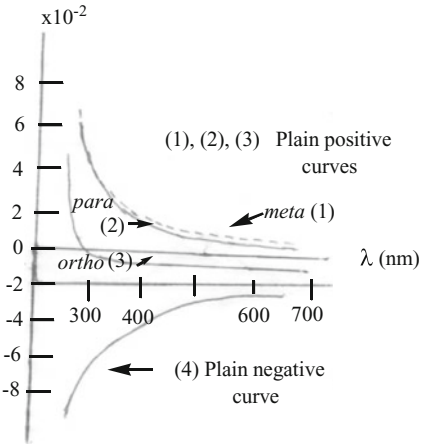


Fig. 2.156 ORD curves α-(*m*-, *p*-, and *o*-iodophenoxy)-propionic acids (*from C. Djerassi "Optical Rotatory Dispersion"*, **1960**, *McGraw-Hill Book Company*). Plain positive and negative curves

2.19.3 Circular Birefringence and Circular Dichroism. Cotton Effect

It has already been mentioned that when the LP rays of different wavelengths pass through a medium having a λ_{max} in the region studied, in addition to circular birefringence, the intensity of emergent light diminishes due to absorption. The two circularly polarized light rays, RCP and LCP, are absorbed to different extents; this phenomenon is termed as circular dichroism. Both these phenomena are wavelength dependent. Absorptions of RCP and LCP rays are maximum at the absorption maximum (λ_{max}). The refractive index increases with the decrease of the wavelength but rapidly falls to a minimum in the region of absorption showing abnormal dispersion. At the absorption maximum there is no effect on refractive index ($n = 1$ for both RCP and LCP rays). Figure 2.157 shows the variation of Δn ($=n_L - n_R$), refractive indices dispersion (I) of LCP and RCP, and of absorbance (A) (II) over a range of wavelength (including the absorption maximum, λ_{max}); Fig. 2.158 shows ORD curve with positive Cotton effect (III) and a positive CD spectrum (IV).

If an optically active compound absorbs in the UV or visible region, the two components RCP and LCP of the LP will be absorbed to different extents (anisotropic absorption), and this phenomenon of differential absorption is called *circular dichroism*, usually abbreviated as CD. The differential absorption is caused by electronic (along with vibrational) transition of energy states associated with a chiroptic chromophore in a chiral molecule. Let A_L and A_R represent the absorbances of LCP and RCP rays, respectively, then in case of CD $A_L \neq A_R$. According to Lambert–Beer's law $A_L - A_R = \Delta A = \Delta\varepsilon cl$, where $\Delta\varepsilon$ represents $\varepsilon_L - \varepsilon_R$, ε_L and ε_R are the molar absorption coefficients for LCP and RCP rays, respectively, c is the

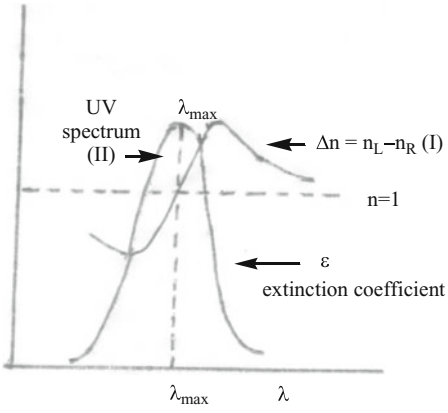


Fig. 2.157 Variation of Δn with λ (curve I). Variation of extinction coefficient (ε) with λ (UV spectrum, curve II)

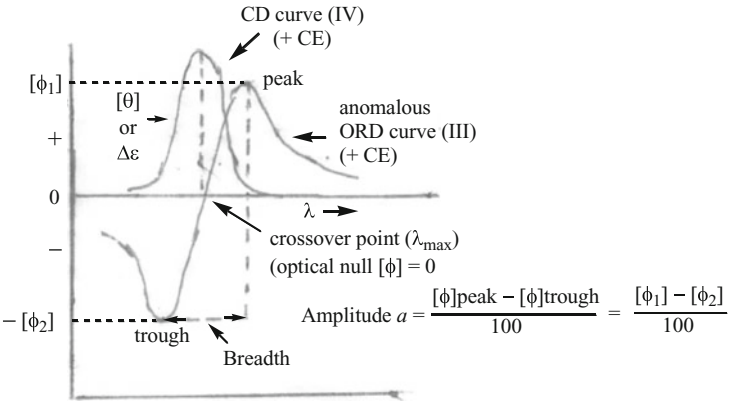


Fig. 2.158 Anomalous ORD curve with a positive CE and molar rotation [φ] as the ordinate (curve III) and a positive CD spectrum with molar ellipticity [θ] as the ordinate (curve IV)

concentration in moles per liter (mol L^{-1}), and l is the path length per cm. The term $\Delta\varepsilon$ is called *differential dichroic absorption*. If $A_L > A_R$ its electric field vector E_L will be smaller than E_R; the tip of the resultant vectors will trace a flattened helix whose projection on xy plane is an ellipse. Such a ray is called elliptically polarized (EP), characterized by a major axis and a minor axis. The elliptical polarization may be right handed or left handed. The major axis of the ellipse traces the angle of rotation α due to also the unequal velocity of RCP and LCP, and the ellipticity ψ is defined by $\tan \psi = b/a$, where b and a are the minor axis and major axis, respectively, of the ellipse. For small difference of A_L and A_R ψ for 1 cm is given by the simplified equation $\psi = {}^1/_4\,(A_L – A_R)$. In general, the major axis $a = (A_L + A_R)$ of this ellipse is much greater than the minor axis $b = (A_L - A_R)$ and may be treated as a plane polarized light for the purpose of measurement. Elliptically polarized light is the most general form of polarized light. In special cases like for linear polarized light, the eccentricity of the ellipse, $(a - b)/a$ becomes 1 since $b = 0$, and for circularly polarized light eccentricity becomes 0 since $a = b$.

Like in case of rotation, a *specific ellipticity* [ψ] and a *molar ellipticity* [θ] may be defined as shown below:

$[\psi] = \psi/c\,l$ in 10^{-1} deg $cm^2 g^{-1}$ and $[\theta] = [\psi]\,M/100$ in 10 deg cm^2 mol^{-1} (*cf.* Fig. 2.13),

where the symbols c, l, and M denote concentration in g/ml, length of the cell in dm, and molecular weight respectively, as in cases of specific rotation and molar rotation (Sect. 2.2.9), (Fig. 2.13).

Using a similar procedure as in optical rotation (Sect. 2.19.2), the specific ellipticity of the chiral medium may be defined for 1 dm path length and concentration c in g/ml as follows:

$[\psi]_\lambda^T$ in degrees $= (\psi.1{,}800)/c\pi$.

Ellipticities and *molar ellipticities* depend upon the temperature, wavelength, and concentration of the sample which should always be specified.

The combination of circular birefringence and circular dichroism gives rise to an important chiroptical phenomenon, namely the Cotton effect. In contrast to the plain curve, an anomalous ORD curve exhibits a peak (maximum) and a trough (minimum). This anomaly is called Cotton effect. The anomalous ORD arises from the superposition of two anomalies, *viz.,* the anomalies of n_L and n_R giving rise to the anomaly of Δn (Fig. 2.157). The Cotton effect is called positive when the rotation first increases with the decrease of wavelength and negative when the rotation magnitude decreases with decrease of wavelength. In other words in an anomalous ORD curve the Cotton effect is said to be positive if the peak is at a higher wavelength than the trough (Fig. 2.158). Conversely, the Cotton effect is termed negative if the trough is at a higher wavelength than the peak. The optical null (crossover point) closely corresponds to the ε_{max} of the UV spectrum in the absence of superposition of two or more close lying electronic transitions.

The *molar amplitude* of the ORD curve is expressed by the expression $a = ([\phi]_1 + [\phi]_2)/100$, where $[\phi]_1$ and $[\phi]_2$ are the absolute values of the molar rotations at the first and second extrema (peak and trough, respectively, in Fig. 2.158 displaying positive Cotton effect). The difference of the wavelengths of the two extrema is called the *breadth* (Fig. 2.158) of the ORD curve.

The chiroptical phenomenon CD is usually observed in the nontransparent regions of the spectrum due to conjugated chromophores of strong absorption with high extinction values. In such regions circular birefringence is not operative.

In the CD spectrum showing positive Cotton effect, only a peak appears near the λ_{max} of the chromophore in the molecule (Fig. 2.158) and conversely, a trough appears in the CD spectrum showing negative Cotton effect. The CD spectra are inherently simpler to interpret. In fact CD has been essentially replacing ORD as the main chiroptical technique in the study of chiral compounds [128].

Classification of chromophores. By ORD and CD measurements, the chromophores may be classified into two broad categories,

1. Chromophores that are *inherently achiral* such as the functional groups and the corresponding λ_{max} in nm in the parenthesis, as follows:

   Ketone (280–300), α,β-unsaturated ketone (330–360, 230–260), carboxylic acid (215–220), α,β-unsaturated acid (~250), ester (215–220), lactone (215–235), conjugated diene (~270), substituted phenyl (250–280), sulfoxides (~210), nitro compound (~270), amides and lactam (220–235).

   Each of the above chromophores when considered separated from the rest of the molecule contains at least one mirror plane and hence are inherently symmetrical but exhibit CE due to asymmetric perturbation exerted by chiral surrounding or by the molecular skeleton itself, *e.g.,* a carbonyl group (of local $\mathbf{C_{2v}}$ symmetry) in a steroid molecule.
2. Chromohores that are *inherently chiral*. In compounds of this category the chirality is built into the chromophore. Examples are helicenes and chiral compounds having skeletons like biaryls, allenes, spiro compounds, cycloalkenes, twisted 1,3-dienes, etc.

Applications of ORD and CD Curves with Cotton Effects *Some empirical rules.* Many chiral natural products are ketones or contain groups which can be converted into ketones (*e.g.,* secondary OH). The carbonyl $n \rightarrow \pi^*$ transition absorbs at 280–300 nm, relatively free from the interference of other chromophores. Moreover, the extinction coefficient is low ($\varepsilon \approx$20–80 near 290 nm) and light is transmitted even at λ_{max} leading to easy measurement of ORD and CD. Several empirical rules have been formulated theoretically to correlate the sign of the Cotton effect with its chiral environment. Several such rules are discussed in the sequel.

2.19.4 The Axial Haloketone Rule and Its Applications

The axial haloketone rule [125, 129] as a special case of the more general octant rule (*vide infra*). The rule is applicable to an axial α-halocyclohexanone moiety (the halogen is Cl, Br or I, but not F). One should view along the O=C bond in the direction of the ring with the carbonyl carbon placed at the "head" of the chair (or boat). If the axial α-halogen (X) appears at the right of the line of view (Fig. 2.159), the compound will show a strong positive CE; if it appears at the left, a strong negative CE will be observed. Presence of axial α-halogen also causes (i) a bathochromic shift of the CE or of the first extremum at higher wavelength (like the λ_{max} in the UV spectrum), (ii) an increase in the amplitude of the CE, and (iii) may cause the inversion of the sign of the CE with respect to the parent cyclohexanone, depending upon the configuration of the chiral atom bearing the halogen atom. The CE sign of the parent compound with known absolute configuration and conformation without any halogen atom can be ascertained by use of the octant rule or by actual experiment.

The sign of the CE depends on the (1) *structure*, (2) *conformation*, and (3) *configuration* of the halocyclohexanone in the neighborhood of the carbonyl group. If any two of these factors and the sign of the CE are known, the third factor will be determined. In case, these three factors are known, the sign of the CE can be predicted. Axial fluorine α-substituents have been shown to have opposite effects when compared with other halogen atoms at axial α-position.

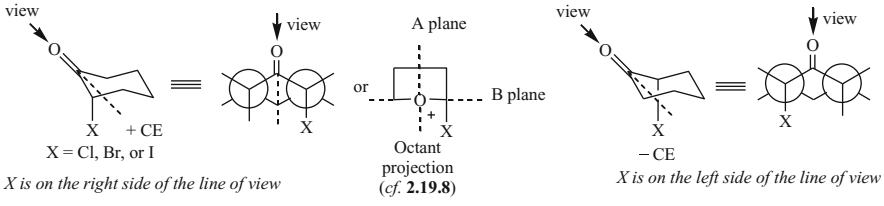


Fig. 2.159 Axial haloketone rule

Based on studies on steroidal ketones, it was revealed that equatorial α-halogen (or acetoxy) substituents on either side of the CO group have little effect on the ORD curve; the sign of the CE at ~300 nm remains unchanged relative to that found for the unsubstituted parent ketone. In cases where the α-halogen is equatorial, no significant bathochromic shift or increase in the amplitude of the CE is observed. This is also evident from the octant projection diagram (*vide infra*) as the equatorial α-substituents fall in the *yz* (B) plane and have no contribution to the sign of the CE.

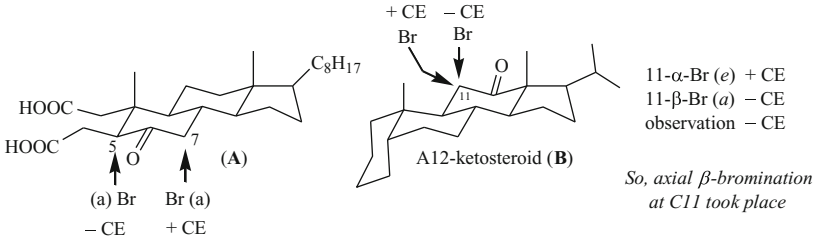
The axial haloketone rule has been later expanded to include SR, SO_2R, NR_2, and other substituents [130].

2.19.4.1 Position of the Halogen Substituent

Two examples of kinetically controlled bromination of nonracemic samples of chiral compounds (**A**) and (**B**) are provided in Fig. 2.160; in each case the constitution and conformation of the parent ketone are known. The bromination product of the compound (**A**), the α-bromo derivative, exhibited negative CE establishing that axial bromination took place at C5. In case of compound (**B**) negative CE of the axial β-bromination took place at C11 [125] (p 123).

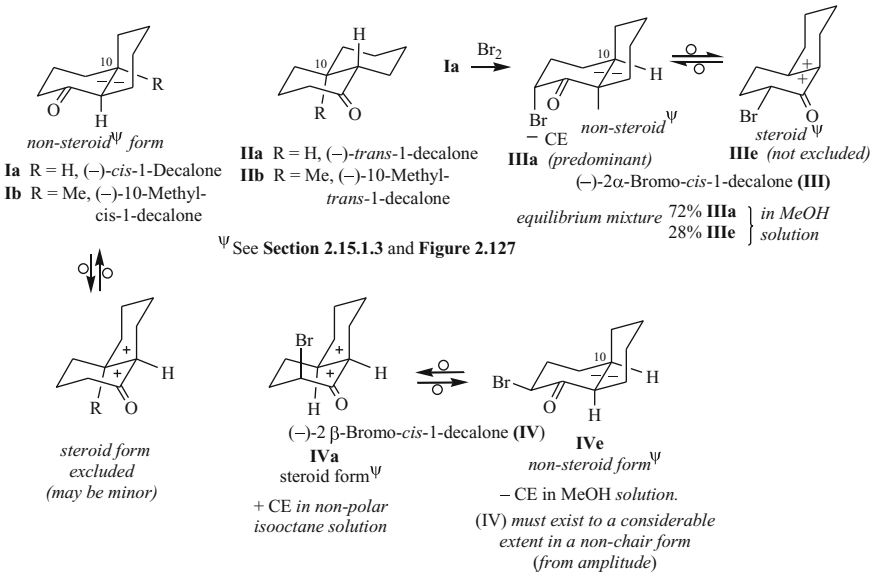
2.19.4.2 Absolute Configuration by Comparison Method

ORD and CD curves showing CEs are extremely useful for determining the absolute configuration by a comparison method: a dispersion curve of a compound of unknown configuration is compared with some compounds of similar structure of known absolute configuration. Some examples have been cited in the sequel. Here, one example is given in Fig. 2.161. The absolute configuration of (−)-*cis*-1-decalone (Ia) and *trans*-1-decalone (IIa) were established by comparing their ORD curves with those of their 10-methyl analogs (Ib) and (IIb) of known absolute configuration [131]. The flipped conformer (steroid form), if present, must have minor contribution in case of **1b** and hence of **1a**.



Note: *Axial orientation of bromine in the compounds established by UV and/or IR.*

Fig. 2.160 Position of bromine substituent in (**A**) and (**B**) by use of axial haloketone rule



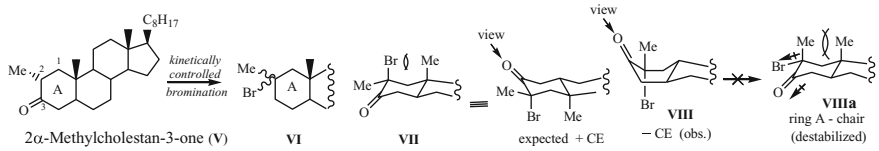
Note: Ψ *The steroid and non-steroid forms seem to be designated [123] in the opposite way by oversight*

Fig. 2.161 Absolute configurations of **Ia**, **IIa** by comparison method and of **IIIa**, **IVa** from axial haloketone rule, and solvent effect

2.19.4.3 Absolute Configuration by Axial Haloketone Rule. Conformational Mobility

Kinetically controlled bromination of (−)-*cis*-1-decalone (**1a**) afforded in pure form all six possible monobromo-*cis*- and *trans*-1-decalones whose ORD curves were fully consistent with the assigned structures [131]. In Fig. 2.161 only the nonsteroid form **IIIa** of the 2α-bromo (axial) derivative and the steroid form (**IVa**) of the 2β-bromo (axial) derivative and their CE by application of axial haloketone rule are shown. It should be noted here that in case of **IVa**, the carbonyl carbon being not at the head or upper layer of carbons, but at the lower layer of carbons of the cyclohexanone ring, and the axial halogen being present at the left of the line of view through O=C (**IVa**) exhibits + CE. The structure **IVa** if turned upside down, the α-axial bromine will be at the right side of the line of view, consistent with the positive sign of the CE.

Measurement of the ORD curves of (−)-2α-bromo-*cis*-1-decalone (**III**) in solvents of different polarities demonstrated the existence in methanol solution of a 70–30 equilibrium between the nonsteroid (**IIIa**) and steroid (**IIIb**) conformations of the ketone (**III**) (Fig. 2.162). The existence of the (−)-2β-Bromo-*cis*-1-decalone predominantly as the axial conformer (**IVa**) in nonpolar isooctane solution showing + CE and as the equatorial conformer (**IVe**) in methanol solution to a



Note: *In* **VII** *the steroid rings including ring A has been inverted to bring carbonyl carbon at the head of the chair.*

Fig. 2.162 Demonstration of boat form by application of axial haloketone rule

considerable extent showing –CE have also been demonstrated. The low amplitude of the ORD of **IV** in methanol solution (compared to that of **III** in methanol) also indicates its existence to a considerable extent in a non-chair form.

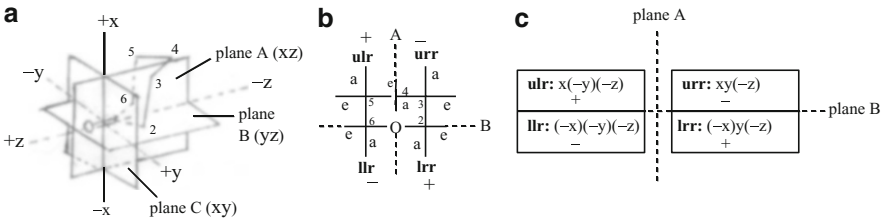
2.19.4.4 Boat Form of Ring A of a Steroid Bromoketone

The axial haloketone rule is also applicable to cyclohexanones existing in a boat conformation, the rule being same as in the case of chair form.

Kinetically controlled bromination of 2α-methylcholestan-3-one (**V**) or its enol acetate leads to 2-bromo-2-methylcholestan-3-one (**VI**), whose spectral properties [λ_{max} ($CHCl_3$) 5.84 μ, λ_{max} 313 nm] indicate an axial bromine atom [132] (Fig. 2.162). The normally expected 2β-bromoketone in the chair form **VII** should exhibit a strong + CE, contrary to the observed—CE. The only structure which conforms to the—CE is the structure **VIII**, a diastereomer of **VII** with ring A in the boat conformation. Bromination takes place by an axial-like electrophilic attack by Br^+ from the α-face through the skew–boat transition state avoiding the syn-axial interaction with 10β-methyl, to form **VIII**. The latter structure does not flip to the chair form **VIIIa** with equatorial Br which is destabilized by 1,3-dimethyl syn-axial interaction and also by the unfavorable dipole–dipole interaction between the equatorial C–Br bond and the carbonyl group.

2.19.5 The Octant Rule and Its Applications

The octant rule is an empirical generalization relating the sign of the Cotton effect of the carbonyl chromophore of saturated cyclohexanones at around 290 nm with the configuration of the chiral centers present in the neighborhood of the chromophore. The octant rule was first formulated by Mislow et al. [133] and Moffitt et al. [134] in 1961. Consider a carbonyl group (say of a cyclohexanone having a dissymmetric part) lying along the *z*-axis, with its attached groups C2 and C6 lying in plane *yz* (plane B), of a left-handed Cartesian coordinate system of two other mutually perpendicular planes *xy* (plane C) and *xz* (plane A), the midpoint of the CO group being at the origin (Fig. 2.163). These are nodal and symmetry planes of



Note: *The projections of A plane and B plane on xy plane are x-axis and y-axis respectively.*

Fig. 2.163 (**a**) Stereoprojection of the cyclohexanone ring (chair form) in the octant projection diagram (opd) (**b**) Projection of cyclohexanone bonds on the *xy* (or C) plane. View facing the CO oxygen with signs of rear octants (**c**) Sign of contribution of a perturber in rear (or back) octants

the orbitals involved in the relatively weak $n \rightarrow \pi^*$ transition of the carbonyl absorption. Thus, it is expected that the effect of an atom at any point P (x, y, z) in inducing asymmetry in the electronic process associated with the CO absorption (270–310 nm) will be equal in magnitude and opposite in sign to that induced by the same atom situated at the reflection of P through any of the planes A, B, and C and will vanish identically when P lies in any one of these planes (the product xyz being zero) (Fig. 2.163). The effect is characteristic of the perturbing atom and is additive of the other atoms.

The coordinate system divides space around the CO group into eight octants or sectors. The principal premise of the very simple octant rule is that the contribution to the sign of the $n \rightarrow \pi^*$ Cotton effect which a given atom at a point P (x, y, z) makes to anomalous rotary dispersion will be determined by the simple product xyz of its coordinates [(C) in Fig. 2.163].

For example: contribution (to the Cotton effect) of an atom located in the lower right rear (**lrr**) octant (Fig. 2.163), whose coordinates are $-x$, $+y$, $-z$, is positive in a left-handed coordinate system. The atoms located in the mirror image (enantiomorphic) lower left rear (**llr**) octant would induce contribution of negative Cotton effect.

2.19.5.1 Determination of the Preferred Conformation

Example 1. Application of the octant rule allows one to ascertain the preferred conformation of a flexible molecule, if the absolute configuration is known. Let us take the example of (+)-3-methylcyclohexanone, known to have *R* configuration (Fig. 2.164). It can exist in an equatorial (**1e**) or axial (**1a**) conformer. Octant rule projections for equatorial and axial conformers show that they would display a positive CE and a negative CE, respectively. Since it actually exhibits a positive CE, the equatorial conformer predominates, consistent with the principle of conformational analysis. Conversely, if the preferred conformation (equatorial) had been established independently (say by NMR), the absolute configuration would have been determined as *R* (Fig. 2.164).

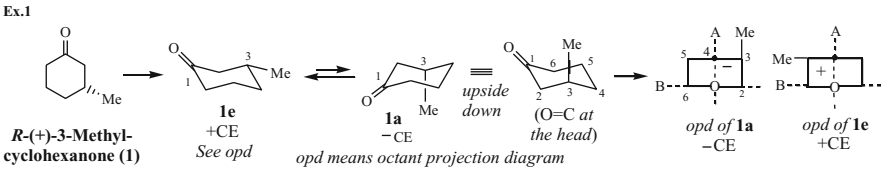


Fig. 2.164 Application of the octant rule to *R*-(+)-2-Methylcyclohexanone

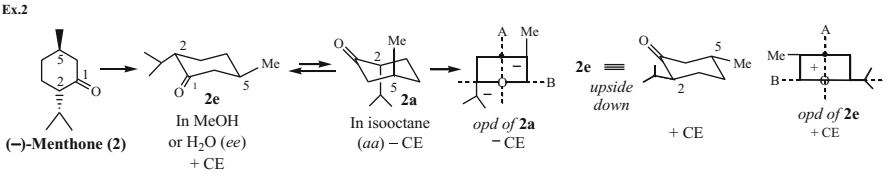


Fig. 2.165 Changes of conformation and CE sign of (−)-menthone with change of solvent polarity in CD spectra

Example 2. ***Conformational mobility of menthone:*** Each conformer of a flexible molecule (−)-menthone like **1** will have its own ORD or CD curve. Change of the conformer population caused by a change of solvent polarity or temperature leads to the change of its ORD or CD curve. (−)-Menthone (**2**) (Fig. 2.165) is a typical example having two conformers **2e** and **2a** in equilibrium. In a solvent of high polarity like water, (−)-menthone exhibits a positive CE (only a peak) in its CD curve due to the predominance of the diequatorial conformer **2e** (probably solvent effect). In methanol (a solvent of moderate polarity) two Cotton effects due to the two conformers appear, one strong positive peak below 300 nm and the other weak negative peak above 300 nm. In isooctane, a nonpolar solvent, a strongly positive CE peak appears at about 300 nm. In solvents of intermediate polarity both the CE's are exhibited in different proportions [135]. Such CD curves having two maxima of opposite signs are called *bisignate* [136]. The diaxial conformer **2a** is stabilized by the synergistic operation of a 2-alkylketone effect in **2a** and 3-alkylketone effect in **2e**, but the function of the polarity is not clear.

That conformational mobility is a function of solvent polarity can be demonstrated by the use of the axial haloketone rule in the following example. Bisignate curves are observed in case of *trans*-2-chloro-5-methyl cyclohexanone (**3**) (Fig. 2.166) obtained from chlorination of *R*-(+)-3-methylcyclohexanone (axial haloketone rule, Sect. 2.19.4.3, Fig. 2.161). The spectral properties of the chloroketone (**3**) indicate that in octane (of much lower polarity) solution it has axial halogen, and the CE in octane is negative establishing its *trans* configuration [137]. The ORD curve in methanol (of much higher polarity) is found to exhibit a positive CE indicating that the conformational equilibrium of the *trans* isomer has changed from the diaxial to the diequatorial form, presumably because in methanol of high dielectric constant the dipole repulsion between carbonyl and adjacent equatorial chlorine is not as serious as in octane of much lower dielectric constant.

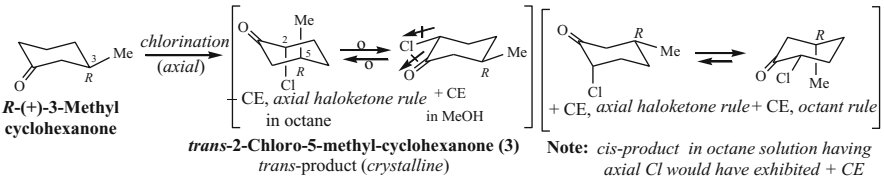
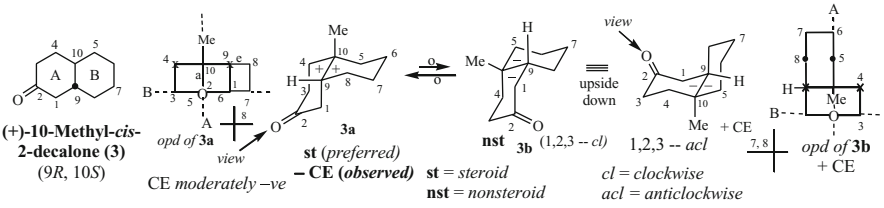


Fig. 2.166 Conformational mobility of *trans*-2-chloro-5-methylcyclohexanone in octane and methanol



Notes: *1) × Atoms are symmetrically disposed: contributions cancel*
2) Atoms in plane A or B: no contribution

Fig. 2.167 Preferred steroid conformation of (**3**) by application of octant rule

Example 3. ***Preferred conformation of cis-decalone derivatives*** can be determined by application of octant rule. (−)-*cis*-10-Methyl-2-decalone (**3**) may exist in steroid (**3a**) and/or nonsteroid (**3b**) conformation/s (Fig. 2.167). By the application of the octant rule, one finds that the steroid form (**3a**) should show a negative CE while the nonsteroid form (**3b**) a positive CE. RD study shows that the compound **3** exhibits a negative CE and hence should have the preferred steroid conformation. Here also the absolute configuration of **3** being known, the preferred conformation can be deduced. The ORD curve of **3** resembles that of coprostan-3-one having rings A/B in rigid steroid conformation (*vide* **2.19.5.3**); for further detailed study see [138].

2.19.5.2 Determination of Absolute Configuration of *trans*-Decalones

A *trans*-decalone of known structure possesses a unique rigid conformation; its absolute configuration can be determined from the sign of the Cotton effect it exhibits in its ORD spectrum, by application of the octant rule. Thus, the absolute configuration of (+)-*trans*-10-methyl-2-decalone can be determined as **4** by its ORD study which shows a positive CE. The mirror image configuration (**4′**) would exhibit a negative CE (Fig. 2.168); for further details see [138].

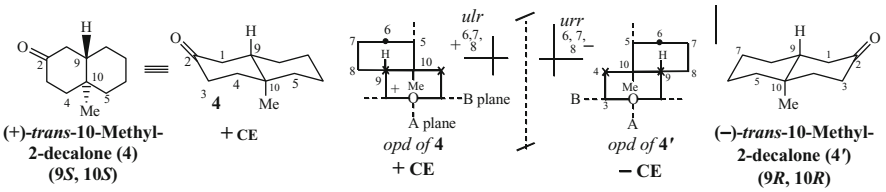


Fig. 2.168 Absolute configuration of (+)- and (−)-*trans*-10-methyl-2-decalone by application of the octant rule

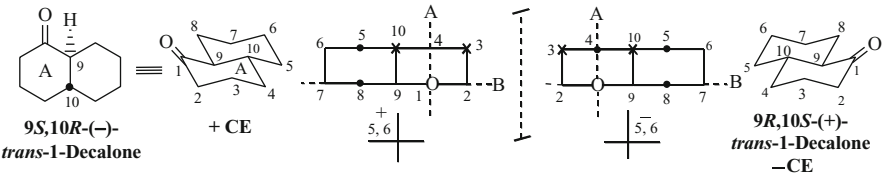


Fig. 2.169 CE signs of *trans*-1-decalone enantiomers by application of the octant rule

Prediction of the CE sign by application of the octant rule. If the structure and absolute configuration of a rigid molecule having a fixed conformation are known, the sign of the CE to be exhibited by it can be predicted by application of the octant rule. Conversely, the absolute configuration of such a molecule with known structure, conformation (fixed), and sign of the CE (by experiment) can be deduced by application of the octant rule. One more example is given in Fig. 2.169. For further details see [138].

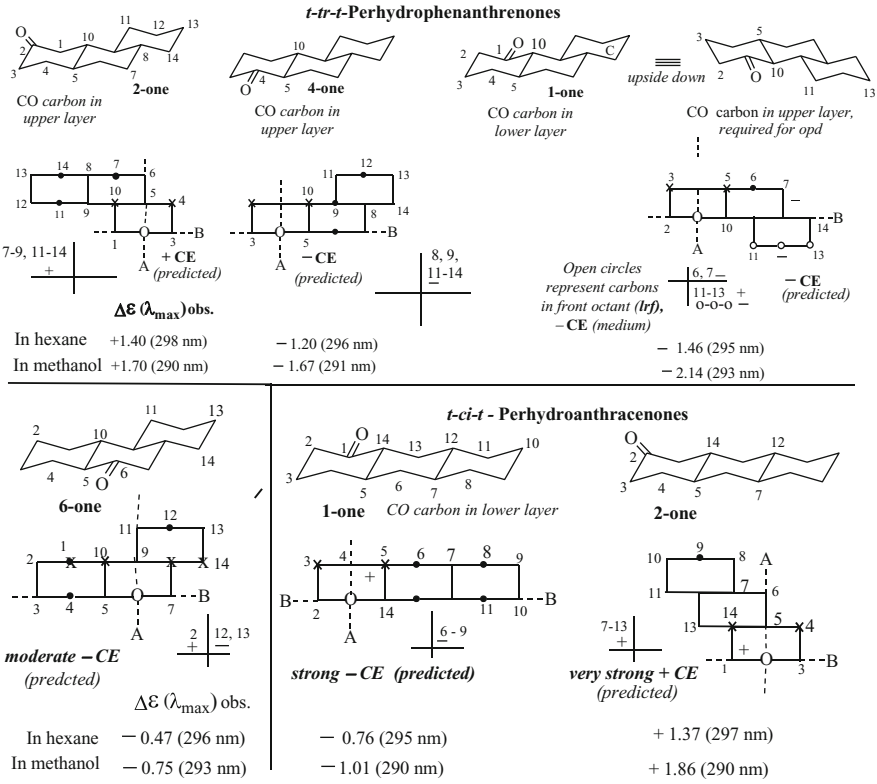
2.19.5.3 Tricyclic Ketones: Perhydrophenanthrenones and Perhydroanthracenones

Only a few *trans-transoid-trans* (***t-tr-t***) perhydrophenanthrenones and *trans-cisoid-trans* (***t-ci-t***) perhydroanthracenones have been studied by CD spectroscopy [138, 139] (see Sects. 2.15.2 and 2.15.3 for nomenclature). Their octant projection diagrams (*opd*) are shown in Fig. 2.170. Steroid numbering has been used for convenience of comparison with corresponding steroid ketones (instead of the usual numbering). All compounds were found to obey the octant rule. The observed Cotton effect sign is same as predicted by octant rule in each case. In *opd* the carbon atoms in a front octant become evident from a watchful examination of Dreiding models.

No other diastereomeric ketones with different ring junction stereochemistry (*cf.* Sects. 2.15.2 and 2.15.3 have yet been investigated by CD; and *this is a potentially rich source for future research*.

2.19.5.4 Tetracyclic Ketones: Steroids

The perhydrophenanthrene skeleton with ***t-tr-t*** and ***c-tr-t*** stereochemistry is found in most steroids, *viz*., cholestane and coprostane derivatives, respectively. Innumerable ORD and CD spectra recorded on such steroid ketones during late 1950s and 1960s formed the basis of the octant rule [125, 133, 134, 140, 141]. Rotatory dispersions of innumerable available steroid ketones and triterpene ketones have been compiled by Crabbe [142] and Kirk et al. [143–145].

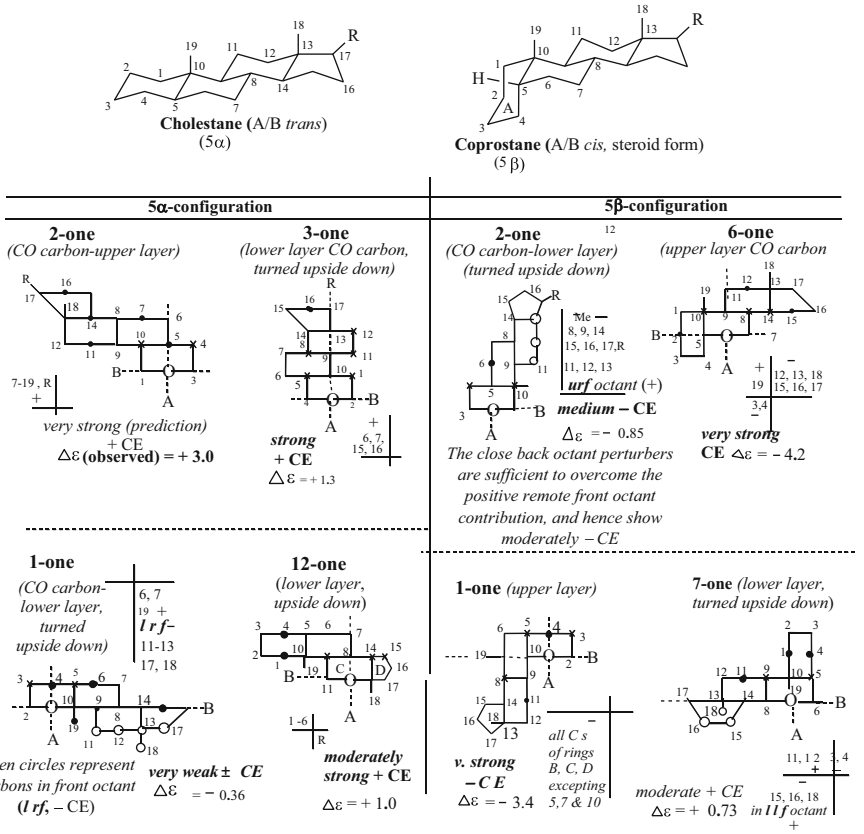


Notes: (i) *The skeletal carbons which are in mirror image positions with respect to A or B plane cancel.*
(ii) *The sign and magnitude of the amplitude of the n□* CD Cotton effect depend upon the contribution of the uncancelled perturbing atoms and their distances from the carbonyl chromophore.*
(iii) *The skeletal carbons which lie on octant planes have no contribution to CE*
(iv) *The skeletal carbons close to the C=O group have strong contributions.*
(v) *Open circles represent carbons in front octant.*

Fig. 2.170 CE signs from octant projection diagrams of perhydrophenanthrenones and Perhydroanthracenones. [Adapted with permission from David A. Lightner and Jerome E. Gurst, *Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy*, Copyright © 2000 by John Wiley & Sons, Fig. 8–2, p. 234.]

Observed $n \rightarrow \pi^*$ Cotton effects of some steroid ketones from ORD and CD spectroscopy, which were consistent with the predicted Cotton effects based on octant projection diagrams (*opd*s), are shown in Fig. 2.171 [146]).

The octant rule correctly predicts the signs as well as the magnitudes of cholestan-1-one (very weakly negative CE), -2-one (very strongly positive CE), -3-one (strongly positive CE), -12-one (moderately strong positive CE), and of coprostane-1-one (very strongly negative CE), -2-one (moderately strong negative CE), -6-one (very strongly negative CE), and −7-one (moderately strong positive



Notes:
- *The skeletal carbons which are in mirror image positions with respect to A or B planes cancel.*
- *The skeletal carbons which lie on octant planes have no contribution to CE.*
- *The skeletal carbons close to the C=O group have strong contributions (adjacent axial position has strongest contribution)*
- *The skeletal carbons remote from CO group are weak contributors.*
- *Open circles represent carbons in front octant.*

Fig. 2.171 Octant projection diagrams and observed/predicted signs of the CE of some cholestanones and coprostanones. [Adapted with permission from David A. Lightner and Jerome E. Gurst, *Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy*, Copyright © 2000 by John Wiley & Sons, Table 8.3, pp. 237–238.]

CE), based on their octet projection diagrams (*opd*s), as delineated in Fig. 2.171 [146]. The carbons in a front octant can presumably be ascertained by carefully projecting a Dreiding model of the molecule in a plane (*xy*) orthogonal to the line of view (*z*-axis) through O=C bond. The ketosteroids showing front octant contributions may complicate the projection as well as prediction of the CE sign. The 15, 16, and 17 ketosteroids, with 5α- and 5β- configurations, which have also been studied, make the projections complicated, but prediction of CE sign has still been correct excepting in case of coprostan-17-one [146]. In making accurate predictions, magnitudes of the individual contributions of the atoms, which depend on their positions as well as distances from the chromophore, should also be estimated from their *opd*s.

2.19.6 Helicity Rule or Chirality Rule

In Sect. 2.19.3 we have mentioned that Cotton effects have been studied in two types of chromophores, inherently achiral and inherently chiral. Molecules consisting of both these types of chromophores have also been treated with success. Inherently chiral chromophores like skewed dienes, unsaturated ketones biaryl atropisomers, and helicenes exhibit much stronger CE than the inherently achiral chromophores present in the molecules. It has been derived theoretically and demonstrated experimentally that the direction of the Cotton effect depends upon the sense of helicity of the inherently chiral chromophores producing a positive effect. Inherently chiral chromophores follow the *helicity rule* or *chirality rule* which states that *P*-helicity (a right-handed helix) or positive chirality gives rise to positive optical rotation and positive CE, and *M*-helicity or negative chirality is correlated with negative optical rotation and negative CE (also see Sect. 2.19.63, last but 2 sentences). Some examples illustrating the helicity rule are shown in Fig. 2.172.

2.19.6.1 Conjugated Dienes and Enones: Steroids

R-α-Phellandrene (**1**) (Fig. 2.172) shows negative CE at room temperature and above. However, on reducing the temperature the magnitude of CE decreases, becomes zero, and down to −160 °C becomes positive [147]. The greater stability of the pseudoaxial conformer (**1a**) at room temperature may be due to higher entropy caused by the free rotation of the pseudoaxial isopropyl group relative to that of the pseudoequatorial conformer (**1e**). This isopropyl group in the latter may be facing some restriction to rotate freely. This thermodynamically preferred conformer is present at low temperature [147, 148].

The contribution of a chiral twisted diene or enone is so high that it usually overrides the contributions of other dissymmetric parts or the chiral center/s present. The helicity rule is applied to the lowest energy $\pi \rightarrow \pi^*$ transition in dienes (around 230–260 nm). The ORD curves of a number of s-*cis* (cisoid)

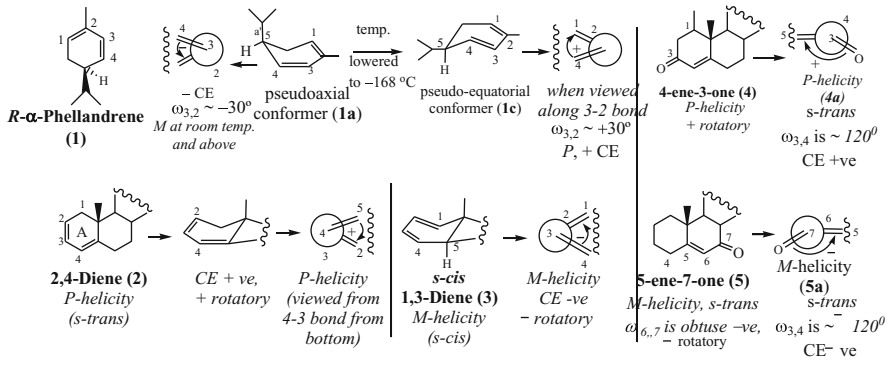


Fig. 2.172 Helicity rule applied to α-phellandrene and steroidal conjugated dienes and enones

2,4-dienes, *e.g.*, (**2**) and 1,3-dienes, *e.g.*, (**3**) (Fig. 2.172) derived from steroids show a strong positive CE and a strong negative CE, suggesting *P*-helicity and *M*-helicity respectively, the two double bonds being at acute torsion angles ($+\omega$ or $-\omega < 60°$). The helicities may be confirmed from the acute torsion angles of the diene portions (Fig. 2.172), as evident from their Dreiding models.

The helicity rule has also been applied to α,β-unsaturated ketones (**4**) and (**5**) (Fig. 2.172), derived from steroids. Such enones are in *transoid* or *s-trans* conformation. Cholest-4-en-3-one (**4**) (in hexane) shows a positive CE at ≈250 nm whereas choles-5-en-7-one (**5**) shows a negatine CE (at ≈250 nm), confirming *P-helicity* and *M*-helicity, respectively [149]. The helicities can be confirmed by the obtuse positive and negative torsion angles (ω) between C=C and C=O bonds, respectively, from a careful examination by Dreiding models.

The homoconjugated enone (C=C.CH_2.C=O) rule has been shown to be compatible with the ketone octant rule, with C=C considered as a substituent, dominating the sign of the CE (generalized octant rule) [150].

2.19.6.2 Biaryl Atropisomers and Helicenes

According to helicity rule the biaryl atropisomers and helicenes having *P*-configuration are expected to show positive rotation and positive CE in their CD spectra, while those with *M*-configuration negative rotation and negative CE. Thus, the biphenyl derivative **B** (new convention) and 1,1′-binaphthyl (**C**) and 8,8′-dicarboxy-1,1′-binaphthyl (**D**) (see Fig. 2.151) possessing *M*-configuration are expected to exhibit negative CE and to be levorotatory, while the biphenyl derivative **A** (see Fig. 2.151) of *P*-configuration (new convention) to exhibit positive CE and be dextrorotatory.

Likewise, the helicenes (**A**), (**D**), and (**E**) of *P*-helicity (see Fig. 2.154) are expected to show positive CE and be dextrorotatory, while the helicenes (**B**) and (**C**) of M-helicity (see Fig. 2.154) are expected to show negative CE and be levorotatory. Helicenes are characterized by high optical rotation.

The absolute configuration of the helicenes can be predicted from chiroptical data only after careful assignment of the transition. In cases of helical molecules during excitation, electron movement takes place in a helical manner. For example, hexahelicene, wherein the electron transition moment direction is identical for either enantiomer, the magnetic moment direction is reversed according to whether the helix is *P* (right handed) or *M* (left handed). The parallel magnetic transition moments for *P*-hexahelicene generate a positive CE, whereas the antiparallel magnetic transition moments for *M*-hexahelicene lead to a negative CE which refers to the longest wavelength of high intensity at about 325 nm, presumably corresponding to the π–π* transition between HOMO and LUMO [126, 151, 152, p. 1012] and [153, p 69].

Thus, the absolute configuration of chiral biphenyls, biaryls, helicenes, and substituted helicenes, determined by CD studies from their observed CE signs, should be confirmed by X-ray or some possible unambiguous method.

Theoretical treatment by Mason et al. [153, 154] gives a more precise correlation of the CD spectra and absolute configuration of certain biaryls; the CD spectra were shown to be a function of the dihedral angle between the two aryl ring planes. 1,1′-Binaphthyl was unambiguously assigned to possess the critical interplanar dihedral angle of 100–110° [153].

2.19.6.3 Correlation of Optical Rotation with Ligand Polarizability: Brewster's rule

Attempts have been made by many scientists to provide a theoretical basis of optical activity during three decades since 1930. It is known that optical rotation at sodium D-line (589 nm) originates from the long wavelength tail of one or more UV Cotton effect/s. Optical rotation at different wavelengths arises because chiral compounds are circularly birefringent. The refractive index is related to the polarizability of ligands (atoms or groups) attached to the asymmetric center/s. Polarizability causes the sensitivity of the ligands to deformation by electric fields and to their relative positions.

In 1959 Brewster formulated a useful model of optical activity of open chain compounds [155] and of saturated cyclic compounds [156]. He suggested that a center of optical activity can usefully be described as a screw pattern of electron polarizability. Brewster approached to calculate the sign and magnitude of optical rotation based on two independent components: (i) contribution to the rotation by the difference in polarizability of atoms or groups attached to asymmetric atoms known as *atomic asymmetry* (or local chirality) and (ii) contribution from conformational dissymmetry (or chiral conformations). Both components lead to chiral screw pattern of polarizability and contribute to rotation in flexible molecules.

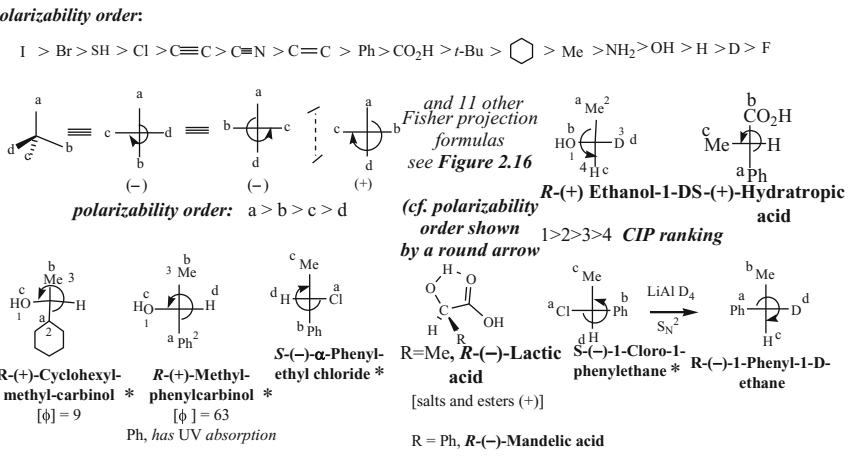
Atomic asymmetry refers to a chiral model (Fig. 2.173) Cabcd, where the ligands a-d are atoms or small groups having average cylindrical or conical symmetry. Such a compound is dextrorotatory, if the polarizability order is a > b > c > d, and the order a > b > c is clockwise, placing "d" at the rear of the line of view [*cf. R,S*

nomenclatures, see Sect. 2.6.2.2, (i) and (ii) with corollary (1), priority sequence order being replaced by polarizability order]; the compound will be levorotatory if the polarizability order is anticlockwise (Fig. 2.173). It is difficult to measure the magnitude of rotation. The contribution of the atomic asymmetry component is small especially when the polarizabilities of two attached atoms or groups do not differ much. The polarizability of an atom or group is derived from the atomic refraction [157]. The order of polarizabilities of several common atoms or group, derived from the atomic refraction [157], is given in Fig. 2.173. The atomic refractions of the attached atoms in C≡C and C=C are taken as half the value of the group refraction.

In case of CN, C_6H_5, and CO_2H a complicated share of the group refraction is assigned to the attached carbon. Polarizability is affected by the nature of the attachment atoms, e.g., NH_2 and OH, when they are α- to a phenyl group, are to be ranked just after the chlorine atom, ahead of groups whose attachment atom is carbon. A conformational dissymmetry contribution to the rotation takes place by intramolecular hydrogen bonding, e.g., lactic acid (Fig. 2.173) or so*me* other type of intramolecular interaction.

If the aforesaid two types of contribution to rotation predict the same sense of rotation, the configuration may be determined accurately. However, if the two components contribute opposite senses of rotation the model may lead to ambiguous results. The usual low contribution of atomic asymmetry to optical rotation is significantly increased, when one or more ligands absorb in UV region, e.g., phenylmethylcarbinol or mandelic acid (Fig. 2.173).

Brewster [155, 156, 158, 159] has developed an empirical approach to the contribution of *conformational dissymmetry* to the sign and approximate magnitude



Note: *The asterisked compounds possess the same sense of polarizability order and the priority sequence order.*

Fig. 2.173 Prediction of the sign of optical rotation in compounds with one chiral center and known absolute configuration and exhibiting atomic asymmetry (Brewster's rule)

of optical rotation which is beyond the scope of the present treatise. Based on major theoretical models of optical activity Brewster concluded [156] that as a general rule a system in which electrons are constrained to right-handed helical path will give a positive Cotton effect and will be dextrorotatory at long wavelength (*cf.* helicity rule, Fig. 2.172).

Atomic asymmetry component has been illustrated by several examples in Fig. 2.173. The absolute configuration of monochiral compounds of known structure can also be determined from the sign of optical rotation.

2.19.6.4 Absolute Configuration of Chiral Allenes: Lowe's Rule

During 1959–1965 the absolute configurations of eight chiral allenes have been determined by either the conversion of an optically active molecule of known absolute configuration into an allene or by converting a dissymmetric allene into a molecule of known absolute configuration by stereochemically unambiguous reaction [160]. Three such allenes (**1**), (**2**), and (**3**) are shown in Fig. 2.174.

Brewster's idea [155] of describing a chiral center as an asymmetric screw pattern of polarizability has been utilized by Lowe to put forward an empirical rule [160] for the absolute configuration of a chiral allene from the sign of its optical rotation at sodium D-line. Lowe's rule can be stated in a clarified general way as follows:

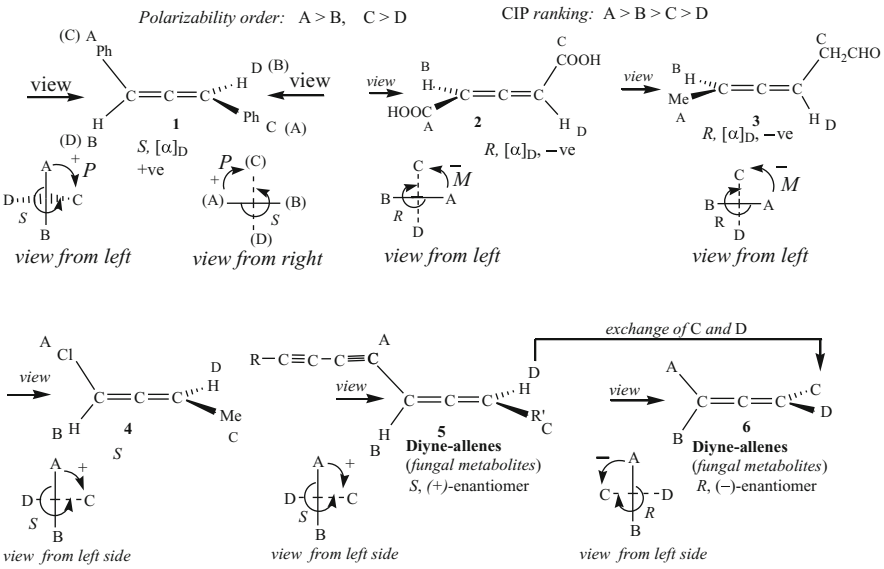


Fig. 2.174 Absolute configuration of allenes of known sign of $[\alpha]_D$ by Lowe's rule

The allene is viewed (as in case of nomenclature of all axially dissymmetric compounds, see Sect. 2.16) along its chiral (molecular) axis to get the ligands A and B (A being more polarizable than B) of the front carbon attached either at the vertical axis or horizontal axis and the ligands C and D (C being more polarizable than D) of the back carbon (attached at the axis orthogonal to that of the front carbon). If the ligand C appears at a clockwise direction with respect to the ligand A, the handedness of the screw pattern of polarizability is also clockwise, the enantiomer should be dextrorotatory at the sodium D-line; if A to C makes an anticlockwise turn, the enantiomer is levorotatory. Levorotation thus results from an anticlockwise screw pattern of polarizability. The rule, *which holds well if any of the ligands A–D in an allene does not exhibit conformational dissymmetry*, is illustrated for allenes **1**–**6** in Fig. 2.174.

Most of the naturally occurring allenes are fungal metabolites. All the dissymmetric fungal allenes contain a rigid diyne-allene system and are represented by the general formulas **5** and **6**. For those metabolites where the group R does not introduce conformational dissymmetry, Lowe's rule predicts that the (+)-enantiomers have the *S*-configuration **5,** and the (−)-enantiomers have the *R*-configuration **6**.

It seems that Lowe's rule may be extended to other axially dissymmetric compounds having similar geometry of the ligands of the end carbon; thus, the absolute configurations of a spiran (with both the double bonds of allene replaced by rings) and of an alkylidenecycloalkane (with one double bond of allene replaced by a ring) should be predictable correctly from their observed sign of $[\alpha]_D$.

2.19.6.5 The Exciton Chirality Method or The Dibenzoate Chirality Rule

As we know, in the UV-visible absorption, an electron moves from an occupied molecular orbital to a higher energy unoccupied molecular orbital, *viz.,* in $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ transition. The movement of an electron causes a dipole or polarization charge known as *electric dipole transition moment*. When two chromophores are in spatial proximity and are so oriented that a chiral array results, and when one chromophore is excited in the CD spectrum, the chromophores may interact with each other to generate distinctive Cotton effect (CE) couplets, known as *exciton coupling*, and the CD spectrum appears in a typical *bisignate* form. This allows the determination of the configuration of the *chiral array*. The term exciton was coined by Davydov in 1962 in connection with his theory of molecular excitons [161]. The energy difference between the common excited states is called *Davydov splitting*, which gives rise to $\Delta\lambda$, the wavelength difference between the two maxima of opposite signs (see Fig. 2.175b).

Harada and Nakanishi in their *benzoate sector rule* [162] utilized the Cotton effect at 225 nm ($\Delta\varepsilon \approx 3.5$) due to π–π^* intramolecular charge transfer band (230 nm, ε 14,000) of the benzoate chromophore. They extended this rule to *the dibenzoate chirality rule* [163], which correlates the chirality of glycols with signs

of the intense and split (bisignate) π–π^* Cotton effects of their dibenzoate derivatives. They later proposed to call this rule as *the exciton chirality method* [164]. The following characteristic features of the exciton chirality method are to be noted:

1. In a typical two-chromophore system (bisaromatic ester) a pair of CD bands (maxima) appears, one band at a longer and the other with opposite sign at a shorter wavelength relative to the absorption wavelength of the monomeric chromophore: thus the CD is bisignate.
2. Davydov splitting causes the appearance of the second CE at a shorter wavelength, the sign of which is opposite of the first CE. Together they resemble an ORD curve.
3. The electric dipole transition moment of each aromatic ester group is oriented collinearly with its long axis (Fig. 2.175a).
4. The sign of the CE at the longer wavelength corresponds to the helicity rule, discussed earlier. The right-handed screw pattern or the positive exciton chirality of the chiral array gives a positive CE, and the left handed screw pattern or negative chirality gives a negative CE.
5. The conformations of the bisbenzoates or bisaromatic esters of vicinal or non-vicinal diols if known, the *chiral array* between the individual electric transition moments (μ) aligned approximately parallel to the alcoholic C–O bonds of the ester groups (Fig. 2.175) will also be known. The dihedral angle (ω) between the two transition moments therefore corresponds to that between the two C–O bonds.
6. For glycol benzoate chromophores the amplitude of the exciton coupling is maximal when the dihedral angle ω between the electric dipole transition moments is about 70° and is zero when $\omega \approx 0°$ or 180° [165].
7. CE couplet should be away from other strong CE bands. Substitution in the benzoate ring especially by a *p*-dimethylamino- or a *p*-methoxycinnamate group displaces the exciton couplet to longer wavelengths ≈311 nm. The *p*-dimethylaminocinnamate group that absorbs at even longer wavelengths (≈361 nm) may also be used.
8. Chromophores with more intense electric dipole transition moments (like a porphyrin chromophore) may be used in order to magnify the CE intensities in long range interactions (*cf.* 5α-steroids **8**–**10** of Fig. 2.175) [166].

2.19.6.6 Absolute Configuration of the 5α-Steroid Diols by Exciton Chirality Method

The benzoate chromophore shows two $\pi \rightarrow \pi^*$ intramolecular charge transfer transitions in the UV region: 280 (ε 1,000) and 230 nm (ε 14,000). These absorption bands have the transition moments along the short axis and the long axis of the benzoate chromophore, respectively. The CD spectrum of 2α,3β-dibenzoyloxy-5α-cholestane (**1**) (Fig. 2.175) like that of glycol dibenzoate gives rise to two strong CEs of similar amplitude but of opposite signs around 233 nm (first CE) and 219 nm (second CE) [163]. The resulting splitting indicates that the two CEs are mainly due

to a dipole–dipole interaction between the electric transition moments of the intramolecular charge transfer band of the two benzoate chromophores, and that the CEs are separated from each other by $\Delta\lambda = 15$ nm due to *Davidov splitting* [161]. Compound (**1**) has a negative chirality of the two OH groups, and in accordance with the *exciton chirality rule* the sign of the first CE is also negative ($\Delta\varepsilon_{234} = -13.9$; $\Delta\varepsilon_{219} = 14.6$) (Fig. 2.175) [163, 164]. As expected, since 2β,3-β-dibenzoyloxy-5α-cholestane (**2**) possesses positive chirality of the two C–OH bonds, the sign of the first CE is positive [164] (Fig. 2.175).

Most 5α-cholestane-diols, whether with vicinal hydroxyls and distant hydroxyls, derivatized as *p*-dimethylaminobenzoates [compounds (**3**) to (**10**)] [166] give bisignate CEs in their CD, originating from exciton coupling and consistent with the exciton chirality rule (Fig. 2.175). In all the examples there is excellent correlation between the observed CEs in the CDs of steroids of known absolute configuration and the predicted positive or negative exciton chirality. The magnitude of the exciton CD Cotton effects decreases with increase in distance between the chromophores. Even in steroid-3,15-diol or 3,17-diol in which the chromophores are, respectively, more than 13 Å or 16 Å apart, quite appreciable Δε values of expected CEs are observed [166].

2.19.6.7 Absolute Configuration of *trans*-Cyclohexane-1,2-diol Enantiomers

The proximity, orientation, and the nature of the chromophores are important in exciton chirality rule. Thus, the CD spectra of bis-*p*-dimethylaminobenzoates of 5α-cholestan-2α,3β-diol and 1*R*,2*R*-*trans*-cyclohexanediol (**11**) (Fig. 2.175), both having the same absolute configuration, and vicinal diequatorial orientation are essentially identical with some difference in $\Delta\varepsilon$ values. The CD spectrum of the 2α,3β-dibenzoate derivative (**1**) has same type of negative exciton chirality (EC) with different $\Delta\varepsilon$ values (but with almost identical $\Delta\varepsilon$ values also of the bis-*p*-dimethylaminobenzoate esters in both cases). The diester of 1*S*,2*S*-*trans*-cyclohexanediol (**12**) possesses positive EC and shows bisignate CE with the first CE positive and the $\Delta\varepsilon$ values equal in magnitude but opposite in sign of (**11**), as expected, the nature of the EC of **(12)** being same as that of the 3β,4α diester **(5)** (Fig. 2.175), showing some difference in $\Delta\varepsilon$ values.

2.19.6.8 Prediction of the First CE Signs of Vicinal and Non-vicinal Dihydroxy-5α-Steroid Diesters

The sign of the first CE exhibited by suitable aromatic diesters of the following 5α-steroids containing two vicinal or non-vicinal OH groups of known absolute configuration (different from those mentioned in Fig. 2.175) *can be predicted* from the sign of the exciton chirality (EC) of each, as is evident from careful

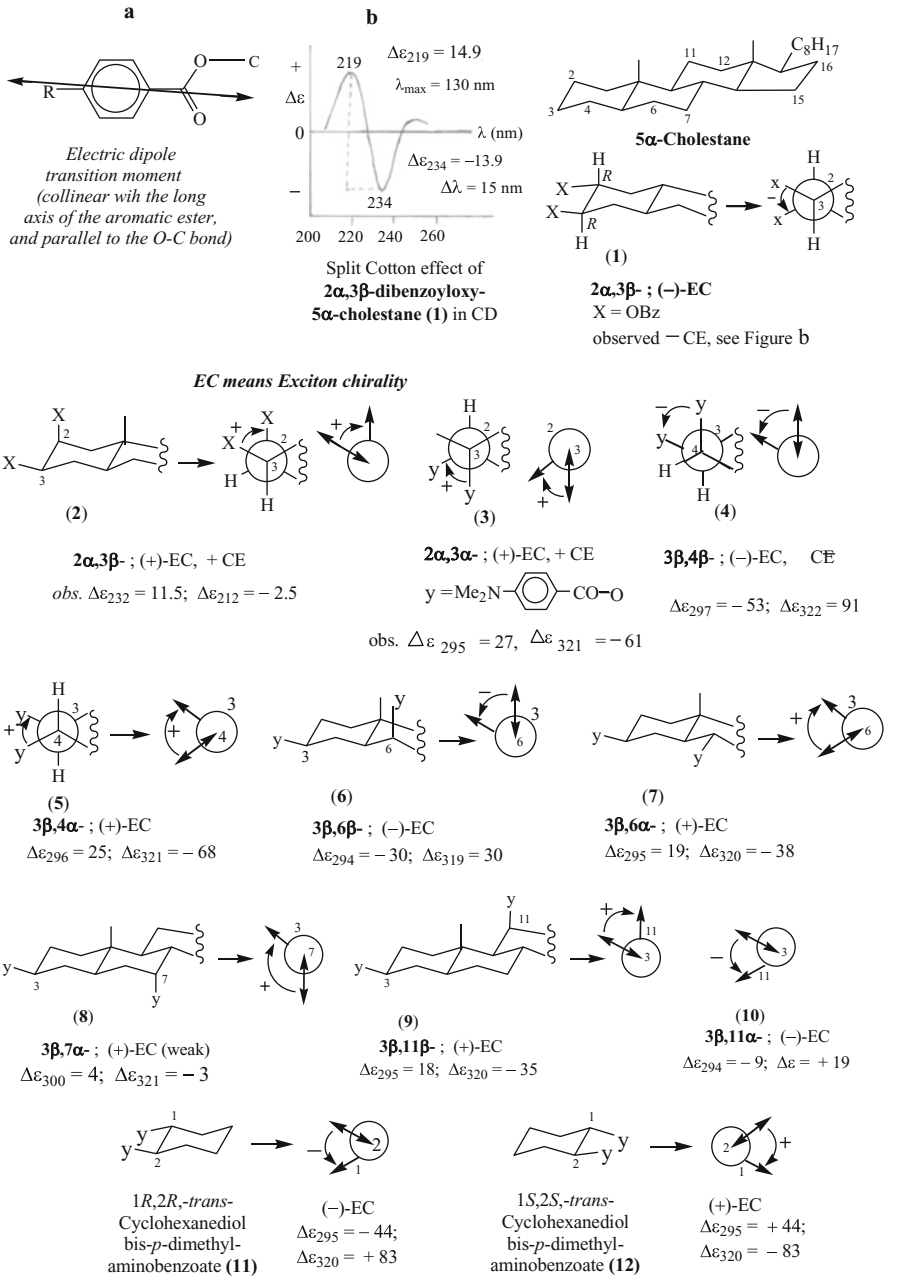


Fig. 2.175 Predicted and observed exciton chirality of bisbenzoyloxychotestane or bis-*p*-dimethylamino-benzoyloxycholestane derivatives and also of (1*R*,2*R*)- and (1*S*,2*S*)-*trans*-cyclohexanediol. [Adapted with permission from David A. Lightner and Jerome E. Gurst, *Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy*, Copyright © 2000 by John Wiley & Sons, the data ($\Delta\varepsilon$ values) of compounds (**3**) to (**10**)from the Table 14.3 (p. 436) and of compounds (**11**) and (**12**) from the Table 14.2 (p.433).]

1β, 2α (*ee*), (+ EC), (+)	6α, 7α (*ea*), (+ EC), (+)	1α, 11α (*ae*), (+ EC), (+)
1α, 2α (*ae*), (– EC), (–)	6α, 7β (*ee*), (– EC), (–)	1β, 11α (*ee*), (– EC), (–)
1β, 2β (*ea*), (– EC), (–)	7β, 8β (*ea*), (– EC), (–)	4α, 7α (*ea*), (+ EC), (+)
3α, 4α (*ae*), (– EC), (–)	11α, 12α (*ea*), (– EC), (–)	4α, 7β (*ee*), (– EC), (–)
4α, 5α (*ea*), (+ EC), (+)	11α, 12β (*ee*), (+ EC), (+)	7β, 14α (*ea*), (+ EC), (+)
5α, 6α (*ae*), (– EC), (–)	11β, 12β (*ae*), (– EC), (–)	9α, 11α (*ae*), (+ EC), (+)

Fig. 2.176 Predicted sign of the first CE of possible vicinal and non-vicinal 5α-steroiddiol bisaromatic esters from the sign of the observed EC (by proper examination of their Dreiding models)

examinations of their Dreiding models. The *a* or *e* orientations of the ester groups, the EC sign and *the predicted sign* of the first CE to be observed in the CD spectrum are shown in successive parentheses for each 5α-cholestanediol diester with the locations and α/β orientations of the ester groups in Fig. 2.176.

The *bichromophoric exciton chirality method* utilizing two different types of exciton chromophores together, which have been selectively introduced at two different types of hydroxyls, has been applied for determining the relative and absolute configurations in acyclic 1,2,3-triols [167].

For more details of CD and other applications of CD, see [166] and [168].

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Chapter 3
Important Biological Events Occurring in Plants

3.1 Photosynthesis

"Photosynthesis is an integrated system in which light harvesting, photo-induced charge separation, and catalysis combine to carry out two thermodynamically demanding processes, the oxidation of water and reduction of carbon dioxide."

- A part of the comment that appeared on the cover of the *Journal of Organic Chemistry,* **2006**, *71, issue no. 14,* (July 7, 2006).

3.1.1 Light Reaction: Formation of NADPH, ATP, and O_2

"Photosynthesis is undoubtedly the most important single metabolic innovation in the history of life on the planet" [1]. It is looked upon as the mother of all biological events of life since essentially all free energy needed for biological systems originates from solar energy *via photosynthesis,* the most successful of all natural energy storage processes. Thus, all forms of life evolved to exploit oxygen for their well-being depend for their energy, directly or indirectly on this phenomenon, which truly belongs to an interdisciplinary field involving radiation physics, solid state physics, chemistry, enzymology, physiology, and ecology. The present discussion is mainly based on chemistry. Photosynthesis is a green plant process, the means of converting light into chemical energy, taking place into organelles called chloroplasts. In this process chlorophyll, the green coloring matter of plants utilizes the solar energy in converting CO_2 and H_2O into carbohydrates, and oxygen and some water are evolved. Bacteria with bacteriochlorophyll can also achieve photosynthesis—bacteria do not evolve oxygen like green plants [2]. Since oxygen is a by-product of the green plant photosynthesis, the latter is sometimes called *oxygenic photosynthesis,* and it is the major source of oxygen in the atmosphere. The chemistry of the green plant or green algae photosynthesis will be discussed briefly. The plant photosynthesis utilizes the solar light. The bacterial photosynthesis utilizes the light in the infrared region. However, photons of far-infrared region

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have too low energies to be utilized for any photochemical reaction. The splitting regions (approximately) of white sunlight, as seen after diffraction through prism or in rainbows (VIBGYOR), are shown in Fig. 3.1.

When white sunlight falls on **chlorophyll**, it absorbs light waves of all the wave lengths except at the region ~480–550 nm; the region is attributed to green color of the VIBGYOR. Because of the nonabsorption and reflection of light of the green region, chlorophyll is green in color and hence the leaves are green. Richard Willstätter (1872–1942) (Appendix A, A30) won the 1915 Nobel Prize for elaborating the structure of chlorophyll. There are two major types of chlorophyll: chlorophyll-*a* (**Chl-a**) and chlorophyll-*b* (**Chl-b**) having the same structural scaffold, differing only in the ring B in having a –CHO group in **Chl-b** in place of a CH_3 group in **Chl-a** (Fig. 3.2). This small change in the substituent causes a difference in energy absorption. The basic structure is named porphyrin possessing a tetrapyrrole skeleton, which contains an 18-membered ring with nine conjugated double bonds. The basic skeleton is thus aromatic and has its two canonical (resonance) forms contributing equally to their resonance hybrid. In Fig. 3.2, one canonical form is shown. However, like aromatic compounds either canonical form is used in the literature to represent the basic skeleton of the compounds shown in Fig. 3.2. Mg^{2+} is present at the center and is covalently bonded with two N atoms, and coordinately bonded to the other two N atoms of the porphyrin. **Chl-a** and **Chl-b**, thus called

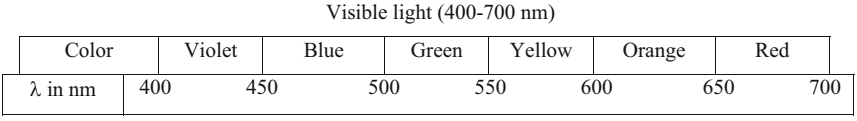


Fig. 3.1 The electromagnetic radiation in the visible spectrum

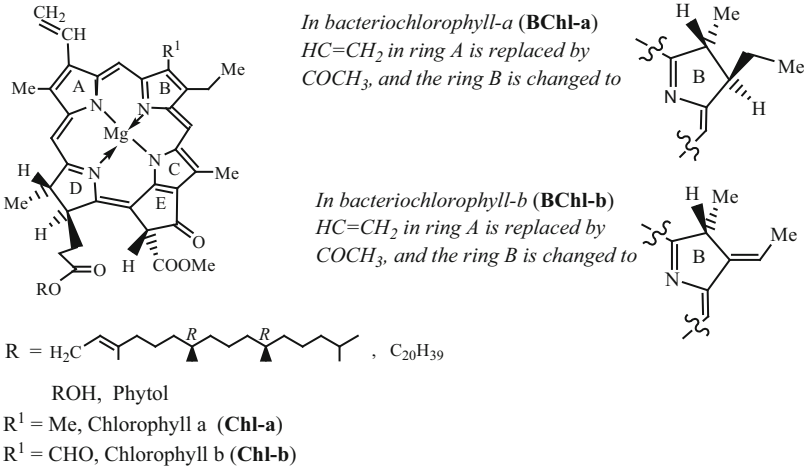


Fig. 3.2 Stereostructures of **Chl-a**, **Chl-b**, **BChl-a** and **BChl-b**

magnesium porphyrins, are present in plants in the ratio 3:1. This ratio as well as their structures has remained the same during evolution, a very amazing feat of Nature. **Chl-a** does not absorb light in a wide range of the visible spectrum. This nonabsorbing region is known as the "green window". The first absorption maxima of **Chl-b** and **Chl-a** are around 460 nm and 430 nm, respectively, and their second maxima are around 650 nm and 700 nm, respectively. **Chl-b** can transfer light energy very efficiently to **Chl-a**. Thus, the absorption gap of **Chl-a** green window is narrowed by the light absorption of **Chl-b**. Hence, the latter increases the efficiency of plants for utilizing sunlight energy.

The antenna protein containing many chromophores absorbs light. **Chl-a** is the central pigment of the plant photosynthesis. The trapped electromagnetic radiation of the sun causes the flow of energy within the photochemical apparatus containing specialized reaction centers, allowing the trapped energy to participate in a series of biochemical reactions. Because of the presence of a number of conjugated double bonds in the chlorophyll skeleton, much less amount of energy (photon of red light) is required to cause excitation of electrons to the lower energy first singlet state (half-life 4×10^{-9} s). The second singlet state requires higher energy (photon of blue light) and is of short half-life (10^{-12} s) to effect chemical reactions. Excited chlorophyll as such directly cannot transfer the energy to the right location. It initiates electron-transfer chain through electron acceptors like *quinones*, *cytochromes,* etc. resulting in a charge separation. In effect, the first singlet state splits water molecule into H and OH radicals. The OH radicals yield some oxygen and some water, while H reduces the oxidized coenzyme **NADP**$^{\oplus}$ to **NADPH** (catalyzed by **ferredoxin** NADP$^{\oplus}$ reductase, a **flavoprotein** with an **FAD** prosthetic group). The excess energy is stored as an energy-rich **ATP**, acting as a temporary source of energy. Its terminal γ-phosphoanhydride bond is hydrolyzed to yield **ADP** with the release of energy to be used in the biochemical processes, which are especially energetically unfavorable. ATP is rebuilt from ADP and Pi with the adequate input of energy. ATP is known as 'energy currency' in the cell. This forms the part of *light reaction* (Fig. 3.3).

The reduced form of the coenzyme NADPH, a two electron donor, thus formed, reduces CO_2 to 3-phosphoglycerate. Inorganic carbon is converted into the first organic molecule of the **carbon cycle** (**Calvin cycle**) to be discussed in the sequel.

The overall green plant photosynthesis reaction is represented by the century-old oversimplified basic expression (3.1) in which we find two thermodynamically demanding processes, oxidation of water and reduction of CO_2 in the combined form.

$$CO_2 + H_2O + h\nu \rightarrow C\,(H_2O) + O_2 \tag{3.1}$$

Priestley in 1770s first showed by performing the following experiment that oxygen is liberated during photosynthesis. He put a mouse under each of two bell jars, one of which contained a plant while the other none. He found that the mouse in the bell jar containing the plant lived much longer than the other mouse. From this observation "...he correctly concluded that the plant, through interaction with

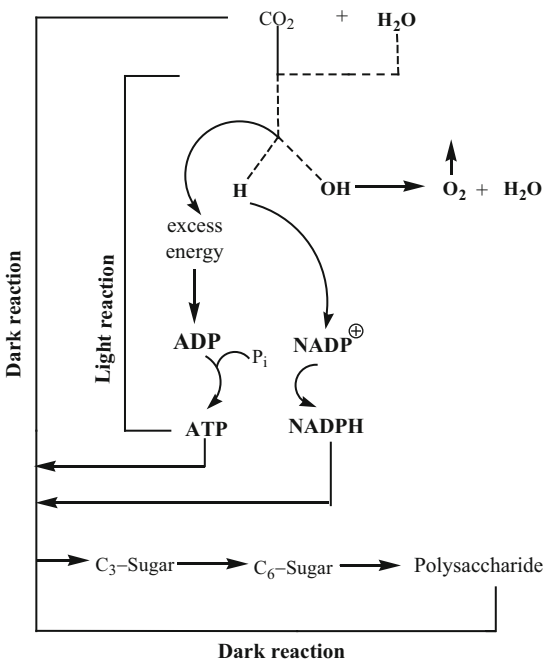


Fig. 3.3 A simplified representation of photosynthesis in green plants

light, was modifying the air by producing a new substance. He set out to prove what this substance was and thereby discovered oxygen" [2]—a gas so convivial to our very existence. Melvin Calvin (NL, 1961) observed that plants/green algae upon irradiation with light in presence of $^{14}CO_2$ produced labeled 3-phosphoglycerate, and he concluded that this C_3-sugar was the intermediate in the fixation of CO_2 to saccharides. The unique enzymatic machinery present in the chloroplasts of green plants catalyzes the conversion of CO_2 into simple organic compounds. This process is called *CO_2 fixation* or *carbon fixation*. The reactions involved make up a cyclic pathway constantly regenerating the key intermediates. The pathway was elucidated by Melvin Calvin in early 1950s and is often called the **Calvin cycle**. These simple products of photosynthesis are converted in plants into complex biomolecules like sugars, polysaccharides, and their metabolites.

Formation of NADPH and ATP takes place by the action of light and is called *light reaction*. In the *dark reaction*, the NADPH and ATP drive the reduction of CO_2 to more useful organic compounds. A very simplified representation of photosynthesis is shown in Fig. 3.3.

To make a glucose molecule, six molecules of CO_2 are needed as shown in the following equation:

$$6\,CO_2 + 12\,H_2O + h\nu \xrightarrow{\text{chlorophyll}} C_6H_{12}O_6 + 6\,O_2 \uparrow + 6\,H_2O$$

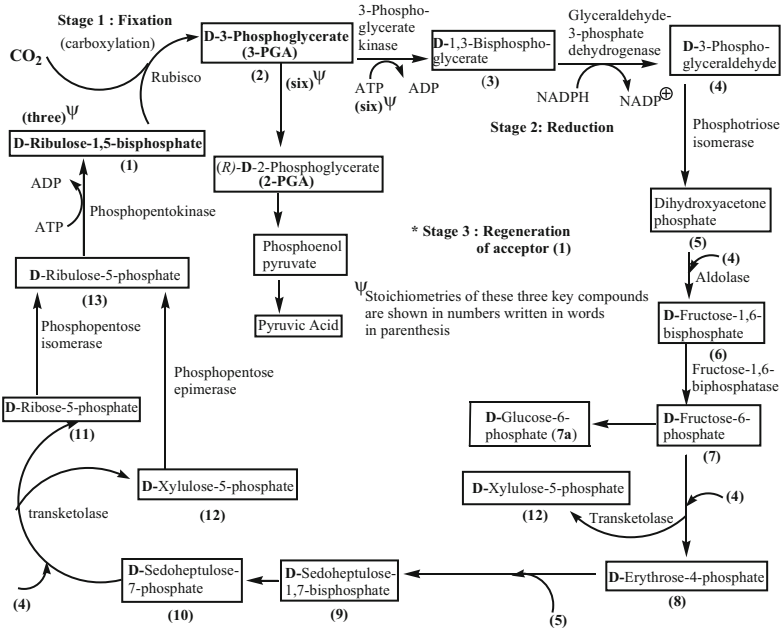
and the overall balanced equation for Calvin cycle may be represented as:

$$6CO_2 + 18ATP + 12NADPH + 12H_2O \rightarrow C_6H_{12}O_6 + 18ADP + 18Pi + 12NADP^+ + 6H_2O.$$

Interestingly, water appears on both sides of the equation. It is due to the fact that OH obtained from H_2O gives oxygen as well as some water molecules.

3.1.2 *Dark Reaction (Calvin Cycle): Formation of 3-, 4-, 5-, 6-, and 7-Carbon Sugars*

Detailed studies by Melvin Calvin (NL 1961) and his collaborators revealed that photosynthetic CO_2 fixation proceeds by a cyclic process which has been named **Calvin Cycle** (Fig. 3.4). Since reduction occurs and pentoses are formed in the cycle, the latter is also termed as **reductive pentose phosphate (RPP) pathway**. The Calvin cycle consists of *three stages* (Fig. 3.4) to be explained in succession.



*** Stage 3 Regeneration of acceptor (1) from (4) via (5), (6), (7), (8) and so on, as shown.**

(*For mechanisms of all conversions see* **Figures 3.5 to 3.10**)

D,L-Nomenclature*: When the Fischer projection formula of the molecule is written placing* ***(i)*** *the more oxidized end of the chain at the top, and* ***(ii)*** *the longest carbon chain vertically, the enantiomer with the OH group at the lowermost (highest numbered) chiral center [e.g.,C2 in* **(2)**, **(3)** *and* **(4),** *C3 in* **(8),** *C4 in* **(1)** *and* **(12)**, *C5 in* **(6)**, **(7)** *and* **(7a)**, *and C6 in* **(9)** *and* **(10)***]* ***on the right-hand side is given*** **D-*configuration*** *- the corresponding enantiomer of each sugar is designated* **L.**

Fig. 3.4 Calvin cycle or carbon cycle: Formation of 3-, 4-, 5-, 6- and 7-carbon sugars in three stages

Stage 1: The Fixation of CO_2 by Carboxylation of a C_5 Sugar to Form D-3-Phosphoglycerate (2) In the fixation of CO_2, the C_5 sugar D-ribulose-1,5-bisphosphate (for D,L-nomenclature *see* Fig. 3.4 and Sect. 2.6.1) acts as the acceptor on which carboxylation takes place in presence of an enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase, commonly known as ***rubisco/Rubisco*** [3], *the most abundant protein in plant, and for that matter on earth. With its 16 subunits, it is one of the largest enzymes in nature. It is a lazy enzyme* and its catalytic rate is 3 s^{-1} only, *i.e.*, it fixes only three molecules of CO_2 per second and causes its rich concentration in *chloroplast* [4]. During the process, an unstable C_6 compound is formed which suffers rapid hydrolysis to yield two molecules of D-3-phosphoglycerate. Incorporation of CO_2 into 3-phosphoglycerate has been verified through the use of $^{14}CO_2$. Rubisco needs bound divalent Mg^{+2} for its activation. Prior to carboxylation, D-ribulose-1,5-bisphosphate undergoes enolization via a complex formation with the enzyme active site, and Mg^{+2}. The enediol form couples with CO_2 when a new carbon–carbon bond is generated. The adduct being unstable undergoes hydrolysis to yield two molecules of D-3-phosphoglycerate (Fig. 3.5).

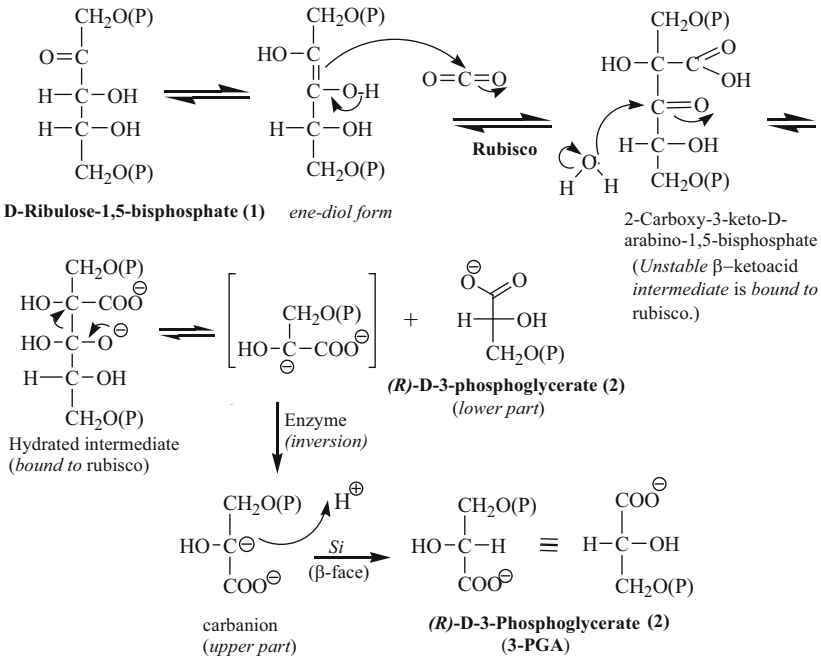


Fig. 3.5 Stage 1 of the Calvin cycle: *Carboxylation*

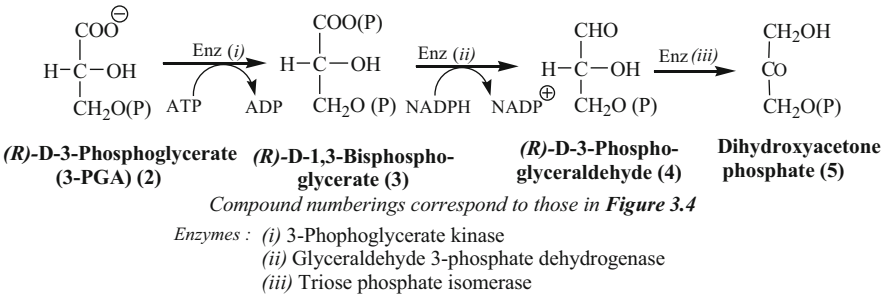
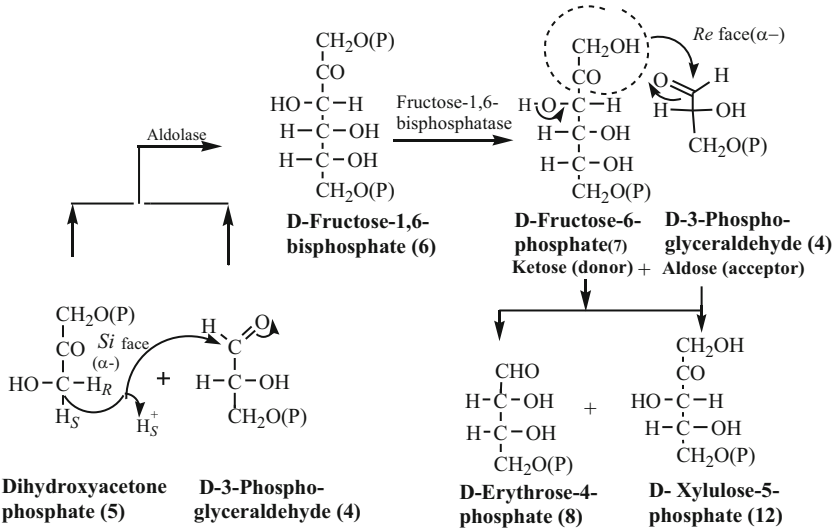


Fig. 3.6 Stage 2 of the Calvin cycle: Formation of D-3-phosphoglyceraldehyde (**4**) and dihydroxyacetone phosphate (**5**)

Stage 2: Formation of 3-Phosphoglyceraldehyde (4) and Dihydroxyacetone phosphate (5), the More Versatile Biosynthetic Precursors, by Reduction of 3-Phosphoglycerate (2) Conversion of D-3-phosphoglycerate (**2**) to D-3-phosphoglyceraldehyde (**4**) takes place in two steps (Fig. 3.6). In the first step, 3-phosphoglycerate kinase catalyzes the transfer of phosphate from ATP to D-3-phosphoglycerate (**2**), yielding D-1,3-bisphosphoglycerate (**3**). In the second step, (**3**) is reduced by NADPH, being catalyzed by glyceraldehydes-3-phosphate dehydrogenase to form D-3-phosphoglyceraldehyde (**4**), which isomerizes to (**5**) in presence of triose phosphate isomerase.

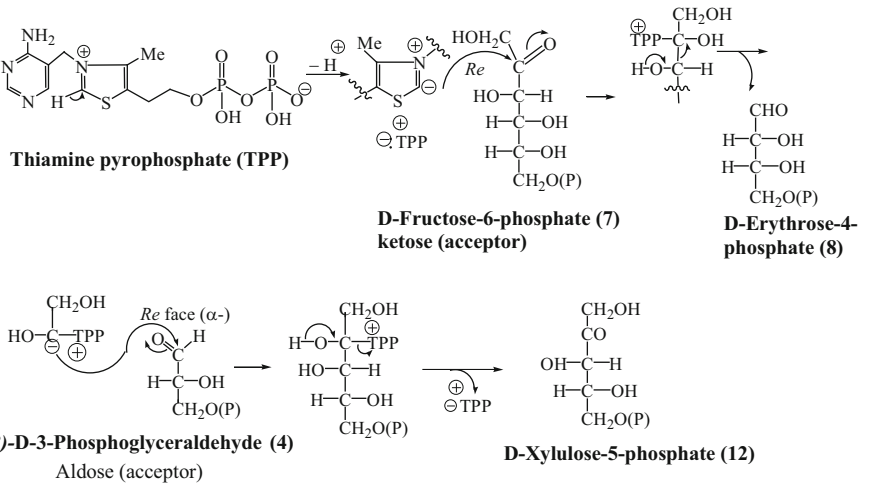
Stage 3: The Regeneration of the CO_2 Acceptor D-ribulose-1,5-bisphosphate (1) The third stage of CO_2 fixation involves the remaining set of reactions of the Calvin cycle. The products of stage 2, *viz*., the triose phosphates (**4**) and (**5**) pass through a series of successive transformations to six-, four-, five-, and seven-carbon sugars, eventually leading to the regeneration of the starting material, D-ribulose-1,5-bisphosphate (**1**) in presence of a specific enzyme in each stereospecific step as shown in Figs. 3.7–3.9. Thus, a continuous flow of CO_2 into carbohydrates is maintained in the Calvin cycle. The H_S of (**5**) attacks on the *Si* (or α-) face of the carbonyl group of (**4**), being catalyzed by aldolase, to produce (**6**). In the next step, the transfer of $CH_2OH–CO–$ of (**7**) to the *Re* face of –CHO of (**4**) is effected by the transketolase enzyme whose prosthetic group, thiamine pyrophosphate (TPP), acts as the carrier. *Regeneration of D-ribulose-1,5-bisphosphate* (**1**) *and its formation from* (**4**) *and* (**5**) *via* (**6**) *to* (**13**) *in succession* are outlined and explained stepwise in Fig. 3.9.

The mechanism of the catalytic function of TPP is shown in Fig. 3.8.



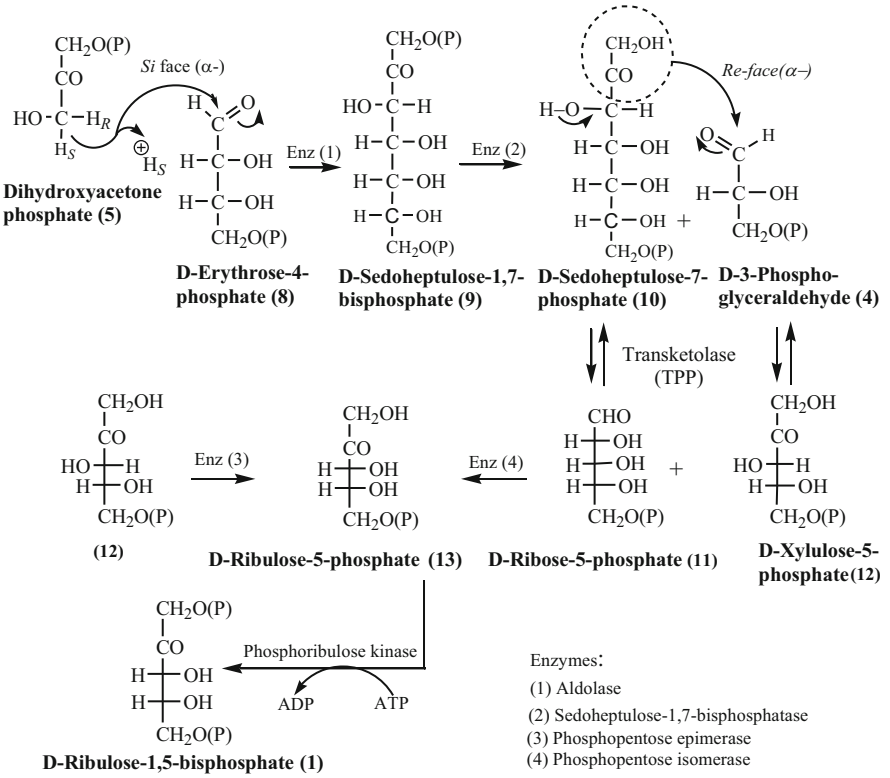
Compound numberings correspond to those in ***Figure 3.4***

Fig. 3.7 Stage 3 of the Calvin cycle (1st part): Formation of D-erythrose-4-phosphate (**8**) and D-xylulose-5-phosphate (**12**)



Compound numberings correspond to those in **Figure 3.4.**

Fig. 3.8 Function of TPP in the conversion of (**7**) to (**8**) and (**12**)



Compound numberings correspond to those in **Figure 3.4**

Fig. 3.9 Stage 3 of the Calvin cycle (remaining part): Regeneration of D-ribulose-1,5-bisphosphate (**1**)

3.1.2.1 Some Comments and Implications Regarding Calvin Cycle Molecules

(i) The reactions can be summarized as follows:

$$C_3\,(\mathbf{5}) + C_3\,(\mathbf{4}) \xrightarrow{\text{Aldolase}} C_6\,(\mathbf{6}) \rightarrow C_6\,(\mathbf{7})$$

$$C_6\,(\mathbf{7}) + C_3\,(\mathbf{4}) \xrightarrow{\text{Ketolase}} C_4\,(\mathbf{8}) + C_5\,(\mathbf{12})$$

$$C_4\,(\mathbf{8}) + C_3\,(\mathbf{5}) \xrightarrow{\text{Aldolase}} C_7\,(\mathbf{9}) \rightarrow C_7\,(\mathbf{10})$$

$$C_7\,(\mathbf{10}) + C_3\,(\mathbf{4}) \xrightarrow{\text{Ketolase}} C_5\,(\mathbf{4}) + C_5\,(\mathbf{12})$$

Adding,

$$5\,C_3 \longrightarrow 3\,C_5$$

$$C_5\,(\mathbf{13}) \longrightarrow C_5\,(\mathbf{1})$$

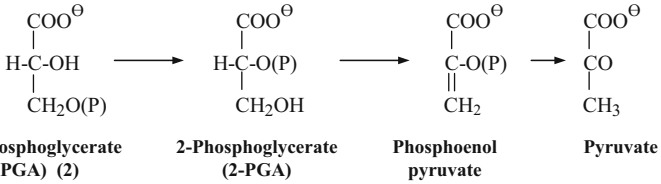


Fig. 3.10 Stepwise conversion of 3-PGA to pyruvate

(ii) Each of these C_3, C_4, C_5, and C_6 molecules plays an important role in the biosynthesis of natural products.

(iii) (*R*)-D-3-Phosphoglyceraldehyde, (**4**), generated in the 2nd stage of the Calvin cycle, serves as the mother molecule for the other sugars (4-, 5-, 6-, and 7-carbon sugars) including the CO_2 acceptor molecule D-ribulose-1,5-bisphosphate (**1**), preserving the stereochemical integrity of the chiral center of (**4**), which becomes the lowermost chiral carbon. Hence, according to IUPAC nomenclature, all the sugars are named D-sugars (Chap. 2). Apparently, in the plant cells, no enzyme is available which can epimerize the designated chiral carbon of the sugars producing an L-diastereomer, although epimerization at other centers in presence of specific epimerases is known.

(iv) Most of the D-glyceraldehyde-3-phosphate (**4**) is recycled to D-ribulose-1,5-bisphosphate (**1**). The remaining (**4**) may be used immediately as a source of energy, converted to sucrose for transport *via* its precursor D-fructose-6-phosphate (**7**), or stored as *starch* for future use. The part of dihydroxyacetone phosphate (**5**), which is not used in Calvin cycle, leaves the chloroplast and can be degraded via glycolysis to provide energy.

(v) 3-Phosphoglycerate (**2**) (3PGA) gives rise to pyruvate at the primary metabolic level as shown in Fig. 3.10.

(vi) 3PGA may also be formed by the breakdown of D-glucose *via* D-fructose 1,6-diphosphate (**6**)

(vii) Pyruvic acid is a very important key compound. It forms acetyl coenzyme A; the latter enters into the formation of fatty acids, polyketides (Chap. 14), and aromatic compounds and also terpenoids and steroids *via* mevalonic acid pathway (Chaps. 5, 5, and 11).

3.1.3 C_4-Plant Photosynthesis, C_3- and C_4-Plants

As discussed above, a C_3-acid, phosphoglyceric acid (PGA) is the primary fixation product in the ***Calvin cycle*** of the carbon fixation process. PGA is formed mostly in the chloroplast present in the leaf *mesophyll* tissues involving *rubisco* having both carboxylase and oxygenase catalytic activities. Because of the latter activity of the enzyme which is less suppressed by comparatively low CO_2 intake from air (CO_2 concentration being much less than that of O_2), a considerable part of fixed carbon

is oxidized and lost as CO_2—a phenomenon known as *photorespiration* resulting into a significant loss of energy. Thus this process is inefficient.

Subsequent to the discovery of Calvin cycle, Hugo Kortschak obtained an unexpected result while studying the photosynthesis with $^{14}CO_2$ in sugarcane (at the Sugarcane Research Institute, Hawaii), in which C_4-acids, viz., oxaloacetate and malate appeared as the primary fixation products. Kortschak did not publish this observation in any journal. Ten years later, a Russian scientist Yuri Karpilov made a similar observation in the maize plant. This observation challenged the universality of the Calvin cycle. However, Hatch and Slack [5, 6] solved this riddle. It was then generalized that in some plants, prior to the Calvin cycle, CO_2 is prefixed as some C_4 acids like *oxaloacetate* and *malate*, as the primary fixation products. It was suggested that such fixation takes place in leaf cells with Kranz (meaning garland/wreath)-type anatomy (a few exceptions) having a wreath type arrangement of cells, with the *inner bundle sheath cells* being surrounded by *mesophyll tissue cells*.

Initial fixation to D-*malate* takes place in the outer *mesophyll* tissues, which lacks rubisco. Atmospheric CO_2 is converted to HCO_3^- catalyzed by *carbonic anhydrase*, which is absent in C_3-plants. The bicarbonate anion reacts with *phosphoenol pyruvate* (PEP), being catalyzed by PEP carboxylase to form oxaloacetate, which is then enzymatically reduced to malate (Fig. 3.11). The metabolic product malate is transferred to *bundle sheath* cell zone through a mechanism involving *plasmodesmata* (intercellular connections), since the bundle sheath cell wall is impermeable to the fixation products. D-Malate forms pyruvate and releases CO_2 to *rubisco* as its substrate to enter into Calvin cycle (Fig. 3.11). The pyruvate moves to mesophyll tissue cells, gets phosphorylated to form PEP, which is reused in the oxaloacetate formation, and the cycle is repeated.

In some plants (e.g., millet and forage plants) glutamate aspartate transaminase concentration being higher, oxaloacetate is transaminated to aspartate (in presence of glutamate), which moves to bundle sheath cells. There it is reconverted to oxaloacetate that in turn enzymatically releases CO_2 and forms PEP (Fig. 3.11). Initially, the concentration of oxaloacetate is not sufficiently high to allow its participation in the metabolic flux to diffuse from mesophyll tissue cells to bundle sheath cells. Hence, it moves via malate or aspartate. This pathway is known as Hatch and Slack pathway.

Thus, two major pathways, C_3-acid pathway (Calvin cycle and RPP cycle) and C_4-dicarboxylic acid pathway (Hatch and Slack pathway and C_4-pathway), are followed in photosynthesis, and according to the primary fixation products the plants are classified as ***C_3-plants*** (most plant species belong to this class) and ***C_4-plants*** (mostly pasture plants, crops, forage plants, wild weeds, desert plants, etc.). In C_3-plants *rubisco* is the primary enzyme while in C_4-plants phosphoenol pyruvate carboxylase (*PEP carboxylase*) is the key enzyme.

In C_4-plants, there is no rubisco in the mesophyll tissue cells, but some rubisco is present exclusively in bundle sheath cells. C_4-Plants need less rubisco, less nitrogen, and also less water for growth. So in warm weather, C_4-plants grow advantageously. However, C_4-plants are affected by chilly weather.

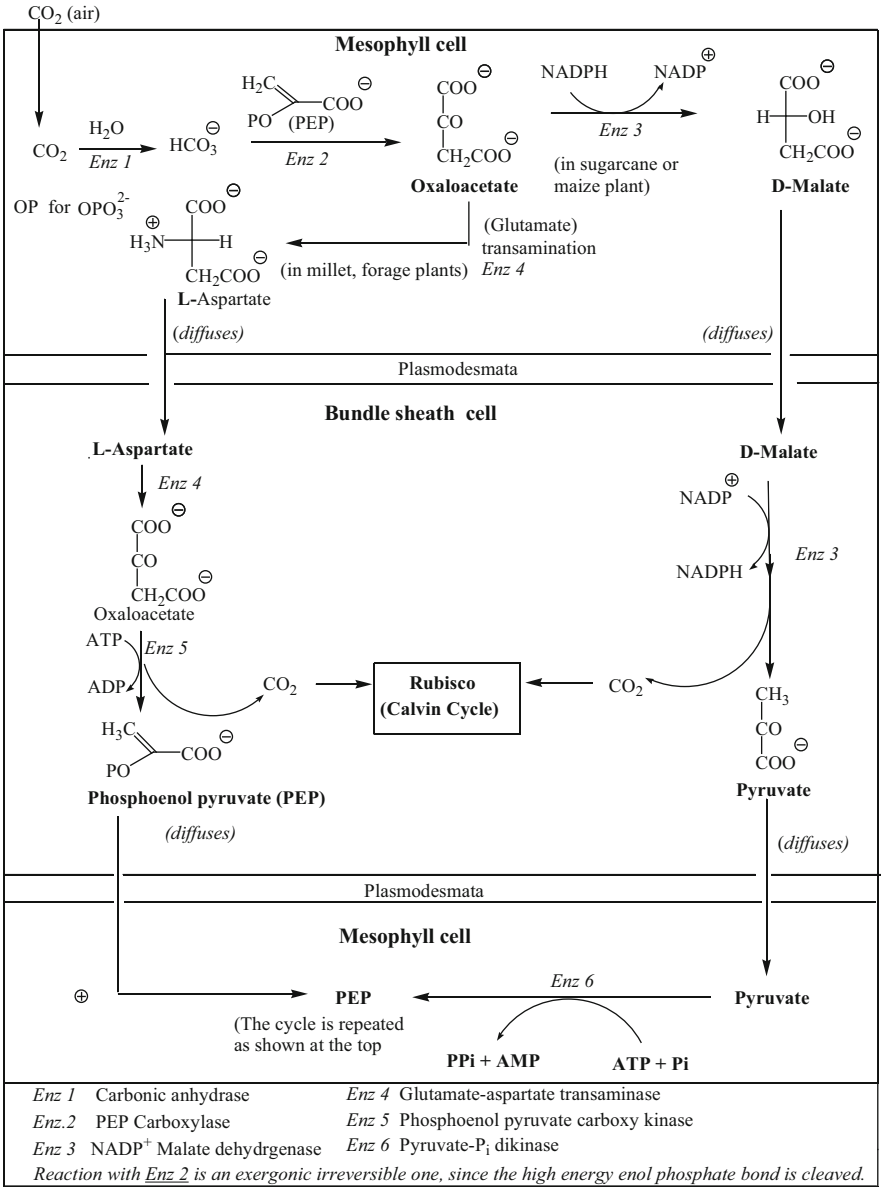


Fig. 3.11 C4- Plant photosynthetic cycle

3.1.3.1 Identification of C_3 and C_4 Metabolism Products by Mass Spectrometry

The natural isotopes of carbon are ^{12}C (98.89 %) and ^{13}C (1.11 %). Because of the kinetic isotope effect rubisco reacts faster with $^{12}CO_2$ than with $^{13}CO_2$ in C_3 plants, resulting in the lowering of $^{13}C/^{12}C$ ratio in the products of C_3 photosynthesis than this ratio in the atmospheric CO_2. In C_4-metabolism, the enzymatically prefixed CO_2 with less kinetic isotope effect reacts with rubisco in bundle sheath cells and thus such lowering of the $^{13}C/^{12}C$ isotope ratio in the photosynthesis products is less. Hence by studying the $^{13}C/^{12}C$ ratio in the product mass spectrometrically, it is possible to predict the metabolism pathway followed to form the product. For example, sugar obtained from two different sources, e.g., sugar beet (C_3-plant) as well as sugarcane (C_4-plant) can be related to its source by its isotope ratio analysis. In C_4 Pathway, photorespiration is almost absent due to high concentration of CO_2 released by the prefixed C_4 products, and the suppression of the enzymatic oxygenase activity of rubisco due to absence of O_2.

3.1.3.2 Crassulacean Acid Metabolism (CAM)

The plants like all cacti and many desert plants growing in arid zones, the succulent ornamental plant *Kalanchoe* and plants growing in tropical rain forests, including half of the orchids manage the extreme shortage of water by keeping their leaf stomata closed during daytime to avoid loss of water, and reduce the area of their leaves. They open their leaf stomata in the night to allow the entry of CO_2 and to mask evaporation. CO_2 is prefixed as C_4-acids and stored in the vacuole until next morning when they are degraded to release CO_2 to rubisco in the chloroplasts; the pyruvate formed is subsequently phosphorylated by pyruvate phosphate kinase. The major difference in this case is the time lag between CO_2 fixation (storage) and its release. The mechanism has been first studied in Crassulaceae plants, and CO_2 is stored as an acid and hence the name crassulacean acid metabolism (***CAM***). Pineapples and the agave sisal, yielding natural fibers are important *CAM plants*.

3.2 Biological Oxidation: Reduction (NADPH ⇌ $NADP^+$)

As mentioned earlier (Chap. 1), there are enzymes which are coenzyme or cofactor dependent in their functions. Enzyme promoted oxidation–reduction in the biological systems uses several coenzymes. The function of one such coenzyme, nicotinamide adenine dinucleotide phosphate [in the reduced (NADPH) (**15**) and oxidized ($NADP^{\oplus}$) (**14**) forms] (Fig. 3.12) will be discussed briefly. They act in a large number of oxidation–reduction reactions. Sometimes, NADPH has been compared to the laboratory reagent $NaBH_4$ by describing it as Nature's $NaBH_4$. However, the

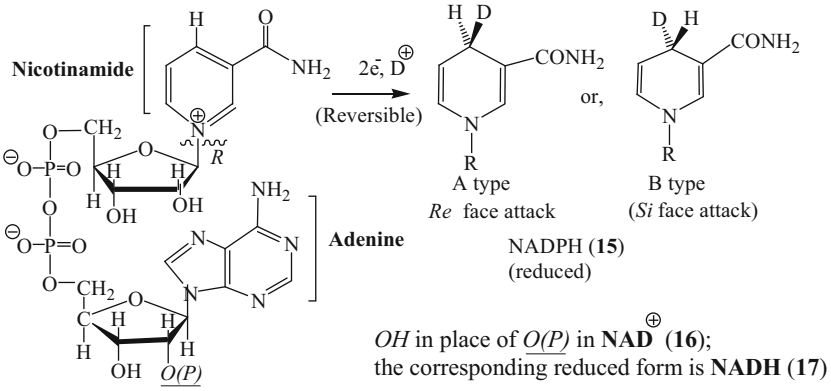


Fig. 3.12 Structures of $NADP^{\oplus}$, NADPH, $NAD^{\oplus}$ and NADH

latter being achiral and incapable of being reversed like a redox system, such a comparison is hardly reasonable. However, Hantzch 1,4-dihydropyridine has been widely used as a biomimetic reducing agent (cf. NADPH) (Chap. 13).

The coenzyme is tightly bound to the enzyme by multiple noncovalent interactions in a dissociable fashion. Many oxidation–reduction involving enzymes carry out electron transfer through the agency of NADPH/$NADP^+$. The nicotinamide part of the coenzyme is responsible for the biological oxidation–reduction reaction and constitutes an example of a biological redox system; its $CONH_2$ part ensures its redox potential; and the R part controls the binding of the coenzyme to the enzyme. The word **'redox'** is the abbreviated form of '**red**uction–**ox**idation'.

The term oxidation finds its genesis in terms of addition of oxygen (or removal of hydrogen) and reduction consists of removal of oxygen (or addition of hydrogen). They can better be explained in terms of electron gain (reduction) and electron loss (oxidation) during the process.

The nicotinamide end of the coenzyme participates in the oxidation–reduction of a substrate molecule by way of a hydride removal and acceptance, at the same time the concerned moiety of the coenzyme undergoing reduction and oxidation (Fig. 3.13). Enzymatic reactions are stereospecific; of the two diastereotopic hydrogen atoms ($H_R = H_A$ and $H_S = H_B$, see Chap. 2) of NADPH (C4 being prochiral), H_R with its bonded electron pair (hydride) is transferred to the substrate to effect its reduction, NADPH itself being converted to its oxidized form $NADP^+$. In the reverse process, i.e., during oxidation of the substrate, the same hydrogen (H_A) with its bonded electron pair is delivered to the *Re* face of $NADP^+$ to convert it to NADPH, and H_A now becomes H_R. Some enzymes use the ***pro-S*** (or H_S) in a similar fashion.

The stereospecificity of several oxidation–reduction processes caused by biological redox system with respect to the substrate and the coenzyme has been

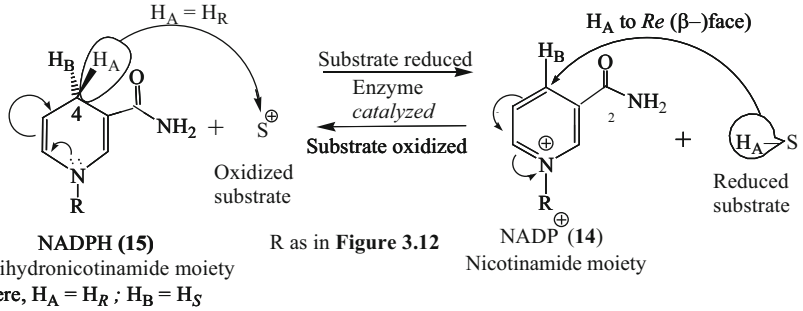


Fig. 3.13 Stereospecific delivery and acceptance of hydride by NADPH and NADP + respectively.

proved by labeling the key hydrogen by deuterium/tritium. The stereochemical information is delivered at the site of attack depending on the *diastereofacial differentiation*, as illustrated in Fig. 3.13.

Incubation of the alcohol dehydrogenase with CH_3CD_2OH in presence of the cofactor NAD^+ (**16**) generates the reduced cofactor (**17a**) (Fig. 3.14). The latter is reoxidized by CH_3CHO to NAD^+ (**16**), the former being reduced to 1*R*-deuteroethanol, demonstrating that the D atom transferred to the cofactor at C4 at its *Re* face is stereospecifically removed in the reverse reaction to attack the *Re* face of CH_3CHO, forming the chiral 1*R*-deuteroethanol. In the reverse reaction (1*R*)-deuteroethanol when incubated with NAD^+ (**16**) in presence of the enzyme regenerates only 4*R*-^{2}H-NADH (**17a**) with complete transfer of D to its *Re* face. Evidently, the rigorous stereochemical control of enzyme catalyzed reactions is brought about by the precise positioning of the substrate and the coenzyme at the enzyme active site.

The interconversion NAD^+ (16) ⇌ NADH (**17a**) and their phosphorylated analogs $NADP^+$ ⇌ NADPH can be monitored in vitro by UV spectroscopy. Both the oxidized forms show one absorption band around 260 nm, whereas reduction to NADH or NADPH produces a new broad absorption band with a maximum of 340 nm. Thus, the production of NADH or NADPH during an enzyme-catalyzed oxidation of a substrate can be conveniently followed by observing the appearance of the λ_{max} at 340 nm. We cite here another example of diastereospecific enzymatic reduction by NADH. It occurs during *souring of milk*.

Glycolysis, a part of *fermentation*, is a ten-step pathway converting one molecule of glucose to two molecules of pyruvate. A lactic acid bacterium uses a direct route to effect an enantiospecific reduction of pyruvate to *S*-L-lactate with NADH in presence of the enzyme lactate dehydrogenase (Fig. 3.15). Here, an electron pair from NADH attacks the *Re* (front) face of pyruvate. *Souring of milk* involves this endergonic reaction.

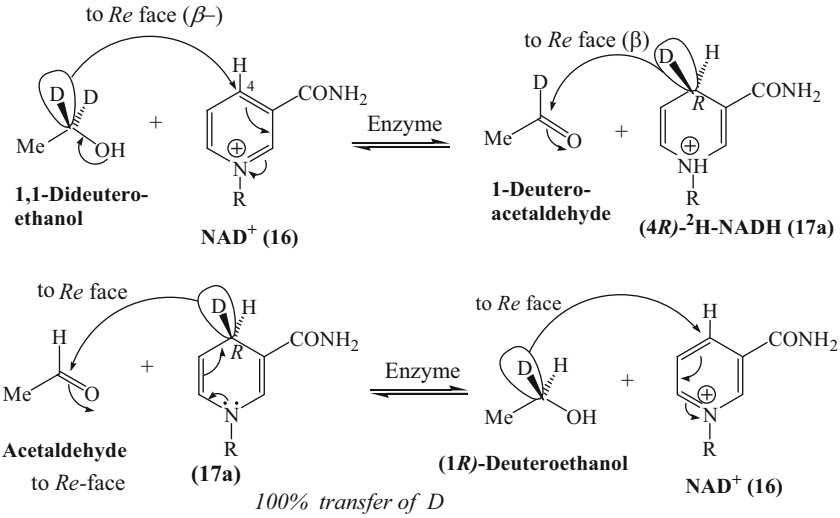


Fig. 3.14 Stereospecifity of alcohol dehydrogenase

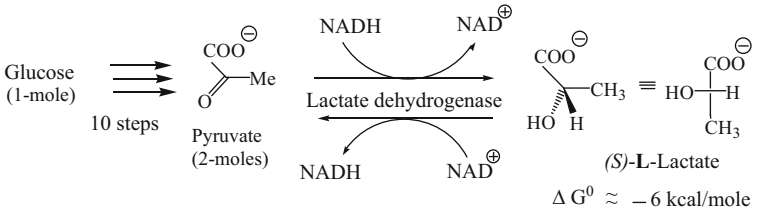


Fig. 3.15 Enantiospecific reduction of pyruvate to L-lactate (during souring of milk)

3.2.1 Flavin Coenzymes

Two coenzymes, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), are derived from vitamin B_2 or riboflavin. FMN is riboflavin phosphate (Fig. 3.16). Functionally both types are equivalent. Some enzymes use FAD while some others use FMN. Highly conjugated tricyclic isoalloxazine ring system, the functional part of both coenzymes, serves as a two-electron acceptor and is responsible for its strong redox character toward diverse classes of compounds. Molecules possessing such a ring system are called flavins. Flavin coenzymes are used by enzymes called flavoproteins (flavoenzymes). The latter bind FMN or FAD in most cases noncovalently but tightly so that the coenzymes can be reversibly dissociated. The flavins undergo two-electron oxidation and reduction reactions. They have a stable species, a semiquinone free radical produced by one-electron reduction that can be detected spectrophotometrically. The oxidized flavins are bright yellow;

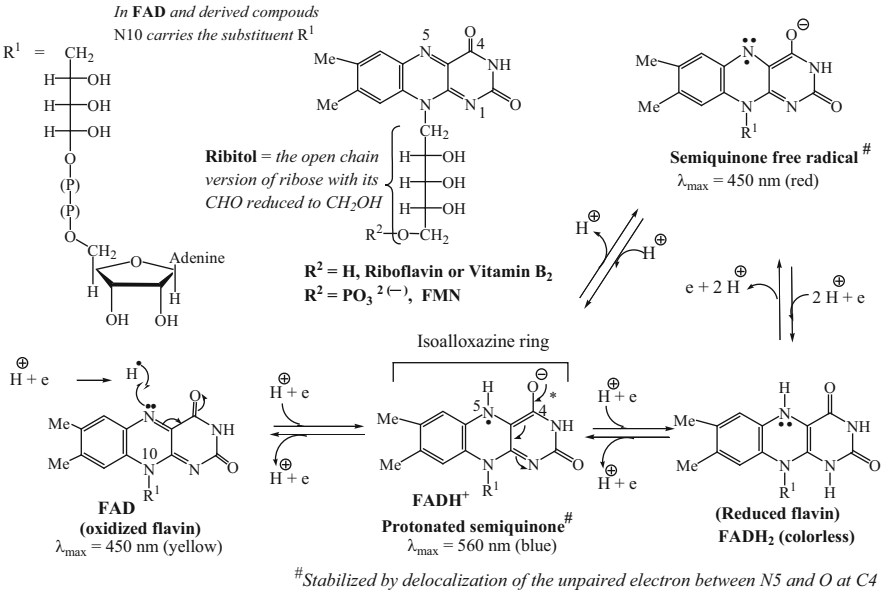


Fig. 3.16 Flavin coenzymes and the mechanism of their redox reactions

protonated semiquinone is blue, the semiquinone free radical is red, but the fully reduced flavins are colorless. The flavins have catalytic versatility more than nicotinamide coenzymes, since they can interact with two-electron or one-electron donor–acceptor pairs. Mechanism of stepwise reduction of FAD to $FADH_2$ via $FADH^+$ (protonated semiquinone) is shown in Fig. 3.16.

3.2.2 Combined Use of NADPH and FAD

There are cases when the substrate molecule is not directly reduced by NADPH (or NADH). Transfer of hydride from NADPH (or NADH) takes place indirectly. Two electrons are delivered from NADPH to a second enzyme cofactor, e.g., FAD in two steps to the N5 in the isoalloxazine ring, accompanied by accepting one proton from the medium in each step to form $FADH_2$. The latter delivers two electrons to the substrate to reduce it, itself being oxidized to FAD (Fig. 3.17).

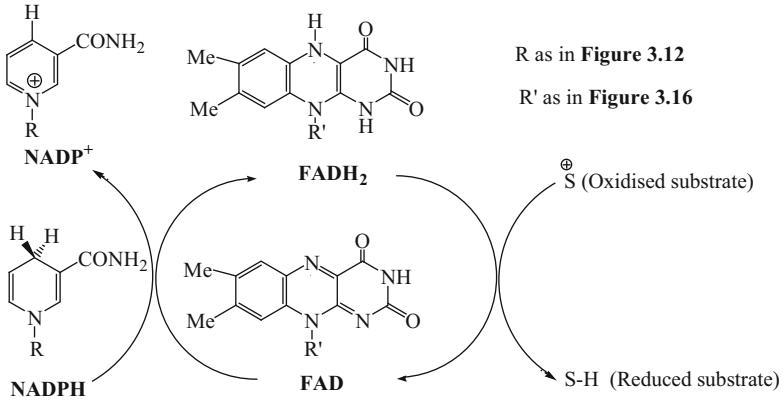


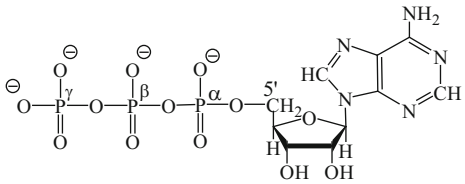
Fig. 3.17 Reduction by NADPH/FAD through $FADH_2$ in presence of enzyme

3.3 Phosphorylation (ATP→ADP) and Regeneration (ADP→ATP)

3.3.1 Function of ATP: Its Conversion to ADP

Adenosine triphosphate (**18**) (Fig. 3.18), abbreviated as ATP, is one of the major cofactors in many metabolic biosynthetic pathways. It acts as the phosphate donor to the appropriate substrates and is biologically a potential source of metabolic energy. It is considered to be the key molecule for capturing and storing chemical energy. It moves through the cells providing energy for all processes of the cells [1]. It contains one ester and two anhydride phosphoryl groups (β- and γ-), and the 5′CH_2 group where nucleophilic attack could take place. Its γ-phosphate group is transferred to various acceptors to activate them for their subsequent participation in various biological events. In unusual cases, attack on C5′ of the adenosyl part takes place, e.g., the formation of *S*-adenosyl methionine (SAM or AdoMet) (see Sect. 3.6).

The function of ATP in various biochemical reactions has been illustrated, whenever necessary (e.g., formation of mevalonic acid and γ,γ-dimethylallyl pyrophosphate, see Chap. 5). It has been observed that during pyrophosphorylation of alcoholic OH group (e.g., mevalonic acid to mevalonic acid pyrophosphate, Chap. 5, Fig. 5.3), two molecules of ATP are needed. Each ATP molecule donates its γ-phosphate group to the substrate and is converted to ADP. Presumably, the incapability of ATP for donating two phosphate groups in succession (ATP→ADP→AMP) is prevented by the spatial incompatibility of ADP to work as coenzyme with phosphorylating enzyme for such a reaction. The ATP can be hydrolyzed to adenosine diphosphate (ADP) (**19**) by shedding off the γ-phosphate.



Adenosine triphospate ATP (18)

5'CH_2 when attached to –O–(P)(P) gives adenosine diphosphate **(ADP) (19)**

5'CH_2 when attached to –O–(P) gives adenosine monophosphate (**AMP**) **(20)**

(P) stands for 

Fig. 3.18 Structures of **ATP(18)**, **ADP(19)** and **AMP(20)**

$$\mathrm{ATP} + \mathrm{H_2O} \rightleftharpoons \mathrm{ADP} + \mathrm{Phosphate}\ (\mathrm{P_i});\ \Delta \mathrm{G^o} = -30.5\,\mathrm{kJ/mol}\ (\textit{exergonic reaction})$$

The negative standard free energy $\Delta\ G^0$ is a measure of the ability of its phosphoryl group transfer potential. However, it has been shown that standard free energy of the hydrolysis of ATP depends on the water concentration. In the absence of water, the above reaction is directed towards ATP formation, and much energy is not needed. The reaction site of ATP formation in the enzyme can exclude water molecules and may proceed without the consumption of energy. Actually, energy requirement is necessary for certain conformational change in the catalytic sites of the enzyme and finally to release the ATP from the binding site.

3.3.2 Conversion of ADP to ATP

Adenosine diphosphate (ADP) can be reconverted to ATP by uniting with inorganic phosphate and with an adequate input of energy. In the leaf cells, during photosynthesis ADP is converted to ATP (photophosphorylation) (Fig. 3.3) by solar energy, and also in almost all cells by aerobic oxidation (oxidative phosphorylation) using chemical energy.

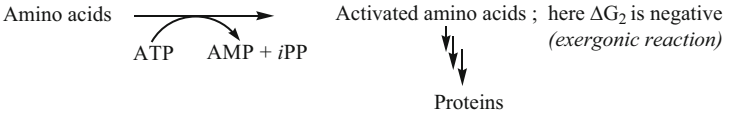
3.3.3 Formation of Proteins from Amino Acids

ATP favors thermodynamically unfavorable reactions like peptide bond formation. Calculations show that polymerization of amino acids to protein in the absence of ATP should be *endergonic, i.e.,* ΔG becomes positive.

$$\text{Amino acid} \rightarrow\rightarrow\rightarrow \text{Protein; here } \Delta G_1 \text{ is positive } (\textit{endergonic reaction})$$

However, ATP activates the amino acid by cellular reaction involving ATP breakdown to adenosine monophosphate (**20**) (AMP), giving more negative ΔG

(−45.6 kJ/mol). The sum total free energy change for the formation of protein becomes negative. Since $\Delta G_2 << \Delta G_1$ (the magnitude of the negative ΔG_2 being much more than the positive ΔG_1), the overall reaction becomes thermodynamically favorable.



3.3.4 Biosynthesis of Starch with the Help of ATP

Similarly in the biosynthesis of starch, polymerization of glucose takes place through the participation of ATP (Fig. 3.19). Different plants perceive a variety of environmental and endogenous influences, and as a consequence various chemical components including starch, differ in the composition. Amylose, one form of starch contains repetitive units of glucose united *via* α-1,4- disaccharide linkage. α-D-Glucose-1-phosphate reacts with ATP reversibly to give ADP-glucose. The pyrophosphate formed is hydrolyzed by the enzyme *pyrophosphatase* and in this way the formation of ADP-glucose becomes irreversible. The glucose activated by

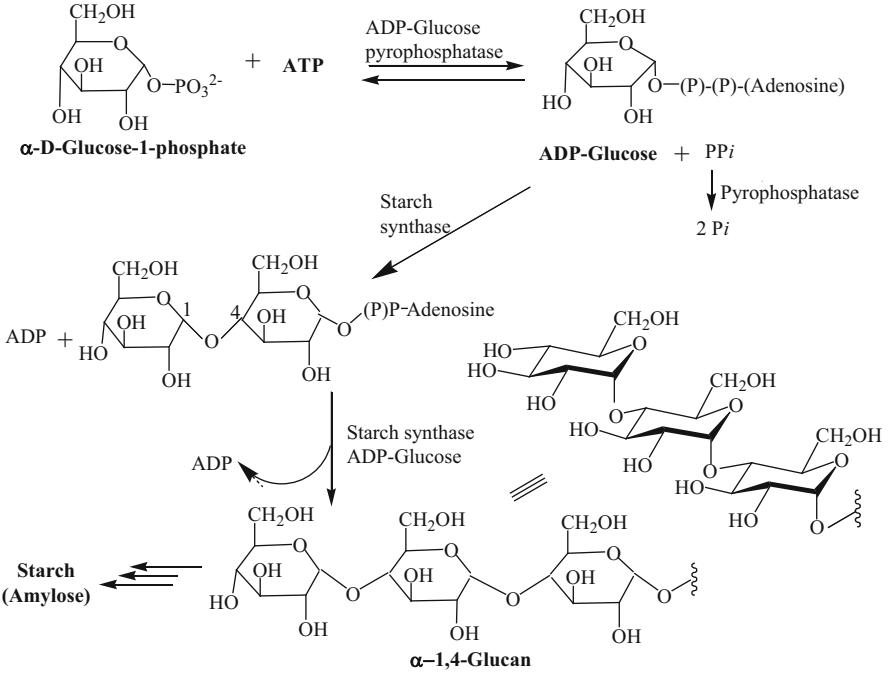


Fig. 3.19 Biosynthesis of starch (amylose) from -D-glucose

ADP is transferred by starch synthase from ADP-glucose to the OH group in the 4-position of the terminal glucose residue in the polysaccharide chain in the formative stage of starch. Similar deposition of glucose residue (α-1,4-disaccharide linkage) continues, catalyzed by starch synthase to form starch.

3.4 Acetyl Coenzyme A

The discovery of the cofactor, acetyl coenzyme A (**21**) (Fig. 3.20), an essential biochemical reagent for many diverse biological reactions, led to a major advance in the field of biochemistry. Structurally, it has three units: β-mercaptoethylamine, pantothenate (vitamin B_5), and 3′-phosphoadenosine-5′-diphosphate units, as shown in its structure (**21**).

Breslow provided evidence (IR, NMR) for the detection of stable thiazolium zwitterions of model thiazolium compounds by deuterium exchange studies in D_2O [7]. He thus suggested a mechanism involving such a zwitterion for the thiamine pyrophosphate (TPP^+) (Fig. 3.21) catalyzed in vivo biochemical reactions, e.g., decarboxylation of α-keto acids like pyruvic acid and benzoyl formic acid.

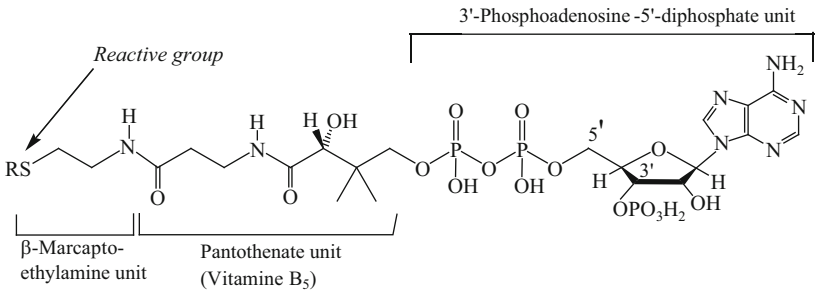


Fig. 3.20 Structures of acetyl coenzyme A and coenzyme A

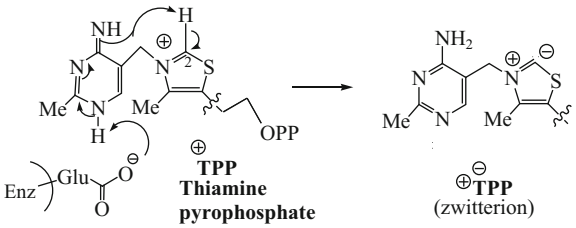


Fig. 3.21 Generation of TPP carbanion

Many other acids are also known to be activated as the corresponding acyl coenzyme A, e.g., malonyl coenzyme A, glutaryl coenzyme A, benzoyl coenzyme A, etc. The acyl coenzymes are responsible for many biological reactions.

3.4.1 *Formation of Acetyl Coenzyme A from Pyruvic Acid*

The stepwise mechanism of the formation of acetyl coenzyme A (**21**) by TPP^+ catalyzed decarboxylation of pyruvic acid is shown in two parts:

(a) ***Generation of TPP carbanion***. It has been shown from NMR and IR studies that the pyrimidine ring of TPP participates in the generation of the carbanion at C2 of the thiazole ring. It is initiated by the abstraction of the proton from the pyrimidine tautomer by the glutamate carboxyl group of the enzyme as shown in Fig. 3.21.

(b) ***Participation of carbanion of TPP (−:$^+$TPP) in the decarboxylation of pyruvic acid***. The thiazole carbanion participates in the nucleophilic attack on the ketocarbonyl of pyruvic acid to form the adduct (Fig. 3.22). The latter then suffers decarboxylation in the presence of the enzyme pyruvate decarboxylase

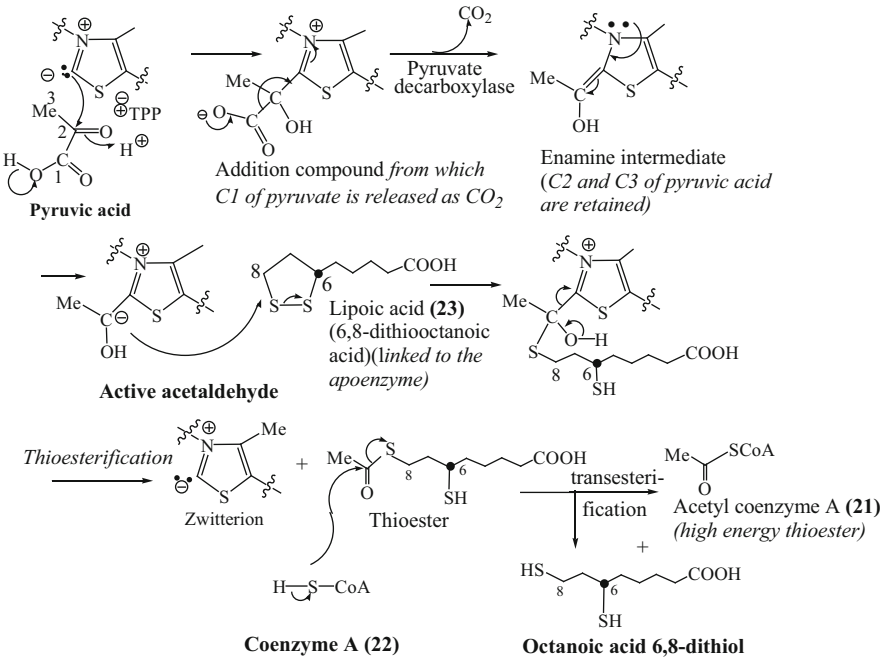


Fig. 3.22 Formation of acetyl coenzyme A from pyruvic acid

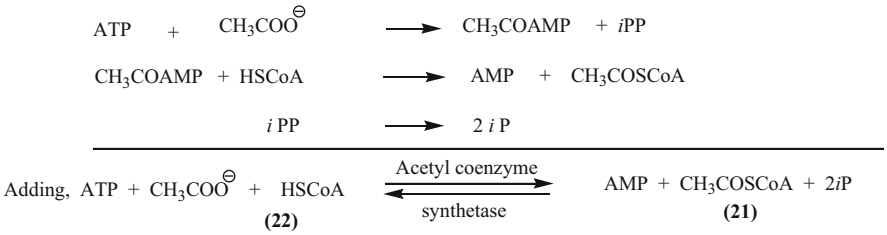


Fig. 3.23 Formation of acetyl coenzyme A (**21**) from coenzyme A (**22**)

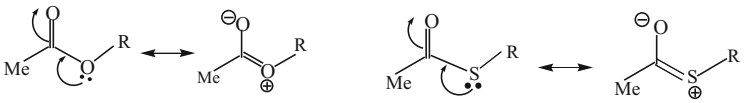
to form an **active acetaldehyde** via an enamine intermediate. The former attacks the disulfide bond of the lipoic acid residue linked to the apoenzyme. The thioester thus formed by transesterification yields acetyl coenzyme A and octanoic acid 6,8-dithiol.

3.4.2 Formation of Acetyl Coenzyme A from Coenzyme A

Acetyl coenzyme A (**21**) is also formed by acetylation of coenzyme A (**22**) by acetate; the reaction is catalyzed by acetate thiokinase as shown in Fig. 3.23. The pyrophosphate (*i*PP) breaks down to inorganic phosphate (*i*P).

3.4.3 Functions of Acetyl Coenzyme

Acetyl coenzyme A (**21**) performs the pivotal role as the starter in the biosynthesis of molecules of different skeletal patterns. It is capable of transferring a C_2-unit, like CH_3CO or CH_2CO moiety to appropriate substrates. It can undergo oligomerization via malonyl coenzyme A to give molecules with active ketomethylene groups, and it finds ways to enter into the *biosynthesis of polyketides, aromatic phenolic compounds and fatty acids* (Chap. 14). It is the key molecule of the several metabolic pathways like *mevalonic acid pathway* (Chap. 5) leading to many terpenoids and steroids and the citric acid cycle.



The reactivity of the thioester part of acetyl coenzyme A is due to two factors: (i) the thioester group is less stable than the ester group since the electron delocalization is more prominent in the latter; smaller volume of oxygen atom and its closer orbital energy than that of sulfur helps the overlapping of the lone pair more efficiently with the carbon orbitals, and hence the double bond character of C–S bond is less compared to the C–O bond in their respective esters. (ii) This makes the C–S bond weaker in addition to the more polarizability of the sulfur atom than oxygen; the thioester group is thus a better leaving group than the ester.

3.4.4 *Enzymatic Conversion of Choline to Acetylcholine by Acetyl Coenzyme A*

Acetylcholine (**ACh**) (**25**) and norepinephrine are important neurotransmitters which use different pharmacological receptors to mediate their end-organ responses. Acetylcholine is formed from choline (**24**) (Fig. 3.24) by the action of acetyl coenzyme A bound to the surface of the enzyme choline acetyltransferase (**ChAT**). The imidazole moiety of the enzyme promotes the removal of the alcoholic proton of choline (**24**) which is also bound to **ChAT**, generating a more nucleophilic center in choline, thus facilitates the acetyl group transfer and produces acetylcholine (**ACh**) (**25**) and coenzyme A (**22**).

High energy transfer potential. Acetyl coenzyme A is a small water-soluble high energy metabolite. It suffers hydrolysis, and the free energy change of the reaction is largely negative:

$$\text{Acetyl CoA} + \text{H}_2\text{O} \rightleftharpoons \text{Acetate} + \text{CoA} + \text{H}^+ \Delta\text{G}^\circ = -7.5 \text{ kcal mol}^{-1}$$

The reaction is thermodynamically favorable. The acetyl coenzyme A has a high acetyl transfer potential, the reaction being exergonic.

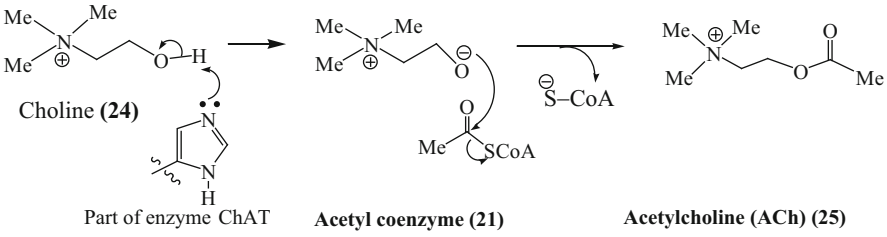


Fig. 3.24 Enzymatic conversion of choline to acetylcholine

3.5 Transamination, Isomerization, and Decarboxylation

Transamination is a very important biological event, a multistep reaction, catalyzed by enzymes called aminotransferases or transaminases. In a transamination reaction, the α-amino group of an L-amino acid$_1$ is donated to the α-carbon atom of an acceptor like an α-keto acid$_2$, *in a stereospecific manner*, leaving behind an α-keto acid, the corresponding α-keto acid analog of the amino acid$_1$. The overall reaction and a specific example are shown in Fig. 3.25. In the transamination reactions, the removal of the α-amino groups constitutes the first step in the catabolism of most of the amino acids. There is no net deamination in such reactions, e.g., α-ketoglutarate becomes aminated, while the α-amino acid is deaminated. The transamination reaction of different L-amino acids with α-ketoglutarate produces L-glutamate. The latter enters either into biosynthetic pathways or into a sequence of reactions producing nitrogenous waste products for excretion.

The aminotransferases are specific for a particular L-amino acid that donates the amino group and are named accordingly. The reactions catalyzed by the transaminases are completely reversible, having an equilibrium constant of nearly 1.0 ($\Delta G^0 \approx 0$ kcal/mol).

The transamination is mediated by the most versatile of all coenzymes (or cofactors), pyridoxal-5′-phosphate (PLP), the vitamin form of which is the corresponding alcohol, pyridoxine (vitamin B_6). The overall established mechanism of the PLP catalysis is delineated in two distinct halves in Figs. 3.26 and 3.27.

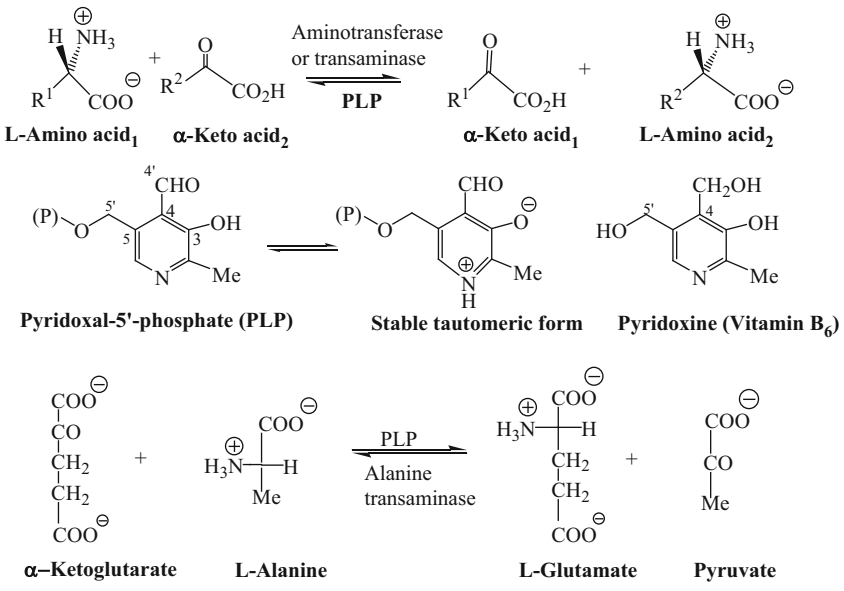


Fig. 3.25 Transamination (Aminotransferase reaction) :

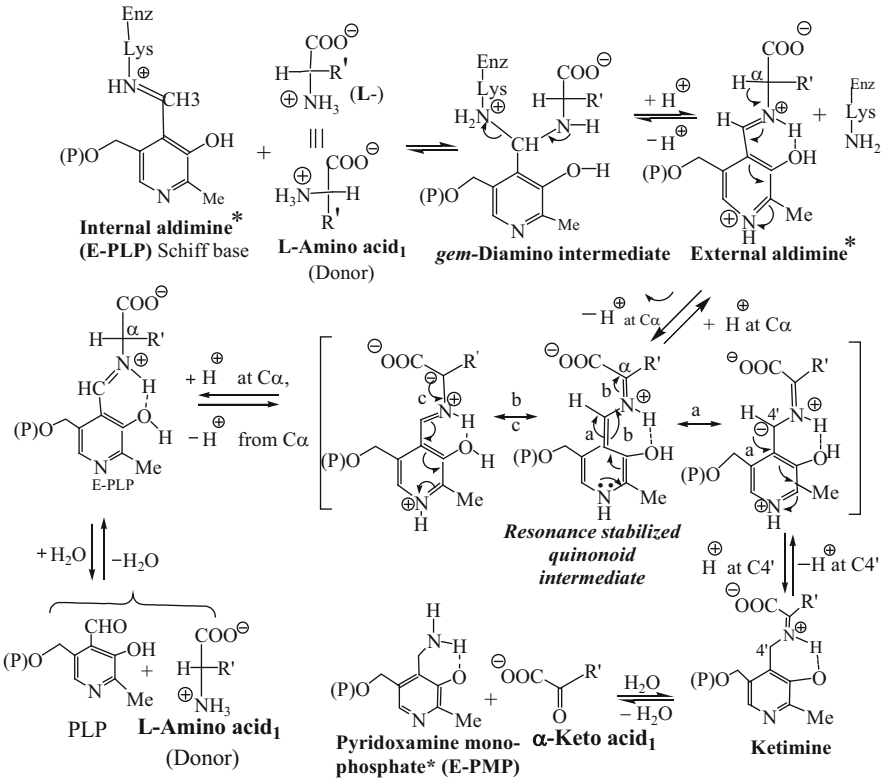


Fig. 3.26 Mechanism of the first half of the PLP catalyzed transamination reaction

The cofactor PLP is initially bound through multiple noncovalent interactions, and also covalently with the active site of the PLP-dependent enzyme through an imine (Schiff base) linkage, and is commonly called *internal aldimine* or E−PLP, by reaction of 4-CHO of the PLP with the ε-amino group of a lysine residue of the enzyme. The E-PLP is then attacked by the α-amino group of the donor L-amino acid (substrate) to form a *gem*-diamine intermediate that rapidly collapses to a new Schiff base of the coenzyme–substrate complex. The new aldimine thus formed is referred to as the '*external aldimine*'. Loss of proton at the α-carbon (of the amino acid), aided by protonation of the pyridine nitrogen (acting as electron sink), gives rise to a resonance stabilized quinonoid intermediate. This species can be reprotonated through addition of H^+ at C4′. Hydrolysis, of the resulting ketimine gives pyridoxamine phosphate (PMP) and a new keto acid (as in the first half of the transamination reaction). Reprotonation of the quinonoid intermediate may also take place at the amino acid α−C to regenerate the catalyst PLP and the original amino acid (Fig. 3.26), through the reformation of E-PMP.

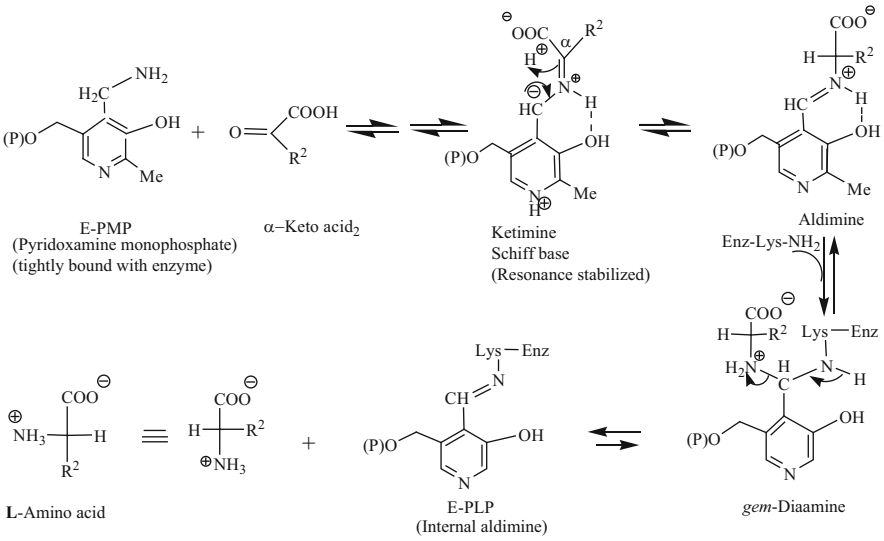


Fig. 3.27 Mechanism of the second half of the PLP catalyzed transamination reaction

Thus the first half of the *transamination reaction* constitutes the following:

$$\text{α-Amino acid}_1 + \text{E-PLP} \leftrightarrows \text{α-Keto acid}_1 + \text{E-PMP}$$

The second half: $\text{α-Keto acid}_2 + \text{E-PMP} \leftrightarrows \text{α-Amino acid}_2 + \text{E-PLP}$

Adding, $\text{α-Amino acid}_1 + \text{α-Keto acid}_2 \leftrightarrows \text{α-Amino acid}_2 + \text{α-keto acid}_1$

Fig. 3.28 Transamination reaction

The second half of the transamination process, catalyzed by the specific aminotransferase is completed in a reversal of the preceding reaction pathway (Fig. 3.27). The second α-keto acid which replaces the released one, condenses with the generated PMP (tightly bound with the enzyme and known as E-PMP) to form the corresponding ketimine Schiff base, deprotonation at C4′—the pyridine ring acting as the electron sink, leading to the formation of the resonance stabilized Cα carbanion, followed by protonation to form an aldimine. The latter gives rise to a different L-amino acid, and regenerates E-PLP, through the intermediacy of a *gem*-diamine (Fig. 3.27). The E-PMP generated in the first half of the transamination is thus recycled to the E-PLP formed in the second half (Fig. 3.28).

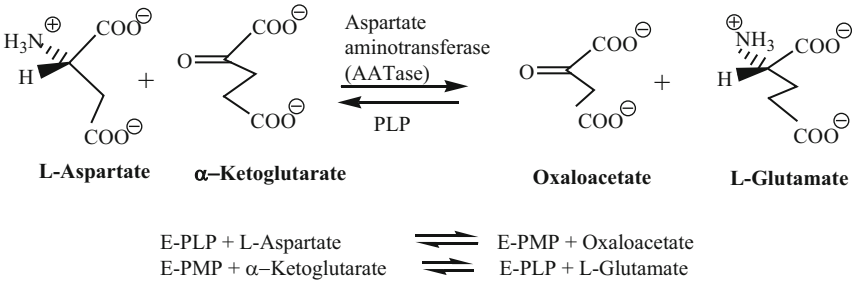


Fig. 3.29 The most studied transamination reaction

3.5.1 Transamination by Aspartate Aminotransferase

Aspartate aminotransferase (**AATase**) is *the most thoroughly studied* of all the PLP-dependent enzymes involved in amino acid metabolism. The reversible interconversion of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate is catalyzed by AATase (Fig. 3.29). The enzyme operates through a mechanism already discussed with two distinct half-reactions constituting a full catalytic cycle as illustrated in Fig. 3.29.

3.5.2 Some Interesting Concepts of The PLP-Catalyzed Transamination Reactions

3.5.2.1 Racemization and Decarboxylation

The external aldimine is a common intermediate in all PLP-dependent amino acid reactions. The pyridinium ring in the Schiff base (external aldimine) (Fig. 3.26), being electron deficient, can act as an electron sink and can trigger the breaking of all the four bonds to Cα, *i.e.,* Cα−H or Cα−COOH or Cα−R or Cα−N, being labilized by PLP-dependent enzymes. Abstraction of the Cα proton by an enzymatic base gives rise to the resonance stabilized Cα carbanion which may also undergo inversion and pick up a proton from the stereochemically opposite face to form the Cα-epimeric aldimine. The latter on hydrolysis gives rise to the corresponding D-amino acid (Fig. 3.30). The racemases thus provide D-amino acids for bacterial cell wall synthesis.

Breaking of the Cα−COOH bond effects decarboxylation and eventually leads to the formation of the amine RCH_2NH_2 as outlined in Fig. 3.30. This type of reaction is physiologically very important for mammals. Decarboxylation of glutamic acid produces γ-aminobutyric acid (GABA), a major inhibitory neurotransmitter. Dopamine, the immediate precursor of the hormone epinephrine, is

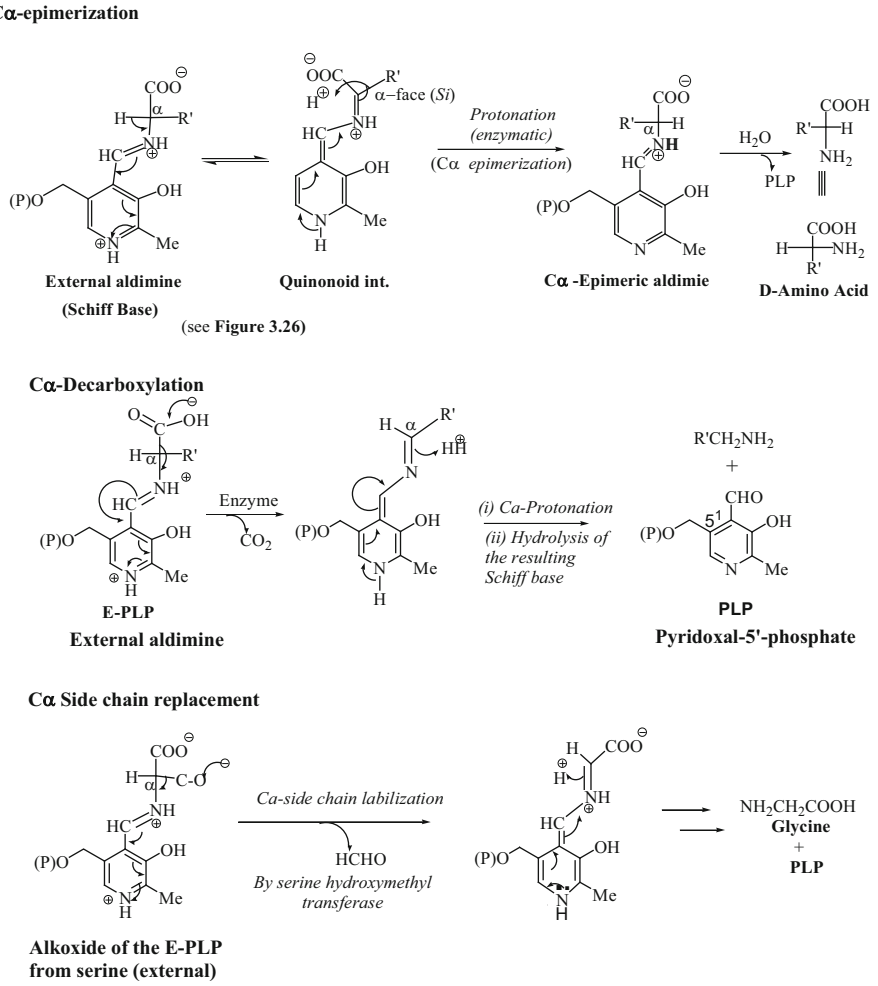


Fig. 3.30 PLP-dependent amino acid Ca epimerization (racemization), decarboxylation and Ca replacement reactions via external aldimine)

formed by the decarboxylation of dihydroxyphenylalanine (DOPA). Likewise, histamine and serotonin, which have important complex biological functions, are formed by the decarboxylation of histidine and 5-hydroxytryptophan, respectively.

3.5.2.2 Cα Side Chain Replacement

An example of such a reaction is that catalyzed by serine hydroxymethyl transferase (Fig. 3.30). Here, the replacement of the hydroxymethyl group by a proton is initiated by the formation of the serine alkoxide ion which collapses to formaldehyde, and the Cα carbanion eventually gives glycine.

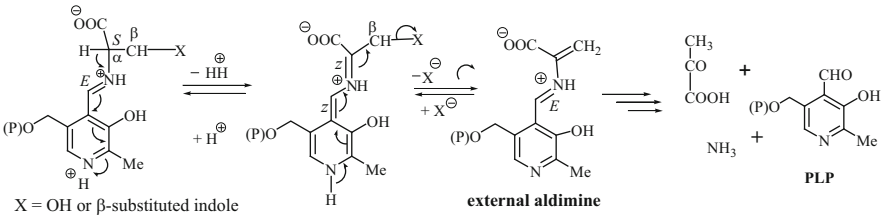


Fig. 3.31 β-Elimination reactions catalyzed by PLP-dependent enzymes

3.5.2.3 PLP-Catalyzed Reaction at β-Carbon Atom of Amino Acids

The catalytic power of PLP is observed in reactions involving groups on the β-carbon (Fig. 3.31). Specific enzyme catalyzed β-elimination takes place when a good leaving group is present. Serine dehydratase or tryptophanase is an example of an enzyme when serine or tryptophane is used. The Cα−H bond is just broken to give a delocalized carbanion, which subsequently expels the leaving group (here H_2O; indole when tryptophan is used) to form the external aldimine. The latter is hydrolyzed to the free enamine which decomposes to pyruvic acid, ammonia, and PLP.

3.5.2.4 Stereochemical Concepts of the Pyridoxal Phosphate (PLP) Catalyzed Reactions

Much work has been done in this area and the salient interesting points are summarized below:

Reaction specificity imposed by the enzyme protein upon the system is achieved by control of the conformation around Cα−N bond of the substrate cofactor complex. The bond to be broken must be orthogonal to the plane of the conjugated π system. In this conformation, the breaking σ-bond achieves maximal orbital overlap with the π system, resulting in a substantial rate enhancement. Thus, the pyridine ring, the C4′ amino nitrogen and Cα of the coenzyme–substrate complex must lie in a plane for resonance stabilization. Hence, the three conformers (A), (B), and (C) (Fig. 3.32) represent the orientations of the complex for the enzyme-catalyzed cleavage of the Cα−H bond, the Cα−COOH bond, and the Cα−Cβ bond, respectively.

The reactions of PLP enzymes take place on one face of the planar PLP–substrate complex—the *exposed* or *solvent* face is the *Si face* at C4′ of the cofactor—the other face being covered by the protein. A chemically intuitive concept is that the enzyme binds the relatively rigid PLP cofactor at the points: pyridine N, and the phosphate, and the third point is probably the single distal carboxyl group on the substrate, resulting in a particular conformation by the Cα−N bond. It has been

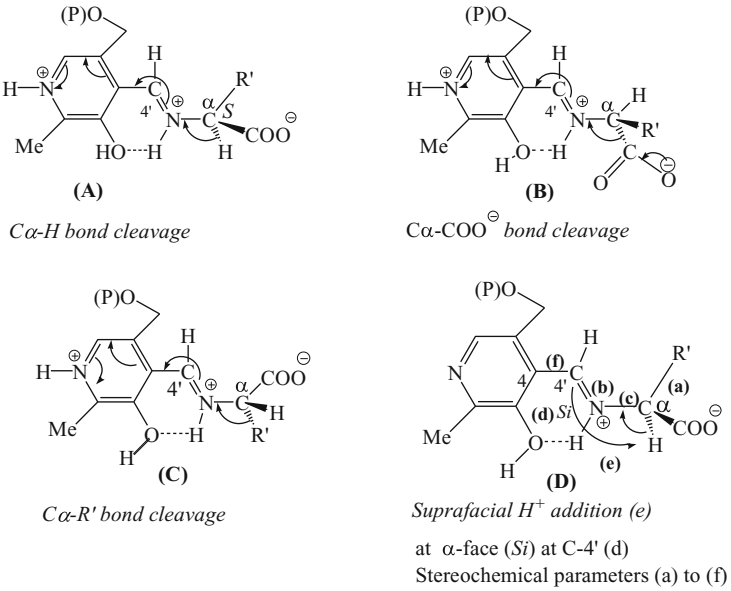


Fig. 3.32 Optimal conformations about the Cα-N bond for cleavage of the Cα-H(A), Cα-$COO^{\ominus}$(B), or Cα-R'(C) bonds of L-amino acid, and suprafacial H^+ addition. Stereochemical parameters of enzymatic transformation (**D**)

shown that group interchanges take place *in a retention* mode and proton transfer with *suprafacial geometry*.

The stereochemistry of the whole transamination reaction, catalyzed by the given specific enzymes and the cofactor PLP, thus involves the following six parameters [(**D**), Fig. 3.32] *established through experiments of many workers.*

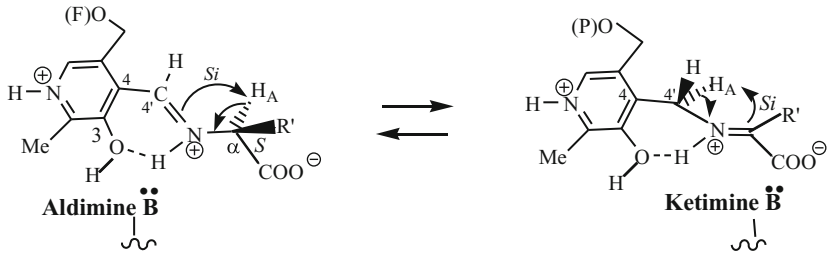
(a) ***Configuration at Cα of the substrate***: Only the L-enantiomer (and not D-) of an α-amino acid reacts.

(b) ***Configuration of the C4′ = N double bond*** must be *E* (or *trans)*: a *Z* (or *cis*) double bond will not be coplanar with the pyridine ring due to steric interference by the adjacent ring substituents (*ortho)*.

(c) ***Conformation around the Cα–N bond***. As discussed earlier, the bond to the Cα to be broken must be perpendicular to the plane of the conjugated π system [as shown in conformers (A), (B), and (C) in Fig. 3.32].

(d) ***Site of proton addition or deprotonation at C4′*** has been shown to be on the *Si* face or α-face of the structure (D) of the coenzyme–substrate or the coenzyme–intermediate complex, as revealed from studies with many specific transaminases.

(e) ***Mode of prototropic shift from Cα of the substrate to C4′ of PLP*** has been demonstrated to be *suprafacial* (not *antarafacial*) by experiments involving internal transfer of tritium and deuterium. Internal proton transfer in aldimine



Note: *Protonation of pyridine N makes the* α-H_A / 4' H_A *more acidic.*

Fig. 3.33 Aldimine ketimine tautomerization in the transamination of an L-amino acid

ketimine tautomerization (Fig. 3.33) strongly suggests that the deprotonation and protonation are mediated by the simple base which necessitates *suprafacial* nature of the process. Thus, for such reaction the conformation around Cα−N single bond must be such that Cα−H is exposed on the side of the complex corresponding to the *Si face* at C4′, *i.e.,* in an L-amino acid–coenzyme complex the carboxyl group and C4′ would be *trans* to each other (Fig.3.33).

(f) ***Conformation around C4–C4′ bond***. Model studies and calculations show that in the absence of the enzyme, pyridoxal Schiff bases prefer a *cisoid* conformation (C4′ = N imine bond is on the same side of C4–C4′ bond as the C3–OH). Of course, during catalytic process a reorientation of the cofactor takes place. X-ray diffraction studies revealed that the cofactor-lysine Schiff base is in the *cisoid* conformation with the C4′ = N roughly coplanar with the pyridine ring, and the *Si* face against a β-sheet of the protein. Moreover, the *cisoid* form of all aldimines and ketimines are expected to be more stabilized than the transoid form, by H-bonding between oxygen of OH and 4′-NH, as shown in the conformers (A) to (D).

3.6 Addition of C_1-Unit with AdoMet (SAM)

3.6.1 Methylation

In the biosynthetic process of C_1-unit addition, the enzymatic transfer of a methyl group takes place from L-**methionine**. The latter, prior to its participation in methyl transfer, is activated by its conversion to *S*-**adenosylmethionine** (**SAM or AdoMet**) by an unusual S_N2 reaction with ATP by the action of methionine adenosyl transferase, in which the nucleophilic sulfur atom of methionine attacks the C5′ on the ribose unit expelling the inorganic triphosphate (*i*PPP) rather than attacking any phosphorus atom. Thus, an unstable *sulfonium ion* is formed having a

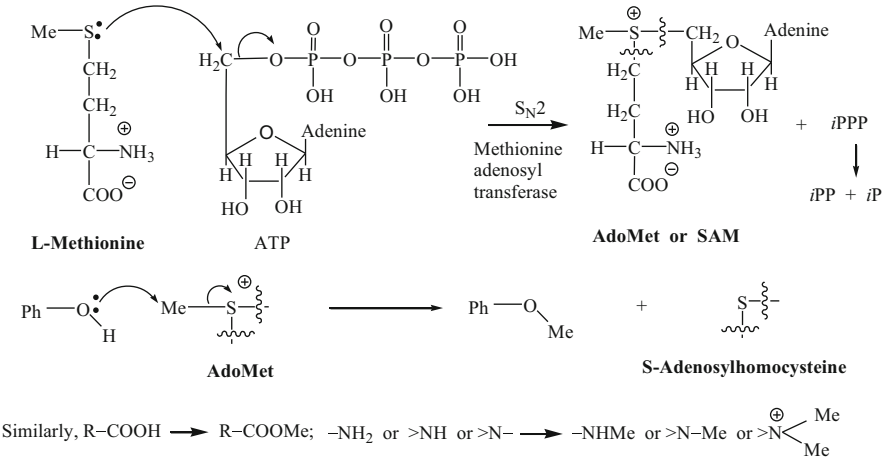


Fig. 3.34 Biogenetic O-methylation or N-methylation by the strong electrophile *S*-adenosylmethionine (SAM or AdoMet)

high thermodynamic tendency to transfer its methyl group and gets neutralized. The expelled triphosphate is enzymatically hydrolyzed to pyrophosphate (*i*PP) and orthophosphate (*i*P) (Fig. 3.34). SAM is a potent strong methylating agent and it undergoes facile attack by oxygen or nitrogen nucleophiles.

3.6.2 Formation of Methylenedioxy Bridge and Its Reductive Opening [8, 9]

The methylenedioxy moiety is formed via regiospecific monomethylation of an *ortho*-dihydroxybenzene system, followed by the removal of a hydride (*cf.* anti elimination of e^- and $H^\bullet$) to form a carbocation, stabilized by resonance by a nonbonding lone pair of the adjacent oxygen (see Fig. 3.35). The *ortho* OH then attacks the carbocation center to form a methylenedioxy bridge, a common functional moiety in many natural products.

Studies with labeled AdoMet showed the stereochemical implication of the transformation of a labeled methoxy into a labeled methylenedioxy group, followed by reductive opening of the latter to a labeled methoxy function, as illustrated in the biosynthesis of *protoberberine type* isoquinoline alkaloids by enzymes from plant cell cultures, *e.g.,* conversion of 2-O-demethyljatrorrhizine (**1**) to labeled *jatrorrhizine* (**4**) via (**2**) and labeled *berberine* (**3**) (Fig. 3.35).

The reaction sequence involves the transfer of a chiral methyl group from SAM to oxygen of a protoberberine alkaloid (**1**) to form (**2**) with inversion of configuration. The latter is then fed into callus cultures of *Berberis koetineana*, which

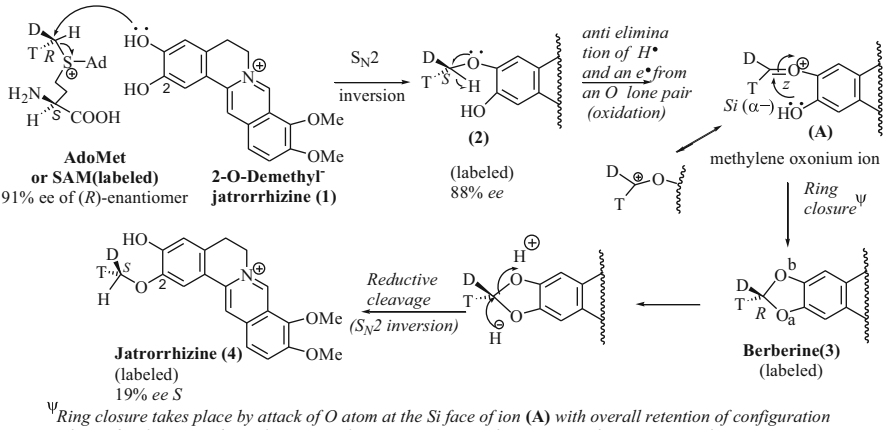


Fig. 3.35 Formation of the methylenedioxy bridge of berberine and its subsequent reductive opening to form the hydroxy/methoxy functions of jatrorrhizine with stereochemical mechanism.

converted it via berberine (**3**) into jatrorrhizine (**4**). By degradation, it was found that the chiral methyl groups in the protoberberine (2) and jatrorrhizine were both having (*S*) configuration.

It follows that probably the oxidative closure to form the methylenedioxy bridge occurs with retention of configuration, and the labeled methylenedioxy bridge reductively opens up with inversion of configuration. Thus, the migration of the chiral methyl from the original oxygen to the adjacent (vicinal) oxygen is formally equivalent to inversion. The methyleneoxonium ion (A) has a significantly lower energy barrier to rotation, and can to a significant extent undergo configurational isomerization prior to attack of oxygen on the methylene carbon, accounting for partial racemization.

3.6.3 N-*Methylation and Formation of a Methylene Bridge Between Nitrogen and Carbon [10]*

The conversion of >NH to –NCH_3 by transfer of the chiral methyl from labeled Ado-Met to the nitrogen proceeds clearly with complete inversion of configuration. The subsequent transformation of the chiral *N*-methyl group into a chiral methylene bridge between nitrogen and carbon takes place in presence of the specific enzyme. This sequence of reactions has been demonstrated to lead to labeled scoulerine, a *tetrahydroprotoberberine* type alkaloid, as shown in Fig. 3.36.

From the tritium NMR analysis of scoulerine formed, it is apparent that the replacement of a hydrogen of the chiral *N*-methyl group by the aromatic ring carbon

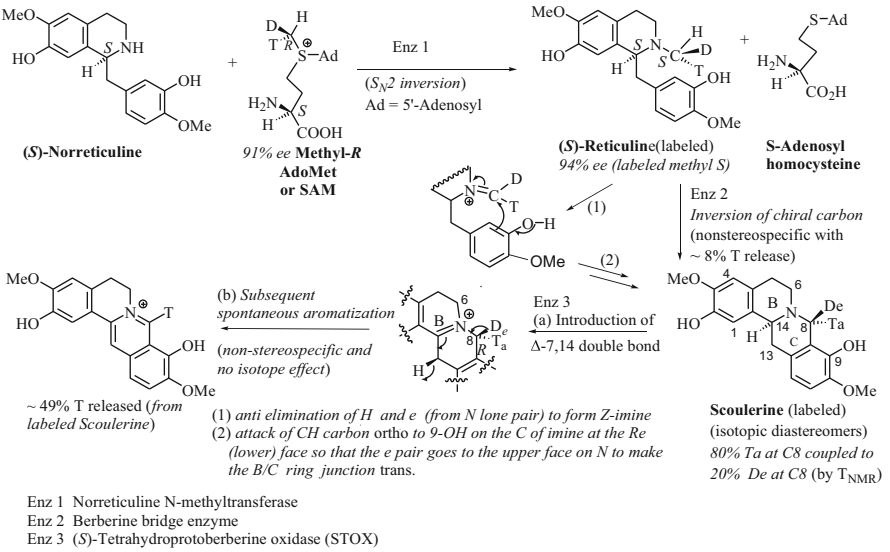


Fig. 3.36 Enzymatic reaction sequence in the N-methylation of norreticuline, and generation of the berberine bridge followed by its further oxidation

has occurred *with inversion*, and that the hydrogen abstraction has an isotope effect k_H/k_D 4. It is observed that in the berberine bridge the enzyme releases about 8 % T from (*S*)-Me group of (*S*)-**reticuline**, consistent with the observed isotope effect of 4. Subsequent addition of the enzyme STOX leads to 49 % T release, nearly half of the remaining T from the two *isotopically diastereomeric* substrates indicating *non-stereospecific nature of the reaction* lacking any isotope effect. Thus it is postulated that STOX only catalyzes the introduction of a $\Delta^{7,14}$ double bond, and subsequent aromatization occurs spontaneously (Fig. 3.36).

3.7 C- and O-Alkylation

3.7.1 C and O-Alkylation of Phenols

Both C- and O-alkylation of phenols are quite prevalent in natural products. The alkyl groups mostly consist of methyl and prenyl groups. SAM (AdoMet) serves as the methylating agent (Sect. 3.6), and various prenyl groups as their pyrophosphates act as the prenylating agents. Figure 3.37 depicts different types of probable C- and O-prenylation and cyclization of polyphenolic compounds like resorcinol.

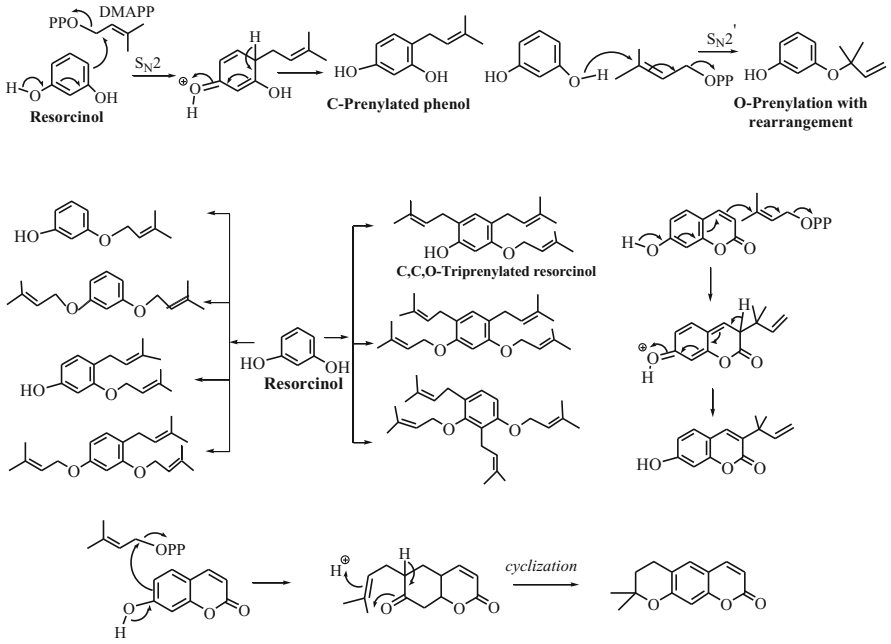


Fig. 3.37 Different types of probable C- and O-prenylations of polyphenols like resorcinol, and cyclization

The formation of C_5, C_{10}, C_{15}, and C_{20} prenyl pyrophosphates has been discussed in detail in Chap. 5. Of all the prenyls, C_5 and its modified forms occur most prevalently. Since the leaving groups of the natural alkylating agents do not vary, their influence on the proportion of the C- and O-alkylation, as observed in the laboratory reactions with different leaving groups, does not occur. Further, the specificity of the catalytic enzymes also plays an important role in the *regiospecificity* of alkylation, as is observed in coumarins, xanthones, flavones, etc. Coumarins provide best examples of such specificity. Figure 3.38 displays some examples of regiospecific methylation, C- and/or O-prenylation/s, prenyl chain modification of *resorcinol*, *umbelliferone,* and *6-hydroxyumbelliferone* to produce natural coumarins [11]. Sometimes prenyl groups are locked in a cyclic moiety (*e.g.*, *pyranocoumarin* or *furanocoumarin* derivatives). In coumarins, perhaps the largest number of biogenetic modifications of the simple isoprene unit occurs, and also quite often they appear in a cyclic form [8]. C-Alkylation takes place at the available *ortho-* position of an activating group like OH before it may undergo O-alkylation [11].

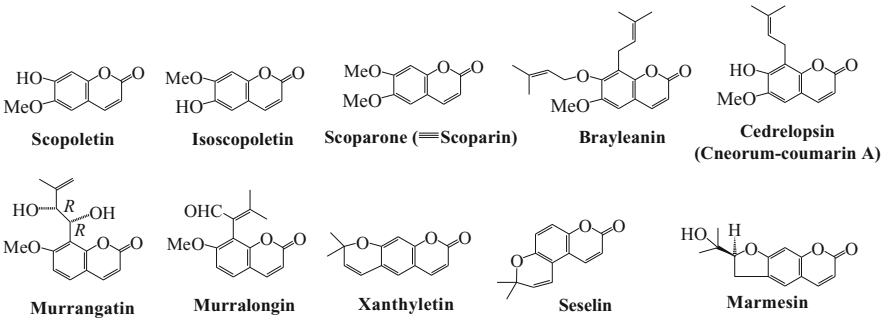
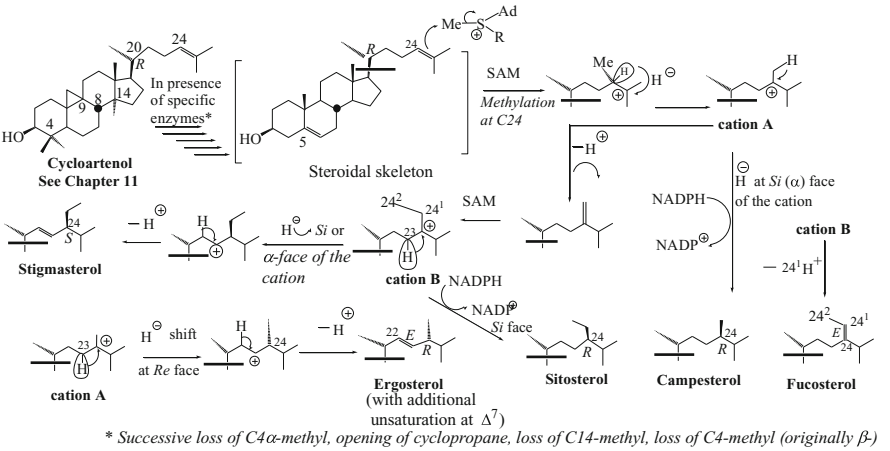


Fig. 3.38 Regiospecific methylation, C- and O- prenylation, prenyl chain modification, cyclization etc. to form some natural coumarins

3.7.2 C-Methylation and Modification of Cycloartenol Side Chain to Form Phytosterols [12, 13]

All phytosterols except cholesterol contain extra carbon atom/s at C24 of the side chain compared to its precursor cycloartenol. Cholesterol occurs mainly in the animal organisms and less abundantly in plant cells. Cycloartenol is metabolized in the plant cells to yield phytosterols including cholesterol (Chap. 11). The presence of a double bond (Δ^{24}) in the side chain of the precursor plays a vital role as an obligatory π-bond nucleophile towards the electrophilic SAM (AdoMet). The electrophilic addition of the methyl group takes place from the *Si* (α-) face of the double bond at C24. Through labeling experiments it has been shown that the resulting C25 carbocation **A** is quenched by delivery of hydride either from NADPH at the *Si* (α-) face of the cation to form **campesterol**, or from the adjacent C23 methylene at the appropriate *Re* face, followed by loss of H^+ from C22 to form **ergosterol** having (24*R*) and *E* stereochemistry of the 22–23 double bond Fig. 3.39). The newly formed sp^3 chiral carbon always appears in one epimeric form—a characteristic of enzymatic reaction. The carbocation **A** may also be quenched to a double bond by loss of H^+ from the methyl group, followed by methylation by SAM to form the cation **B**, which upon delivery of hydride by NADPH at the *Si* (α-) face of the cation forms **sitosterol**. Alternatively, the cation **B** may be quenched by a hydride shifted from C23 giving rise to the C23 cation, followed by loss of the appropriate proton to form **stigmasterol** having (24*S*) and *E* stereochemistry of the 22–23 double bond. Thus, in cases of ethyl group (C_2) containing sterols (sitosterol and stigmasterol) two methylations by SAM take place (Fig. 3.39).



** Successive loss of C4α-methyl, opening of cyclopropane, loss of C14-methyl, loss of C4-methyl (originally β-) and formation of 5,6 double bond.*

Each step in this Figure is catalyzed by a specific enzyme present in plant cells.

Fig. 3.39 Side chain methylation and modifications to form different phytosterols

3.8 Other Important Biological Events

Many reactions/rearrangements like Wagner–Meerwein rearrangement, Baeyer–Villiger oxidation, Michael addition, Markovnikov addition, less likely anti-Markovnikov addition, diradical coupling reactions, etc., and rarely 3,3-sigmatropic rearrangement do occur in plant cells, being catalyzed by suitable enzymes during the biosynthesis of natural products with various skeletal patterns. These have been discussed in the biosyntheses of pertinent natural products in different chapters.

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Chapter 4
Natural Products Chemistry: A General Treatment

"*The plant kingdom is a virtual goldmine of new chemical compounds waiting to be discovered.*" [1]

"*Isolation of secondary plant metabolites constitutes a chemical research project with significant potential for future benefit to mankind.*" [1]

4.1 Introduction. Isolation

The most important part of the natural products chemistry work is the isolation of the constituents in the pure state from their source/s. Since natural products possess different structural patterns and properties the procedures for their isolation need a lot of manipulations and modifications over the usual general procedures (Figs. 4.1, 4.2, 4.3, 4.4, and 4.5) given as models. Further, with the advent of new isolation techniques and refinements of the old ones, it is now possible to isolate most of the secondary metabolites including the trace ones present in the crude extract. This was almost an impossible task till the mid-twentieth century when only the major components could be isolated. The earlier laborious procedures have now been reduced to some time-saving efficient procedures. The crude extracts of the plant materials should first be fractionated adopting some suitable procedures, and the different fractions may then be subjected to easily available classical separation techniques, such as column chromatography (CC) (Sect. 4.1.6), flash chromatography (FC) (Sect. 4.1.7), and thin-layer chromatography (Sect. 4.1.8), as needed. These techniques have been discussed in some detail, based on the experience of the authors in sixties to eighties. These details will be handy and useful, especially to those who are working in different corners of the world with profuse plant resources, but lacking the expensive materials/tools needed. The fractions remaining after isolation of the major constituents may then be subjected for isolation of minor or/inseparable constituents to sophisticated expensive separation techniques like HPLC, MPLC, GC-MS, etc., in laboratories where these are

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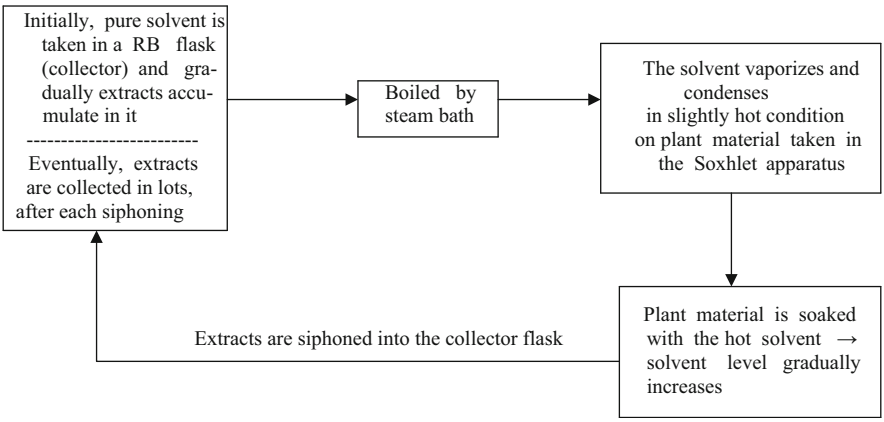


Fig. 4.1 Recirculating system of solvent extraction in a Soxhlet apparatus

available. The high-resolution NMR spectroscopy if available in any organic chemistry laboratory may preferably be used for monitoring the constituents (natural or synthetic) starting from their crude states.

4.1.1 Herbarium Specimen. Voucher Specimen

Prior to the commencement of the isolation work, a *voucher specimen* of the plant under chemical investigation, preferably with flowers, the latter being a better marker, should be preserved after establishing its identity by comparing it with an authentic *herbarium specimen*. *Herbarium* is a collection of specimens of dried and pressed plant materials (usually leaves and flowers), properly mounted on sheets arranged systematically. Each sheet carries the botanical name and family of the plant and place, season, and date of its collection. The plate should record particularly the characters which may be lost after drying and should be housed in a botanical museum in such a way that it can be preserved for centuries. Such specimen sheets are used for authentic identification of plants. The factors affecting the ecology of plants, e.g., the place or location and the time of collection, and the maturity of the plant under investigation (if known) should be recorded in the voucher specimen. These factors should be mentioned while publishing the work. The maturity of the plant is usually not recorded in the literature.

4.1.2 Ecological Influence on Plant Constituents. Plant Names. Plant Parts

Ecological factors mentioned above are important because the chemical constituents of plants (natural products) are quite often the function of these parameters. The chemical constituents of the same plant grown at different places and collected in different seasons might vary in kinds and yieldwise. The maturity of plants, especially in cases of higher plants, usually causes variations in natural products contents: some unreported compounds may be present, and some isolates obtained earlier may be missing. That the geographical parameters quite often play an important role in the formation of the secondary metabolites is evident from an example of a plant, namely, *Vinca rosea* (syn. *Catharanthus roseus*). The plants grown in India and Madagascar and other places vary remarkably in their alkaloidal content [2]. To cite an example: dramatic changes due to seasonal variations have been observed in the monoterpene constituents of the oil from both *Rosmarinus officinalis and Salvia officinalis* [3, 4].

Plant names The botanical name of a plant has two parts. It starts with the genus (e.g., *Vinca*—conventionally the name of the genus always starts with a capital letter), followed by the name of the species (e.g., *rosea* which always starts with a small letter), and they appear in italics in print (*Vinca rosea*). The name is underlined singly when written by hand (e.g., Vinca rosea). Sometimes two or more different names appear for a particular plant, identified independently by different botanists. Even after establishing their identity, these names are retained in the literature as synonymous (syn.) (e.g., *Vinca rosea* Linn., syn. *Lochnera rosea* (Linn.) Reichb. f., syn. *Catharanthus roseus* (Linn.) G. Don [3, 5]), for convenience of researchers. This nomenclature known as "Binomial nomenclature" introduced by a Swedish Botanist Carolus Linnaeus (Carl von Linne', 1707–1778) first appeared in Linnaeus' "Species Plantarum" in 1753 and then in a book *Systema Naturae* written in Latin language. This nomenclature is applicable to both plants and animals. Initially Latin names were introduced. Later Greek names have also been used. By this practical method a plant could be quickly placed in a named category. After the botanical name of the plant usually the name/s or short name/s or initial/s of the identifier/s appear/s. The name of the family to which the plant belongs should also appear in parentheses, not italicized, and starts with a capital letter (e.g., *Vinca rosea* Linn.) (Apocynaceae)—the suffix "ae" generally appears at the end of the name of the family. Some examples are cited in Table 4.1.

Plant parts The part/s of the plant (leaves, flowers, stems, stem-bark, roots, trunk bark, root-bark, and heart wood (in cases of trees); fruits, exudates, seeds), with which the chemical work is done, should be recorded since the chemical components of different parts of the same plant, especially a tree, vary in kinds and concentrations. In cases of small plants like herbs, creepers, and sometimes even small shrubs, the whole plant materials are conveniently used for chemical work.

Table 4.1 Names of some plants and their families [5]

Name of the plant	Family
Conium maculatum Linn.	Umbelliferae
Jatropha curcas Linn.	Euphorbiaceae
Lawsonia alba Lam	Lythraceae
Alstonia scholaris R.Br.	Apocynaceae
Stephania glabra (Roxb.) Miers	Menispermaceae
Cinchona calisaya Wedd.	Rubiaceae
Aegle marmelos Correâ	Rutaceae

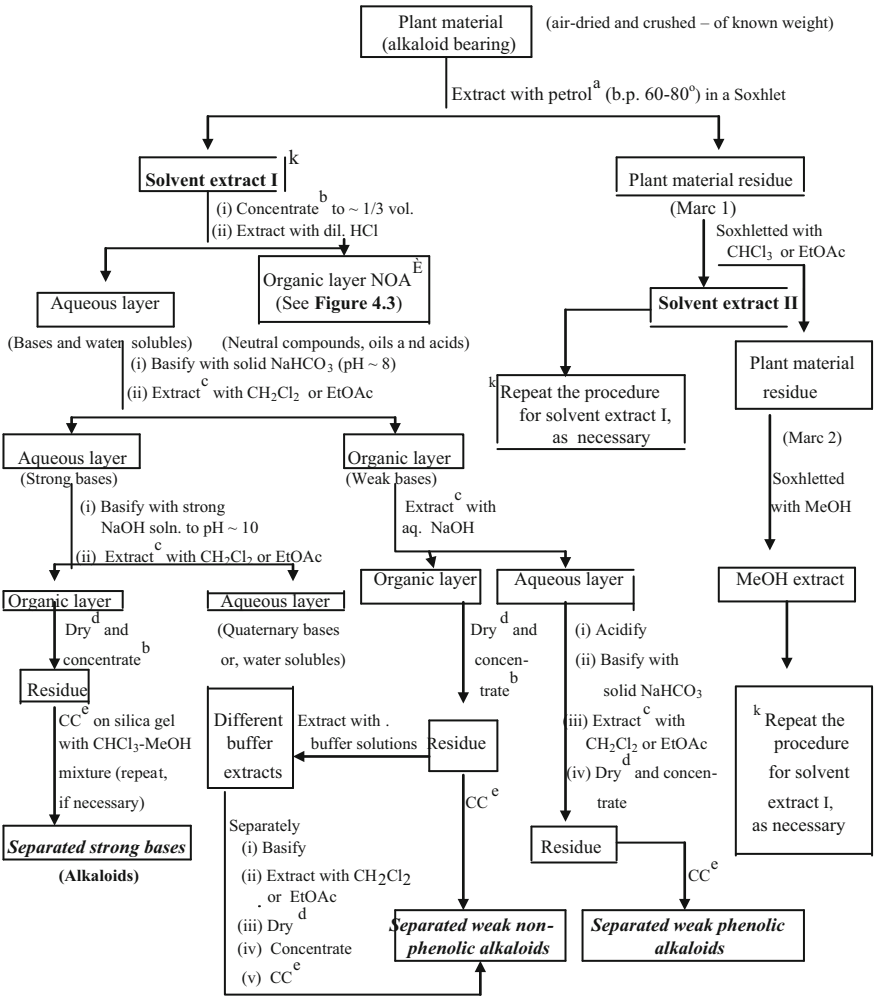
Part/s of the plant, one has worked with, should always be mentioned for precise information and reproducible data.

4.1.3 Literature Survey. Phytochemicals. Chemotaxonomic Significance

One essential element of any research is the search of relevant literature. Thorough literature survey on the chemical studies of the plant/s under investigation is a must. In cases of chemically virgin plants, work on other species of the same genus and sometimes on other genera of the same family should be searched to know the structures of their chemical constituents. This will be of immense help in the manipulation of the isolation process (Figs. 4.2, 4.3, 4.4, and 4.5) and also for easy identification of the known compounds. Literature survey, especially the biosynthetic knowledge of the compounds already reported from the plant species of the same or different genera of the family, helps largely for the structural elucidation of new compounds: the plant species of the same genus are likely to elaborate compounds with same or biogenetically related skeletal patterns on genetic grounds. Chemical studies on innumerable plants have shown compatibility between taxonomical classifications and the phytochemicals produced in the same genus or allied genera within the family. A few such observations are cited in the sequel.

Rutaceae plants are well known for elaborating coumarin derivatives (Sect. 13.2), which are absent in Apocynaceae plants. Again indole alkaloids occur abundantly in a number of genera of Apocynaceae plants, while Rutaceae plants do not produce them. Likewise, many plant families may be correlated with the types of compounds they produce—and a chemotaxonomic relationship is established. In some cases the taxonomical classification (genus or even family) has been revised based on the class of the phytoconstituents.

With the help of new analytical and instrumental techniques such as CC, preparative TLC and HPLC, GC-MS, ^{1}H and ^{13}C NMR (one and two dimensional), EIMS, HRMS, and FABMS spectra, etc., a large number of individual compounds can be isolated from a plant and can be characterized unambiguously in a relatively



[a] Usually $CHCl_3$ and EtOAc extracts and not petrol extract may respond to Dragendorff's reagent. In case of nonalkaloid bearing plants of some families, or alkaloids not extractable by petrol, subsequent procedures for alkaloids are omitted.

[b] Concentrate always in a flash evaporator under reduced pressure at ~ 40 °C.

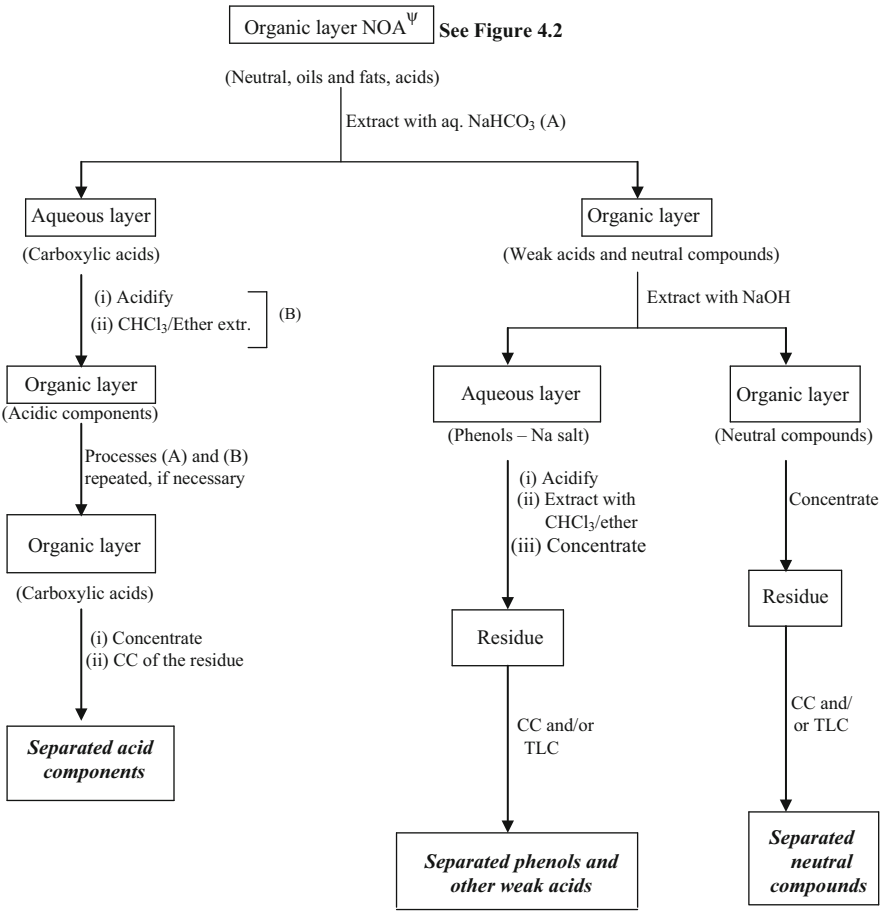
[c] Extract >2 instalments with ~ 1/3 volume of the phase being extracted.

[d] Organic extracts should be dried over anhydrous Na_2SO_4 or $MgSO_4$ to get the residue for CC and/or TLC.

[e] CC (column chromatography), followed by preparative TLC and/or HPLC/MPLC, as required.

Fig. 4.2 A general scheme for separation of strong bases, weak non-phenolic and phenolic bases and the quaternary basic fraction from petrol, $CHCl_3$/EtOAc, and MeOH extracts of plant material

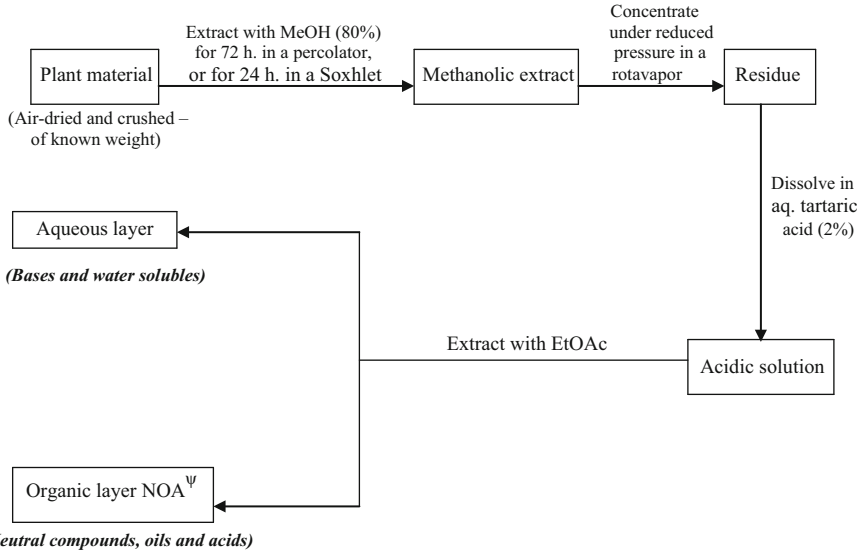
short period of time. The chemical knowledge gained not only helps the taxonomists but also stimulates interest of the chemists and biochemists involved in biosynthetic work. The occurrence of a given compound or its congeners in two related species often gives evidence of common steps in biosynthesis.



All footnotes excepting 'a' of **Figure 4.2** are applicable in relevant steps.

Fig. 4.3 A general scheme for separation of *neutral compounds, acids, phenols*, and other *weak acids* from petrol and $CHCl_3$ or EtOAc extracts of plant material

Extensive studies on the chemotaxonomic significance of various types of natural products have been carried out. Only a few of such studies will be briefly mentioned. Part 18 of the series, "Phytochemistry and Chemotaxonomy of the Convolvulaceae," concerns the occurrence and distribution of 74 tropane (including 4 new) and 13 biogenetically related pyrrolidine (including nicotine) alkaloids in 18 *Merremia* species (Convolvulaceae) [6] of tropical occurrence. The extensive GC-MS study with aerial parts as well as with roots led to the isolation and structure elucidation of four novel 3-acyloxytropanes. Each species, *excepting two*, included in this study is capable of producing simple tropanes, and thus the presence of such



Treat the aq. layer for different types of alkaloids (as are present), as outlined in ***Figure 4.2*** *and the organic layer NOA$^{\Psi}$ for neutral compounds, acids, phenols and other weak acids (as are present), as outlined in* ***Figure 4.3.***

Fig. 4.4 Direct extraction of plant materials by MeOH (80 %) and isolation of alkaloids, neutral compounds, and acids

metabolites is a common trait in this genus. Those *two species* are segregated from the genus and transferred to a novel one, *Xenostegia*. This unique observation in lacking tropanes thus supports this transfer chemotaxonomically.

The chemotaxonomic significance of the isolated compounds from the water-soluble part of extracts of two *Digitalis* species and some other related species of Plantaginaceae family (N.O. Digitalideae) has been discussed [7]. Recent extensive chemosystematic investigations of the family Scrophulariaceae have led to significant changes in its limitation. Many former members of the family have been assigned to a largely expanded Plantaginaceae. Within this family the chemotaxonomy of the genus *Plantago* has been recently reviewed [7]. Thus, enormous amount of accurate chemical work is to be done using modern techniques for the analysis of all possible isolated constituents of different species of the same genus or related genera for getting significant chemotaxonomic information and for revealing the many closely interwoven biosynthetic routes. Hence the use of the chemical constituents of plants as an aid to their classification is now a familiar concept. However, a few fortuitously isolated substances cannot always give much phylogenic information. In the end, the presence or absence of the enzyme systems involved and the alterations in their substrate specifications and catalytic activities will provide the most illuminating information.

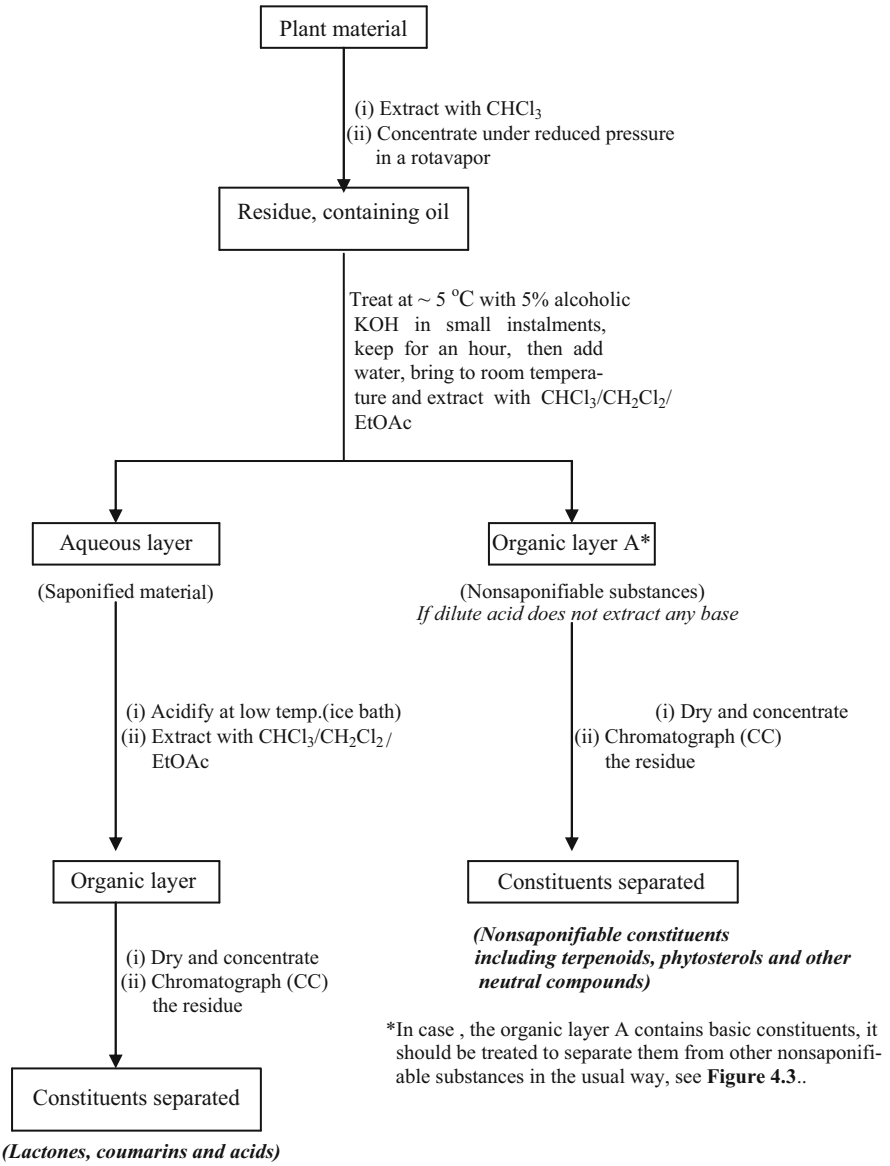


Fig. 4.5 Separation of saponifiable constituents of plant extracts from nonsaponifiable ones

4.1.4 *Isolation of Plant Constituents: Solvent Extraction. Buffer Extraction. Thimble Extraction. Steam Distillation*

The plant materials are usually sun-dried, crushed to powder in a grinding machine, and weighed. The weight is necessary for calculating the percentage yield of the constituents of the plant materials. A few general methods of isolation will be outlined (Figs. 4.1, 4.2, 4.3, and 4.4) briefly in the sequel. *These are just the skeletal procedures for the specified types of compounds*. For better results one can make a lot of modifications, designed by experience, and by trial and error methods. Though the present generation of chemists is fortunate to have more sophisticated and time-saving equipments for the effective separation of the natural products from their sources, the old methods responsible for the present developments are worth inclusion in the current books on natural products of plant origin in order to get an idea of the principles involved. They may be useful for simple separation of the major components.

Extraction in a Soxhlet apparatus The dried and powdered plant material is extracted in a suitable Soxhlet apparatus [8, 9] successively with organic solvents of increasing polarity, e.g., petrol (b.p. 60–80 °C), chloroform or ethyl acetate, and methanol (or ethanol) to effect some sort of separation during the initial extraction process. The Soxhlet apparatus is a device for repetitive use of nearly the same volume of solvent to extract the plant materials through boiling, condensing, soaking the plant materials, and siphoning the solvent extract. Somewhat hot extraction (due to the vapor of the solvent) takes place. The Soxhlet apparatus, made of glass, of different sizes (5 l, 3 l, 2 l, 1 l, 500 ml, 250 ml, 100 ml, 50 ml) are available. It is a recirculating system and its operation may be represented as shown in Fig. 4.1.

Extraction in a percolator The dried and powdered plant materials (known weight), if available in kgs, can also be extracted in a metal percolator (with a lid at the top and a tap at the bottom) at room temperature, by keeping the plant materials soaked thoroughly with a less volatile polar solvent (e.g., rectified spirit/methanol, etc.) for a week or so and then taking out the extract completely by opening the tap at the bottom. The extraction is repeated. Fresh (undried) plant materials (usually leaves) are sometimes used for extraction at room temperature, especially for light and heat sensitive constituents. However, in case of fresh leaves, the extracted chlorophyll interferes with the separation procedure. If the plant material is available in less quantity (0.1–0.5 kg), suitably designed small metal/glass percolatorsand volatile solvents like chloroform or ethyl acetate may be used.

General schemes for the isolation of weak bases, strong bases, and quaternary basic fractions (Fig. 4.2) and for the isolation of acids, phenols, and other weak acids and neutral compounds (Fig. 4.3) from solvent extracts of plant materials are presented. At every stage one should monitor the organic layer by micro-TLC (*vide*

infra) and proceed accordingly. The schemes may be simplified if constituents of basic or acidic nature are absent. *These procedures may be followed for work-up of chemical reaction mixtures also, as needed.*

Buffer extraction Complex mixture of alkaloids (as evident from Dragendorff test[1] and micro-TLC), present in the basic fraction of the plant extract, can be successfully separated by extraction with buffer solutions of different pHs, taking advantage of the different basicities of the alkaloids present, as indicated in the lower part of the Scheme in Fig. 4.2. For this purpose, a countercurrent distribution apparatus may be conveniently used.

The following procedure (Fig. 4.4) may be conveniently used for extraction and isolation of alkaloids from different parts (leaves, twigs, stems, stem-bark, trunk-bark, roots, or root-bark) of plants bearing alkaloids (positive Dragendorff test).

Lactones and coumarins can be separated from the nonsaponifiable components by saponification. This procedure is generally followed to isolate the lactones or coumarins from plant oils (Fig. 4.5).

Components of different extracts Petrol (b.p. 60–80 °C) or hexane generally extracts less polar monoterpenoids, sesquiterpenoids, di- and triterpene hydrocarbons, carotenes, fats, and waxes. Chloroform usually extracts sesquiterpene lactones, diterpenoids, less polar triterpenoids, sterols, oxygenated flavonoids, coumarins, alkaloids, etc. Ethyl acetate extracts more oxygenated di- and triterpenoids, sterols, and alkaloids. Methanol (90 %) or ethanol (90 %) extracts highly oxygenated (hence polar) di- and triterpenoids, flavonoids, steroidal glycosides, and alkaloids, etc. Finally, water extracts highly polar substances like glycosides, free sugars, and amino acids, etc. Solvents may be arranged in order of increasing polarity and "solvent power" toward polar functional groups as stated in the sequel, under '*Eluting Solvents*'.

Thimble methods *In a more convenient procedure* the dried and powdered plant material (weighed) is extracted with ethyl acetate and/or 90 % aqueous ethanol/methanol. The crude residue (may be solid, resinous, or tarry), obtained by concentration/evaporation of the extracts in a rotavapor under reduced pressure at a temperature <40 °C, is thoroughly mixed with a requisite amount of celite. The mixture is taken in a suitable thimble (made from good thick filter paper sheet by rolling, stapling, and closing one end), which is then put into a Soxhlet apparatus of appropriate size, and successively reextracted with petrol (b.p. 60–80 °C), or hexane, chloroform, and ethyl acetate, and finally with 90 % methanol to effect broad fractionation. Different solvent extracts are collected and concentrated. The residues obtained are processed to separate the acidic, basic, and neutral fractions in the usual way; each fraction is then chromatographed to isolate the pure constituents.

Various essential oils containing monoterpenes, sesquiterpenes, and some other volatile compounds as components are obtained from plant materials by water

[1] A small crystal of potassium bismuth iodide when added to an acidic solution of an alkaloid taken in a watch glass, generally a yellow to orange precipitate is obtained.

distillation, by steam distillation, and sometimes by a combination of the two, when the components are codistilled.

Direct steam distillation Plant materials, cut into small pieces (generally flower petals), are heated in water for some time in a distilling flask and then distilled. Here the steam is produced in situ. As the steam is removed with volatile compounds, water is added dropwise from the separatory funnel fitted with the flask. The collected distillate contains essential oils and other volatile compounds. During efficient distillation for a longer period, sometimes the esters, if any, may get hydrolyzed.

Live steam distillation Crushed plant materials are arranged on grids of a vessel from the bottom of which steam as such or under pressure is allowed to pass through the grids. The steam comes in contact with the plant material and carries with it the volatile components, which are collected in the distillate. Geraniol, citral, menthol, caryophyllene, etc., are obtained by this method. Here steam is used as one of the immiscible phases and the mixture of immiscible liquids boils at a constant temperature lower than the boiling points of the pure constituents present in the mixture and of water.

Quite often grids containing the plant materials are preheated by steam coils and then are allowed to come in contact with steam directly. Steam carrying the essential oils is collected in the distillate. In the coil, the steam is generally allowed to pass under low pressure.

Another efficient method is the *supercritical fluid extraction method* in which liquid carbon dioxide is used for the extraction of the essential oils.

4.1.5 Chromatography: Different Techniques [10–14]

Chromatography is the most powerful tool for the separation of two or more components present in a mixture. It is most widely used in chemical laboratories as well as in chemical industries for effecting isolation and purification. Since its invention, which is a great boon to chemical sciences, its full potential has been explored and utilized in basic research, industry, and medical sciences with great success. It is a physical method of separation effected by the distribution of the components between two phases—one of which, being in the form of a porous bed or bulk liquid layer or film, is immobile (*stationary phase*), while the other one is a fluid (mobile phase) that percolates over or through the stationary phase on which the sample is applied.

Various chromatographic techniques are in use. The common ones are conveniently classified based on the nature of the phases employed for the separation (Fig. 4.6) [10, 14]. Two distinct phases are required to set up the distribution resulting from repeated adsorption/desorption events during the movement of the sample components along the stationary phase in the direction of the flow of the mobile phase. Thus, gas–gas chromatography does not exist and liquid–liquid chromatography is restricted to immiscible solvents. It is interesting to understand the mechanism of the different chromatographic techniques (Fig. 4.6).

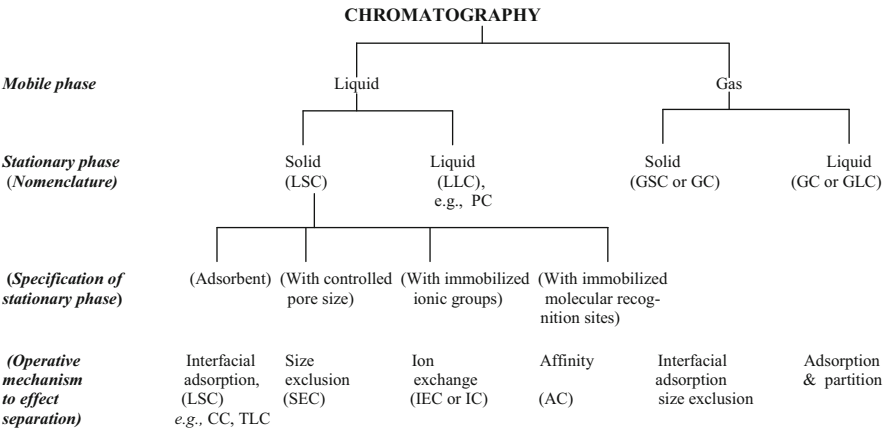


Fig. 4.6 Classification of the common chromatographic techniques

If the mobile phase is a gas, the stationary phase may be a solid or a liquid, and the separation techniques are called *gas–solid chromatography* (**GSC**) and *gas–liquid chromatography* (**GLC**), respectively. The simple term **GC** implies both these techniques, but it usually means GLC (unless otherwise specified), since this is the most commonly used technique. Here separation is caused by differences in interfacial adsorption and gas–liquid partitioning. In **GSC** the retention mechanism depends upon interfacial adsorption. If a solid of controlled pore size, such as zeolite, is used as the stationary phase, the size-exclusion mechanism operates.

If the mobile phase is a liquid, the stationary phase can be any one of the following:

(i) A solid (*liquid–solid chromatography,* LSC) with interfacial adsorption as the dominant distribution process.

(ii) A solid of controlled pore size (*size-exclusion chromatography,* **SEC**); here, the ratio of the solute size to the dimensions of the stationary phase pore sizes determines the distribution constant.

(iii) A solid with immobilized ionic groups coming in contact with the solutes in the mobile phase having electrostatic interactions operative as the dominant distribution process (*ion-exchange chromatography*, **IEC**, or *ion chromatography*, **IC**);

(iv) A solid possessing immobilized molecular recognition sites when in contact with the solute in the mobile phase (*affinity chromatography*, **AC**); here, the dominant distribution process is the three-dimensional specificity of the molecular recognition between the receptor sites and the solute (a technique more applicable in biotechnology)

(v) a porous solid coated with a thin film of immiscible *liquid* (*liquid–liquid chromatography*, **LLC**); here, the dominant distribution process is partitioning.

We will briefly discuss only a few chromatographic techniques which find extensive use in the separation and purification of small molecules like secondary metabolites or organic reaction products from their mixtures. Details of operations, operational data, and the instrumental components may be found in a number of books [10–14].

*The genesis of the term "**chromatography**"* appears to be interesting [15]. The first compounds separated by Tswett (Appendix A, A-27) using his technique were plant pigments, which appeared as colored bands on the column. He used the term ***chromatography*** in his first paper on the subject in 1906. Tswett coined the term for this technique from the Greek words "*chroma*" (color) and "*graphein*" (to write), as if, the results were *written* in *color* on the column. Some speculated that in Russian "*Tswett*" means color; perhaps Tswett named the technique after his own name (Tswett's writing ≅ '*chromatography*'). However, the term *chromatography* was known throughout the nineteenth century in connection with artists' material, especially artists' colors and pigments. George Field was the foremost *color man* of the nineteenth century; he published his treatise on *chromatography* in 1835. "It is interesting that a term, originally only used by artists, is now almost exclusively in the province of scientists" [15].

4.1.6 Column Chromatography

Adsorption Column Chromatography (LSC) It has been used most extensively for separation of simple to complex mixtures of natural products from different fractions (Figs. 4.2, 4.3, 4.4, and 4.5) or of reaction products and also for purification of individual compounds. *It has profound influence on the progress of natural products research and hence the parameters affecting the separation and some other important aspects will be treated somewhat in detail.* The method depends upon reversible adsorption of the substances in different degrees at a solid surface (adsorbent) and also upon their solubility in the solvents or solvent mixtures being used. Hence the substances are displaced from the adsorbent by the eluting solvent of varying increasing polarities at different rates, leading to their separation.

Adsorbents The most commonly used *adsorbents* are silica gel, SiO_2, xH_2O (used for polar to less polar substances), and alumina, Al_2O_3, xH_2O, obtainable in different activities (used for a range of nonpolar or less polar substances). More restricted adsorbents include charcoal (for sugars, amino acids), sucrose (for chlorophyll), calcium hydroxide (for carotenoids), etc.

Deactivation of adsorbents Anhydrous alumina or silica gel is highly activated. They can be *deactivated* by adding water to different extents. Water binds very tightly to the adsorbent taking up sites on the particles which could otherwise be used for equilibration.

Anhydrous neutral alumina (grade I) (purchased or obtained by heating alumina at 200 °C for 3 h with occasional stirring) is deactivated by adding small amounts of

water: 3 % (grade II), 6 % (grade III), 10 % (grade IV), and 15 % (grade V). Deactivated alumina can be prepared by adding the requisite amount of water to a clean big beaker, swirling it to distribute the water over the inner walls, and adding the adsorbent immediately, while continuing the swirling motion. The adsorbent should then be transferred to a distilling flask of a flash evaporator and rotated and blended for about an hour.

Capillary tube standardization process This can be used for determining the activity of alumina or silica gel produced. A m.p. capillary tube *is filled* to the top with each grade of adsorbent and a drop of benzene is put on the adsorbent at the open end. The closed end of the tube is broken and the other end (wet) is immersed in a 0.5 % solution of *p*-phenylazoaniline in benzene for a moment. The tube is then immersed in a shallow layer of benzene taken in a small jar/beaker. Migration of the solvent by capillary action is allowed to a point near the top of the capillary column. The tube is then removed and the R_f value of the dye band for each activity grade is measured. The R_f value stands for "**R**atio to **f**ront" and is defined by the following fraction, expressed as a decimal. The R_f value is characteristic of a compound under some specific conditions (solvent, adsorbent, and thickness of the layer)

$$R_f = \frac{\text{the distance traveled by the spot/dye-band}}{\text{the distance traveled by the solvent front}}$$

Both distances are measured from the center of the applied spot/band. The distance traveled by the dye is measured up to the center of the band/spot. The standardization process can also be done using microscopic TLC slides (*vide infra*).

R_f values for the dye on alumina are *approximately* as follows: grade I 0.0; grade II 0.13; grade III 0.25; grade IV 0.45; and grade V 0.55.

Silica gel may be regarded as the most versatile of all adsorbents and can be used with all solvents. *Activity grade I silica gel* (anhydrous) can ordinarily be prepared by heating it at 150–160 °C with occasional stirring for 3–4 h. Deactivated silica gel grades II–V are made by adding water to a concentration of 10, 12, 15, and 20 %, respectively, following the same way as described in case of deactivated alumina. The capillary tube standardization process, already described for alumina, or microscopic TLC slide standardization (*vide infra*), can be used for determining the activity of silica gel. Activity grade I has an R_f value of 0.0 and grade III has an R_f 0.65 (approx.). Usually adsorbents of lower activity, grade II or III, are used to avoid too strong adsorption or any possible rearrangement.

For column chromatography the ***particle size*** of alumina is generally of 60–120 mesh and that of silica gel is 40–100 mesh. These are commercially available from different manufacturing firms. The **mesh number system** *gives a measure of the number of openings per linear inch in a screen*. This can easily be determined, as screens are made from wires of standard diameters; however, opening sizes can vary slightly due to wear and distortion. Some mesh numbers and their equivalent opening sizes in mm are given below (reproduced from website):

Mesh no.	Opening (mm)	Mesh no.	Opening (mm)	Mesh no.	Opening (mm)
20	0.841	80	0.177	200	0.074
42	0.354	100	0.149	250	0.063
60	0.250	150	0.105	400	0.037

Some solid **adsorbents** can be arranged as follows *in order of increasing strength of binding interactions,* as given below, toward polar compounds or solutes called **eluates** or **elutants**:

Silica gel, florisil, alumina, activated charcoal.

Eluting solvents The common solvents used are called **eluents**. They are arranged *in order of increasing polarity and "solvent power" toward polar functional groups* as follows:

Petrol (b.p. 40–60 °C or 60–80 °C), hexane, cyclohexane, carbon tetrachloride, benzene, chloroform, methylene chloride, diethyl ether, ethyl acetate, acetone, ethanol, methanol, water, acetic acid.

The elution sequence (fastest to slowest) ***of elutants*** of different kinds are approximately as follows:

Hydrocarbons, olefins, ethers, aromatics, ketones, aldehydes, esters, alcohols, amines, acids, strong bases.

Hydrocarbons will be eluted with a nonpolar solvent (like hexane or petrol) whereas acids or strong bases will be eluted with a polar solvent. Of course, the molecular weights and the number and kind of functional groups of the natural or reaction products also play a vital role in this regard. Generally one starts elution with a nonpolar solvent to **elute** (bring down) relatively nonpolar compounds from the column and then gradually increases the solvent polarity to **elute** the compounds of increasingly greater polarity. The polarity of the eluent can be increased gradually by adding suitable greater percentages of a more polar solvent. The eluent to be used can be determined by running micro-TLC (*vide infra*).

Principle of separation. Interactions and distribution equilibrium Intermolecular forces of varying strength cause organic molecules to bind to the adsorbents. Strengths of different types of interactions in the decreasing order are as follows [12]:

Salt formation > coordination > H-bonding > dipole–dipole interaction > van der Waals forces.

The first three types are direct interactions and are typical of polar organic compounds. Salt formation may take place between acids and alumina or between bases and silica gel. Coordination interaction may occur between bases and Al of alumina. Dipole–dipole interactions may occur between polar molecules and alumina or silica. Nonpolar compounds weakly bind to the adsorbent using only van der Waals forces. Of course, compounds of extremely high molecular weights, even

if nonpolar, may bind strongly by such multiple weak forces. In general, the more polar the functional group, the stronger will be the bond to the adsorbent.

Solubility also plays a significant role: nonpolar solvents dissolve the nonpolar compounds best, whereas polar solvents dissolve polar compounds more effectively than nonpolar solvents. Thus any compound with some carbonyl function, adsorbed on an adsorbent, may not be eluted by hexane, but may be completely eluted by methylene chloride, chloroform, or ethyl acetate. Because of the competition for the solutes a distribution equilibrium is set up for each solute between the adsorbent and the solvent. Thus many such equilibria in series are set up as a consequence of the solutes in the solution coming in contact with the adsorbent down the column. These ultimately result in the difference in the partitioning of each solute from the other, leading to their differential elution by different eluents, and thus achieve separation.

Columns Cylindrical tubes of various types, usually made of glass, are used for column chromatography. They are packed with the adsorbents and clamped *exactly vertically*. The upper end is open and the lower end is fitted with a stopcock (preferably made of teflon) for controlled exit device (like a burette). Alternatively, a piece of flexible polyethylene (inert in most solvents) tubing is often attached to the bottom of the column and a screw clamp is used to stop or regulate the flow. Rubber or tygon (which dissolves in many solvents) should not be used. For large-scale chromatographic separation it is often convenient to use a solvent reservoir made by fusing the top of the column to the bottom part of a round-bottomed flask. The eluting solvent may also be added from a long stem capped separatory funnel, adequately opened and well inserted into the column of the eluent, filling the space above the adsorbent column to make the column *self-filling*. The available volume at the top of the column must be large enough to prevent any overflow.

Column preparation For efficient separation of the mixtures the column should be prepared properly. The column is partially filled with a nonpolar solvent like hexane or petrol (b.p. 40–80 °C), or the solvent mixture to be first used during chromatography. A loose plug of glass wool (or cotton) is pushed down softly into the bottom of the column with a long glass rod, and all entrapped air is forced out as bubbles. Clean, white sand is poured into the column to form a thin layer. The surface of the layer is leveled by tapping the column. Then the column is best prepared by either wet-packed (or slurry) or dry-packed method.

The slurry or wet pack method The slurry is prepared in a container by adding the adsorbent, a little at a time, to a quantity of the solvent (not in the reverse order). Enough adsorbent is added to the solvent, with swirling, to form a thick but flowing slurry. The swirling is continued to make it homogeneous and free of entrapped air bubbles. The column is now half filled with the solvent and the stopcock is opened to drain the solvent slowly into a large beaker. The slurry is then poured in portions through a funnel into the top of the draining column. The column is then tapped gently and constantly on the side with a plastic or wooden rod or a piece of thick pressure tubing until all the material has settled. The process is continued until the

desired column height is obtained. The collected solvent in a conical flask should be recycled through the column to make it firmly packed. *The column should never be allowed to run dry during packing or subsequent elution.*

Dry pack method The column is filled with a less polar solvent, which is allowed to drain slowly. *The dry adsorbent* is added from a beaker through a funnel, a little at a time, while the column is tapped constantly, as in the previous method. Addition is continued to a desired height of the column. An evenly packed column will thus be obtained. Solvent should be recycled a few times through the column before starting elution.

Alternatively, the column may be packed dry without any solvent. The adsorbent is introduced in small quantities, tapping constantly, as described before. After packing, the solvent to be used initially is allowed to percolate down the column. *This method may lead to uneven packing, cracking, and some entrapped air bubbles and hence is not recommended,* especially when the solvent, having an exothermic heat of solvation with the adsorbent (silica gel or alumina), is used.

Column size and adsorbent quantity The amount of adsorbent should usually be 20–50 times (by weight) the amount of material to be separated by chromatography. For separation of complex mixtures, or for separation of the components (having close R_f values) of a simple mixture the adsorbent–solute mixture may be raised up to 200:1. Monitoring the crude mixture by micro-TLC may give an idea about the suitable ratio to be used. Moreover, the column should have the height:diameter about 10:1. Difficultly separable compounds may require larger columns and more adsorbent. A smaller column and less amount of adsorbent may be enough for easily separable compounds.

Sample application The sample containing the mixture of solutes is dissolved in a minimum volume of the solvent to be used first for elution, if it is quite soluble. If not, it is dissolved in minimum amount of a polar solvent (like chloroform), since the mixture should form a *narrow band* on the top of the column, *ideal* for optimum separation of the components. The concentrated solution of not very thick consistency (which might choke the column head) or the neat liquid (if the sample mixture is liquid) is added carefully with a small pipette or a long dropper touching the inside of the column close to the surface of the adsorbent, and slowly draining it, not disturbing the surface. The small layer of liquid or solution is then drained into the column until the top surface just begins to dry. Some eluting solvent (taken in a test tube) is carefully added with the pipette or dropper. The small layer of solvent is now drained into the column until it touches the upper surface. Another small layer of the solvent is added and the process is repeated till the sample is expected to be adsorbed. The level surface of the adsorbent may then be protected by carefully filling some solvent, covering the top with a circular filter paper and sprinkling clean white sand, which settles down and forms a small protective layer on the top of the adsorbent. Separation is often better if the sample is allowed to stand for a short time on the column before elution, allowing a true equilibrium to be established. Elution is then started.

Alternatively, to a solution of the sample mixture in a minimum amount of a polar solvent like chloroform or methanol taken in an evaporating dish, a small quantity of the adsorbent is added The solvent is evaporated, first by stirring with a spatula under a fan and then in a vacuum desiccator. The dry powder thus obtained is added carefully to the wet column having a big layer of the solvent at the top. Elution is then started after putting a small protective layer on the surface in the manner described above.

Elution The starting eluting solvent or eluent should be determined by running a micro-TLC (*vide infra*) of the mixture. The minimum polar solvent or solvent mixture showing a spot of $R_f \sim 0.3$ should be used first. Usually the starting eluent is hexane or petrol (b.p. 40–60 °C). If this eluent shows more than one spot having close R_f values, the adsorbent:mixture ratio should be increased. The polarity of the eluent can be increased gradually by adding to petrol or hexane successively greater percentages of a more polar solvent, benzene/ethyl acetate/chloroform (e.g., 10 %, 15 %, 20 %, 30 %, 50 %, 75 %), or the more polar solvent alone, or by adding successively greater percentages of methanol to ethyl acetate/chloroform (e.g., 1 %, 2 %, 5 %, 10 %, 20 %, etc.), as needed. The transition from one solvent to another should not be too rapid in most eluent changes, especially when they differ greatly in their heats of solvation in binding to the adsorbent, since the generated heat may crack the column. *Automatic gradient elution technique* may be conveniently introduced. Most organic compounds can be separated on silica gel or alumina using hexane–benzene mixtures followed by mixtures of benzene and chloroform, ethyl acetate, or methylene chloride, and then by pure chloroform, ethyl acetate, or methylene chloride. Increasing percentage of methanol in chloroform, ethyl acetate, or methylene chloride may elute more polar compounds.

Rate of flow The effectiveness of a separation depends upon the *rate of flow* of the solvent through the column. It should be optimum—not too rapid; then the solutes will not have sufficient time to equilibrate with the adsorbent as they pass down the column. If the rate of flow is too low, or stopped for a period, the solute band may diffuse in all directions. In any of these two cases the separation will be poor. Usually, *the optimum rate of flow will be approximately 5–50 drops of effluent per minute, depending upon the diameter of the column.* The running of the column should not be stopped or set aside overnight, or for any prolonged period of time to avoid diffusion of the bands. In this respect FC, to be dealt with next, is much better. The bands, if not colored, may be seen under UV lamp (if they fluoresce), and may be collected separately as they elute from the column. A common method of monitoring separation of a colorless compound is to collect fractions of constant volume in preweighed flasks, to find out the weights of the residues after complete evaporation under *vacuo*, and to plot the weights against fraction numbers. The volume of each fraction collected (e.g., 10 ml, 25 ml, 50 ml, 100 ml, 250 ml, or 500 ml) depends on the size of the column and the ease of separation. Fractions may be collected continuously by setting the column stem directed to a tube of an *automatic fraction collector* (carrying many tubes in a concentric circular fashion) and adjusting for collecting the eluate fractions of some fixed volume, each.

Fractions showing the same spot/s in micro-TLC slides, when developed in the same solvent/solvent mixture, are combined for further separation, if necessary, and purification. *Visualization of the spots is routinely done using microscopic slides, to be described later, for monitoring the collected fractions.*

Chromatogram A chromatographic experiment gives the information contained in the *chromatogram*. The latter consists of a plot of concentration or weight profile of the sample components as a function of the flow of the mobile phase (fraction numbers) or as a function of time. The developed and spotted slides or plates (in TLC) are also usually termed chromatograms.

4.1.7 Flash Chromatography [16–18]

FC, first introduced [16] in 1978, provides a very powerful and rapid technique for separating compounds with similar polarities. It is mainly applied for (i) laboratory scale purification of mixture of organic compounds, (ii) isolation of target compounds from different fractions of the extracts of natural products, and (iii) separation of geometric isomers and diastereomers. It is also used for simplification of mixtures prior to preparative high-pressure liquid chromatography (HPLC), when needed. For low molecular weight neutral organic compounds relatively fine silica powder is used, with a smaller particle size range (e.g., May and Baker Sorbsil C 60, 40–60 μm, i.e., ≈600–400 mesh or Merck C 60, 40–63 μm, type 9385). Such finely powdered alumina is also used occasionally. The finely powdered silica (or alumina) gives better surface contact leading to more effective adsorption than ordinary column chromatography (CC). The latter operating under gravity and atmospheric pressure is slow and leads to band dispersion reducing the resolution. In flash chromatography modest pressure of less than 2 atm., with some sort of pressure-release valve, is applied from the top of the column to accelerate solvent flow resulting in fast and increased resolution between bands with reduced tailing. Labile compounds, susceptible to degradation, or rearrangement in the chromatographic system, can be isolated in higher purity because of the shorter contact time. *Every separation is different, and like any chromatographic technique one gains expertise by experience.*

Column preparation Glass columns of suitable length (because of the limited operating pressure) usually 25 cm, sometimes 10–15 cm for small amount of the mixture, are used. Originally long columns were used, but these are difficult to load. A set of four or five columns ranging in diameter from about 5 mm to 50 mm should be on hand, since a ratio of ≈20:1 (silica:mixture) should be sufficient; for more difficult separation of compounds ranging by ~1 % in their R_f values in TLC, this ratio may be increased up to 100:1. The more spots the mixture shows in TLC, the greater proportion of silica is to be used for separation of each component.

The column, containing at its base a circular glass frit of suitable size, or a small glass wool or cotton wool plug and a layer of pure sand, is partially filled with the

adsorbent using the slurry-packing technique. At first, the column is partially filled with a small volume of a weak solvent. A dilute suspension of the adsorbent in the same solvent is then added slowly, the excess solvent being drained out slowly, putting small pressure on the adsorbent bed periodically.

Sample application Usually a solution of the sample in a *minimum volume* of the weak solvent like hexane or petrol (b.p. 40–60 °C or 60–80 °C) is added to the column and forced into the adsorbent bed to form a narrow sample zone. For samples of low solubility in weak less polar solvents a solution of the sample in a minimum volume of a strong more polar solvent like $CHCl_3$ is added to a small amount of the adsorbent (1–2 g of the adsorbent per g of the sample) taken in a standard joint r-b flask to make a slurry. The solvent is then stripped completely in a rotavapor under reduced pressure and finally under high vacuum to give a free-flowing powder. The latter is then added to the solvent on top of the column. Finally, a thin layer of pure (acid-washed) sand, glass wool, or cotton wool is added to the top of the column so that the column bed is not disturbed during addition of the eluent. *(It should be ensured in both column and flash chromatography that the column should always be under solvent during column packing, sample application, and the elution sequence; and the top of the column should never be disturbed, and never be allowed to run dry.)*

Elution and fractionation Eluting solvent or solvent mixture is chosen by use of TLC of the sample mixture employing the same adsorbent. If several spots/zones appear in the TLC (after developing in the iodine chamber), the solvent strength should be adjusted so that the central zone or the zone of interest has an R_f value of ~0.3. As for example, ethyl acetate–petrol mixture may be started with. The impurities will be usually very polar having R_f ~0.1, or very nonpolar (fatty material) moving with the solvent front, which may be ignored. The individual components may be effectively separated by using stepwise gradient elution, the optimum mobile phase velocity being ~5 cm/min. Well-packed columns are expected to provide about 5–20 theoretical plates per cm of bed height. For samples containing components of wide polarity one can start with hexane or petrol (b.p. 40–60 °C or 60–80 °C) and add to it increasing volumes of strong polar solvent such as ethyl acetate, methylene chloride, or acetone, and finally with increasing proportions of methanol. The number of fractions to be collected at each step is determined by monitoring the composition of each fraction. The fractions are subsequently combined, based on similarity of their composition as monitored by TLC or GLC. *TLC on microscopic slides is mostly used since it is quick and inexpensive compared to various detectors like UV, visible, or IR spectrum, or refractive index which may be online, continuous, and adequate for this purpose.*

Since compressed air or gas is used for pressurizing the flash columns, some sort of pressure-release valve should be incorporated so that unsafe pressure buildup cannot occur.

When flash chromatography lacks the resolving power needed to separate the components, it can be repeated to get the desired separation by use of the expertise gained through experience. The fractions containing the components of interest, isolated through flash chromatography, if needed, should be subjected to high-resolution techniques, such as *medium-* or *high-pressure liquid chromatography* (MPLC or HPLC) for isolation of the pure components.

Miniature flash chromatography For rapid isolation of pure compounds (having similar polarity, as evident from TLC) from their mixture (~10–50 mg), or for rapid purification of crude sample (10–50 mg) obtained as some reaction product or as some natural product isolation fraction, a *miniature form of flash chromatography* may be conveniently used.

For this purpose a Pasteur pipette or a long pyrex glass dropper may be employed as a column. The pipette/dropper containing a cotton wool plug at the bottom is half to three-quarter filled with silica gel, the quantity being decided as stated before. A weak solvent like hexane or petrol is added to the top of the thin column at its inner wall from a pipette or dropper. The column may also be prepared by adding the solvent system (eluent) (petrol–ethyl acetate mixture) showing the $R_f \sim 0.3$ of the main component or components in TLC. When the eluent appears at the bottom running through the column under gravity, pressure is applied using a pipette teat to force the eluent to pass through at a faster rate. Two column volumes of the eluent is passed through the adsorbent. The sample is then applied to the top in the usual way, preferably by the slurry method, already described. A small layer of glass wool or pure sand is put at the top to protect the adsorbent from any disturbance. Pressure is again applied using the pipette teat. Here, of course, the pressure applied is not constant and the eluent does not pass through at a constant rate, which apparently does not affect the separation or purification.

4.1.8 Thin-Layer Chromatography [11–13]

This technique is being extensively used since mid-1960s for identification of mixtures of two or more organic compounds and for separation on a small scale. This technique is comparatively inexpensive and much less time-consuming. It involves interfacial adsorption/partition between liquid (mobile phase) and solid (stationary phase), like CC. This technique may also be termed **LSC** (Fig. 4.6). In this case the mobile phase is allowed to ascend a thin layer of the adsorbent (stationary phase), coated on the surface of a glass plate or microscopic slide (or a standard aluminium sheet which can be purchased) and dried—called *thin-layer plate* or *thin-layer slide*.

The adsorbents usually used for TLC are silica gel G (silicic acid) or alumina G (aluminium oxide). "G" stands for gypsum, $CaSO_4$, $\frac{1}{2}H_2O$ (better known as plaster of Paris), and is present in the adsorbent in ~10–13 % by weight, as a binder. In the presence of water or moisture it sets in a rigid mass, $CaSO_4$, $2H_2O$, and binds the

adsorbent to the backing support (plate or slide). The particle size of the adsorbent is small (200–300 mesh), compared to that (usually 60–120 mesh) used for CC. Silica gel is normally used for separation of neutral and acidic compounds, whereas alumina is frequently used for separation of bases. TLC is useful as an adjunct to column chromatography.

Microscopic slides Small TLC plates are made from microscopic slides (~7.5 cm × 2.5 cm). They are extremely useful for (i) identifying the components in a mixture, (ii) determining the appropriate solvent for a CC separation, (iii) monitoring the eluate fractions of a CC and also the course of any reaction, and (iv) ascertaining the identity of two compounds or purity of a compound.

The microscopic slides should always be handled by the top edge to avoid fingerprints on the slide surface—before coating, which may not allow uniform coating, and also to avoid disrupting after coating. A pair of new or clean microscopic slides is carefully held together by external air pressure, generated by pressing them together with a micro drop of water (from a fine dropper). They may be held with a strong forceps or by hand with the thumb and the forefinger at the top sides. The pair is smoothly dipped into a slurry of the adsorbent, already prepared in a wide-mouthed screw-cap bottle, ~6.5 cm—leaving ~1 cm at the top of the slides uncoated. They are then withdrawn slowly and steadily. The slurry should be shaken immediately before dipping. *The dipping operation should be done within 2 s*. After withdrawing the pair of slides the cap should be replaced on the bottle, and the slides should be held for a few minutes for evaporation of most of the solvent. The coated slides may then be separated and kept on their backs (uncoated side) for complete drying. Several pairs of slides, *thus evenly coated and dried*, are kept ready for use.

Slurry preparation The slurry is prepared in a 4 oz or 125 ml (approx.) wide-mouthed screw-cap bottle. The silica gel G should be added to methylene chloride or chloroform, used as solvent, with constant swirling. The reverse addition may cause formation of lumps. Amounts of the adsorbent and solvent (usually 1 g of silica gel is required per 3 ml of the solvent) should be adjusted so that the depth of the slurry in the bottle becomes ~7 cm. After addition the cap should be tightly secured on the bottle which is then shaken well by a swirling motion to ensure thorough mixing and stored for future use. Evaporation loss may require addition of requisite amount of solvent from time to time. Each time just before using the slurry should be shaken vigorously by a swirling motion.

Thin-layer chromatography plates These are larger than microscopic slides and are usually of the size approximately 20 cm × 5 cm, 20 cm × 10 cm, or 20 cm × 15 cm. The TLC plates must be coated by an applicator (a commercial spreading device) with a uniform layer of the adsorbent, usually of 0.25 mm thickness for analytical purposes and of up to 2.0 mm thickness for preparative purposes. With the Stahl–Desage applicator, model S 11, the thickness of the adsorbent layer may be varied from 0.25 mm to 2 mm by proper adjustment. With such applicator the

glass plates of uniform thickness must be placed beside one another. The more expensive models available now have designed plate holders leveling all plates, and thickness to ~±0.01 mm is guaranteed. Instructions for preparation of slurry and use of the applicator are given by the manufacturers and are found in the literature. The coated plates of silica gel G or alumina are allowed to stand for 30 min, activated at 110 °C for a minimum of 1 h, and stored in a dry box or desiccator until used. The layers have activity II–III.

Application of the sample A solution of the sample in a volatile solvent (chloroform, methylene chloride, or acetone), as less polar as possible, is applied on the plate or slide by means of a capillary micropipette (prepared from a drawn m.p. capillary tube) by briefly touching it to the adsorbent surface at about 1.5 cm from the edge. This is popularly known as *spotting*. The spot should be as small as possible. The spotting may be done several times, if needed, after allowing the solvent to evaporate. Up to three spots, suitably apart, may be applied to a microscopic slide.

Development chamber For development of microscopic slides a coupling jar or a wide-mouthed screw-cap bottle should be used. The inside of the bottle should be lined with a filter paper cut to such a length as to keep a vertical opening of ~2.5 cm for observation. The filter paper should be thoroughly moistened with the chosen developing solvent. Thus the chamber is kept saturated with the solvent vapor leading to the increase of the development speed. The level of the solvent in the bottle is adjusted to a depth of 0.5 cm; the bottle is capped and can be used when needed. Special tanks, used to develop large TLC plates, are available in the market.

Choice of developing solvent Hexane or petrol (b.p. 40–60 °C) with varying proportions of benzene will be a good solvent for least polar compounds like hydrocarbons. For compounds containing a wide variety of functional groups benzene with varying proportions of methylene chloride (or chloroform) will be suitable. For more polar compounds methylene chloride or ethyl acetate with varying proportions of methanol gives satisfactory separation.

A suitable solvent is rapidly determined in the following way. Several sample spots are applied on a coated microscopic slide at a minimum distance of 1 cm from each other. A different solvent or solvent mixture (already available on the rack) is gently touched to the centre of each spot by a microcapillary, as already stated. The solvent front and the compound will advance outward *in concentric circles around each spot*. The solvent front should be marked with a pencil by dotted lines since it will disappear when the adsorbent gets dried. *From the appearance of the rings the suitability of the solvent or solvent mixture may be judged*. The compound rings, if colorless, will be seen under UV light or will stain in iodine chamber (*vide infra*).

Development of the chromatogram After application of the spots and selection of the solvent the slide is carefully placed in the chamber containing the solvent for development so that the coated portion does not touch the filter paper liner and the

solvent level in the chamber is 5–8 mm below the applied spots. The cap is then replaced; the solvent rapidly rises up the slide by capillary action. One should watch carefully and the slide should be removed from the bottle when the solvent has traveled to within 5 mm of the top end of the adsorbent layer. The position of the solvent front is immediately marked gently with a pencil/needle as a dotted line. After the slide has dried, the spots (after visualization) are outlined on the slide with a pencil. Visualization methods are discussed briefly in the sequel.

The R_f for each spot (compound) is measured as described earlier. It varies from zero (if the compound does not move) to unity (if the compound moves with the solvent front) and will be a decimal fraction (when the compound moves). It serves for qualitative identification under a given set of conditions (vide *supra)*. Two compounds may have, by coincidence, same R_f value under a given condition, just as two compounds may have the same m.p. In such a case, different solvent systems should be tried, and the solvent system showing different R_f values may effect their separation by **CC** using same adsorbent and similar solvent system. The relative R_f values of the compounds, obtained in microscopic slides under some specified condition, may still be published since these give some idea about their relative mobility and may be helpful for separation/identification.

Visualization Separation by a TLC experiment can be followed visually for colored compounds. Many organic compounds are, however, colorless. The separated components must be made visible by some method or reagent, called visualization method or reagent, which may be *nondestructive* or *destructive*.

Nondestructive methods

(i) ***Exposure to iodine***. Most organic compounds (except saturated hydrocarbons and alkyl halides) react with iodine to form unstable yellow or brown complexes. The developed and dried TLC microscopic slide is placed in a 4-oz wide-mouthed screw-cap glass bottle containing some crystals of iodine. The capped bottle is mildly warmed on a steam bath when the former is filled with iodine vapor, and the spots begin to appear. After the spots become quite intense, the slide is removed from the bottle, and the spots are immediately outlined by a needle/pencil. On keeping for some time, the spots in most cases fade, as the iodine sublimes off leaving the compounds unchanged.
Documentation. Microscopic slide chromatograms can best be recorded in a lab. notebook in the following way. A cellulose tape is pressed onto the visualized layer. The tape and the portion of the layer, which adheres to it, are then removed. An additional layer of tape is placed, covering the layer. The resulting "tape sandwich" is then placed in a lab. notebook. Alternatively, one can draw a picture of the chromatogram in a lab. notebook.

(ii) ***UV method***. The most common method of visualization is by a UV lamp, when compounds often look like bright spots. Some types of compounds fluoresce under UV and become very bright. The bright spots under UV should be marked with a needle/pencil.

Destructive methods Several chemical methods are available as stated below for plates using adsorbent with binder:

(i) The slides or plates can be sprayed with conc. H_2SO_4 and heated to 100–110 °C in an oven for a few minutes. Organic compounds get destroyed and charred and appear as black spots.

(ii) There are chemical methods, specific only for particular functional groups, permanently altering the compounds through reactions. Preparation and use of the spray reagents, e.g., H_2SO_4–$Na_2Cr_2O_7$, H_2SO_4–$K_2Cr_2O_7$, $HClO_4$ (organic compounds appear as black spots), $SbCl_3$–$CHCl_3$ (steroids, steroid glycosides, etc. → various colors), 2,4-DNPH (for aldehydes and ketones → yellow to red spots), bromocresol green (carboxylic acids → yellow to green spots), Dragendorff's reagent (alkaloids and organic bases → yellow to orange), ferric chloride (phenols → various colors), ninhydrin (amino acids, amino sugars → blue), etc., are available [11].

Some more chromatographic methods which are quite involving, but useful, especially HPLC and MPLC, and also paper chromatography (PC), gel chromatography, reverse phase chromatography (RPC), etc., are briefly discussed in the sequel, only to give an elementary idea about the techniques and their principles. For detailed information, the relevant chapters of the books [10–14, 17] may be consulted.

The recent versions of the apparatus/equipments used for the different chromatographic techniques, as discussed in this chapter, including their operating manuals, etc., are available with their manufacturers. They are also available in advanced organic chemistry laboratories (research institutes and universities) of many countries.

Preparative TLC This technique was being used extensively for separating small quantities of compounds (up to 1 g) of similar polarity until late 1980s. However, it has been largely superseded by the advent of flash chromatography and preparative HPLC and MPLC.

Large plates (20 cm × 20 cm, 20 cm × 15 cm, or 20 cm × 10 cm) are coated with silica gel G or alumina by an applicator with a thickness of 1–2 mm, as required—depending upon amount of the material. The sample to be separated is applied in a thin line on one side of the dried and activated layer, about 1 cm from the layered bottom of the plate. A number of TLC plates, as needed, may be used. The plates are then developed as usual by dipping in a suitable chamber containing about 0.5 cm of the desired eluting solvent to resolve the mixture into bands. The bands are visualized by a nondestructive method already described. The adsorbents in those bands are separately scraped from the glass plates and the adsorbed material is extracted with a polar solvent. Filtration removes the adsorbent and evaporation of the solvent from the filtrate gives the separated components admixed with some adsorbent. Further purification of the latter by repeated preparative TLC and crystallization from a suitable medium often becomes necessary to get the components in the pure state.

Impregnated silica gel as an adsorbent Silica gel impregnated with different inorganic salts like silver nitrate [19] and mercuric acetate or with an organic compound like trinitrobenzene has been found to be selective; it shows discriminating adsorption behavior toward closely related compounds with special reference to olefinic compounds. In the latter cases, this selective behavior depends on the configuration, steric location, and the number as well as conjugation of the double bonds present in the concerned molecules. An adduct is formed with each of the constituent olefins endowed with different absorptivity and hence could be eluted from the column separately. From the adduct the olefin could be isolated by steam distillation, when it is steam volatile, or by treatment with ammonia. Silica gel is treated [19] with silver nitrate solution in a rotavapor when silver nitrate solution gets uniformly smeared on silica gel surface, thus making the silica gel ready for the chromatographic column. TLC plates or slides can also be drawn with a slurry of silver nitrate impregnated silica gel of TLC grade.

4.1.9 Paper Chromatography

Though apparently PC appears to involve solid (paper)–liquid (mobile) phases, in practice it involves liquid–liquid phases and is mechanistically termed as LLC. The paper is made up of high-quality cellulose which absorbs water from the atmosphere and retains it in the pores. The absorbed water thus serves as the stationary phase rather than the cellulose (solid) itself. The sample is applied at one end and the mobile phase is allowed to act either by the ascending way or by the descending way in a chamber specific for this purpose. The compounds of the sample get partitioned between the two liquid phases and a series of distribution equilibria is set up; the latter being different for different components for a particular pair of phases, the separation could be achieved.

Two-dimensional PC run in an orthogonal direction of the first development by the same ascending or descending method makes better resolution of the components.

Mostly, ionic compounds or polar compounds like sugars could be effectively separated. Spots are developed on the paper by spraying different reagents and the choice depends on the nature of the compounds separated. The mobile phases used are polar and in most cases water is present as a cosolvent.

4.1.10 Gas Chromatography

In this technique the components of the mixture to be separated are partitioned between the moving gas phase and the stationary liquid phase. Here the column is usually made up of copper or stainless steel or glass tubings of various diameter and length. A high boiling, less volatile liquid (low vapor pressure) or a low melting

solid is supported generally on uniformly crushed firebricks (common supporting material) in the following way. The former is taken in a low boiling volatile liquid (CH_2Cl_2, $CHCl_3$) and mixed with firebrick particles using a rotary evaporator. When the solvent is removed, firebricks are uniformly coated with the liquid or the low melting solid acting as the stationary phase. With these uniformly coated particles, the tubing is packed carefully and uniformly. The column is then coiled and placed in a gas chromatograph oven. One end of the column is used for gas entrance and the other end as outlet. The latter is connected to a detector. The sample is injected in the form of either liquid or solution. The injected material is vaporized and the components will travel through the column at different rates due to difference in their partition coefficients; the components are collected at different times and get detected by the detection device. *Retention time* is constant for each component for a particular column system. Retention time of a compound is defined as the time between the sample injection and the appearance of the maximum peak shown by the concerned compound, as pointed out by the deflection of the pen. Outcoming components in the gaseous state are collected in a cooled trap; the coolant may be liquid nitrogen (b.p. −196 °C), liquid helium (b.p. −260 °C), dry ice-acetone (−65 °C), etc.

The area of the gas chromatographic peak is proportional to the amount of the sample that appeared. Nonpolar, nonionic, water-insoluble hydrocarbons and multifunctional compounds could be separated by this method. Other equivalent terms for this process are *vapor phase chromatography* and *gas–liquid partition chromatography*.

4.1.11 High-Performance/Pressure Liquid Chromatography

It is one of the most successful and routinely used chromatographic techniques. Unlike GC, its application is not limited by the sample volatility or thermal stability. It is capable of separating a wide variety of compounds that include macromolecules, natural products, ionic compounds, polymeric compounds, and also labile and multifunctional compounds. It is the most versatile tool of modern days for both qualitative and quantitative analyses. It allows many types of interactions operative within the sample, mobile phase, and stationary phase, which in turn result in effective separation. At one end of the column sample injection device is present, and at the other end a detector is attached. Compounds are mostly detected from their refractive indices. The packing material and the length of the column are chosen carefully. Sometimes separation is more effective at high temperature which decreases the viscosity of the mobile phase, increases the sample solubility in the mobile phase, and causes effective mass transfer; the net effect is a better resolution. Generally the internal diameter of the tube is 4–5 mm and the packing material particle size is 3–5 μm.

At the beginning the sample material stays at the top of the column. With the beginning of elution, the strength of the mobile phase (eluent) gradually increases and the migration of the components starts; the components are collected in the

liquid mobile phase. Solvents with low viscosity are always preferred for HPLC. *High-grade dry solvents should be used.*

Several modes of HPLC other than the partition chromatography or interfacial adsorption chromatography, *viz.,* size-exclusion chromatography, ion-exchange chromatography, etc., are also in use today.

HPLC and GC coupled with mass spectrometry (MS) deliver more information about the compound. Sometimes MS acts as the detector for collecting fractions (having same M^+) for further study—especially for bioactive fractions.

Various solvent extraction methods and different chromatographic techniques, e.g., capillary electrophoresis, micellar electrokinetic capillary chromatography, centrifugal partition chromatography, and two-dimensional PC, have been employed for the separation and isolation of specific coumarins [20].

4.1.12 Medium Pressure Liquid Chromatography

MPLC, in principle, is quite close to flash chromatography and HPLC, but is more efficient than flash chromatography in separating the constituents from their mixture. It is capable of handling larger quantity and is economical since the column could be reused several times. However, the column should be cleaned before reuse from the residual leftover from the previous use. The most vital part of MPLC is its pump to be operated at 100 psi with a controlled flow-rate system and a provision for not building up high pressure during operation. Columns are made up of teflon or good quality glass and are coiled. The total length of the column may be tailored according to the need. The columns may be packed ordinarily with flash chromatography-quality silica (40–60 μm) and for difficult separations with 15–20 μm silica. Other packing materials include alumina, ion-exchange resin, and sephadex. The chromatogram is connected to a fraction collector and the fractions may be identified by a refractive index or a UV detector. The flow rate depends on the diameter of the column tube. The sample is injected with a good syringe at a reduced flow rate to avoid the building up of the pressure in the system. The columns can be purchased from the manufacturers. *Solvents used must be of high grade*.

4.1.13 Reverse Phase Chromatography

The name itself suggests the order of elution of the components. The most polar compound is eluted first, and the other components are eluted in the order of decreasing polarities. Accordingly hydrocarbons are eluted last. The packing material of the column is generally hydrophobic bonded material, usually with an octadecyl (C_{18}) or octyl (C_8) functional group and a polar mobile phase, often partially or fully aqueous. Polar compounds dissolve in the polar mobile phase first and will move faster than the nonpolar components. The solvents like methanol,

acetonitrile, etc., which are available in high-grade purity, are used along with water, as the mobile phase.

4.1.14 Gel Permeation Chromatography

In this chromatographic technique the stationary phase commonly uses cross-linked polymer having sieve-like structure. In this method the molecules get separated according to their shapes and sizes, and the dominant distribution process is SEC. When a mixture is allowed to pass through such a column used for SEC, small molecules stay in the pores, while the big molecules, which the pores fail to hold, move and elute faster than the small molecules. Sephadex (cross-linked dextrans of microbial origin) is commonly used for this purpose. It has an ability to absorb water, and as a consequence, the material swells and expands, and holes are created in the matrix. Equivalent terms of the process are *gel filtration, gel chromatography*, *or molecular sieve chromatography*. Biomolecules are mostly separated by this method.

4.1.15 Bioassay-Guided Investigation

From time immemorial the plant kingdom remains a major source of various remedial measures against physical ailments of man. Today, search for bioactive molecules from plants has been one of the major thrusts of natural products chemistry research. The objective is to isolate bioactive molecules having fair to profound medicinal values and to discover lead molecules directed toward the synthesis of comparatively simple molecules with improved medicinal properties and economic viability. In this endeavor, during fractionations of crude plant extracts, each fraction should be monitored by ^{1}H NMR and GC-MS. Fractions showing the presence of good signals and peaks for organic compounds are collected, and their biological activities are studied. Fractions showing promising bioactivity are collected in amounts, monitored each time during collection by HPLC and/or GC-MS. Both structural work as well as different biological activities in detail on the compounds isolated from the bioactive fractions should be carried out to reach the goal of discovering a drug.

4.1.16 Homogeneity and Physical Constants of the Isolated Compounds

Homogeneity of an isolated compound is the primary requirement for its characterization. Assessment of homogeneity basically needs the application of various

chromatographic techniques; the following applicable characterizations are to be observed for this purpose:

(i) Appearance of a single spot on TLC plate/slide in different solvent systems
(ii) Appearance of a single molecular ion peak in its mass spectrum
(iii) In case of a polar compound a single spot on paper chromatogram
(iv) A single peak in GLC/HPLC
(v) A sharp/consistent m.p. in case of a crystallizable solid
(vi) In case of a chiral compound, optical purity may be established if there is no significant change of $[\alpha]_D$, after repeated purification
(vii) For a liquid, refractive index measurement, till there is no significant change after repeated purification by GLC or HPLC.

4.2 Structural Elucidation

4.2.1 General Approach

After the establishment of the homogeneity of the isolate, some of its physical properties, *viz.,* m.p./b.p., molecular formula, solubility in common solvents, and optical rotation if any, are studied, followed by its structural elucidation. In fact, structural elucidation is a prerequisite for any further research in natural products chemistry.

Optical rotation The chirality (mirror image nonsuperposibility) of a natural product is exhibited by its ability to rotate the plane of polarized light. The observed sense of rotation of a chiral natural product—if clockwise—identifies it as the dextrorotatory (+) enantiomer, or if anticlockwise—as the levorotatory (−) enantiomer. Usually optically pure natural products would reveal the sense and magnitude of rotation of one enantiomer only. *When the absolute configuration is determined, it is mandatory to tag it with the sign of rotation, i.e., it must be mentioned whether it is the absolute configuration of the (+) or (−) enantiomer. The magnitude of rotation depends upon the solvent and concentration. Sometimes even the sign of rotation also changes with different solvents, as well as with the concentration in the same solvent, e.g., nicotine* [21]. For a detailed discussion of optical rotation, see Sects. 2.2.8 and 2.2.9.

The various strategies developed for structural elucidation during the nineteenth and twentieth centuries will now be discussed through brief illustrations. We will find, how the tedious and time-consuming degradative methods and various chemical reactions, once practiced with great success although taking many years, have been gradually replaced by IR, UV, and NMR spectroscopic and mass spectrometric analyses [22–66]. The spectral analyses have manifold advantages, as stated below, over the earlier degradative and other classical methods.

(i) It requires a very small amount of the material and much less time.
(ii) It is nondestructive in nature (except mass spectrometry which needs less than a mg), and the sample may be recovered and reused, if necessary.
(iii) Presence of some chromophore/s and some functional group/s can be detected immediately from the UV and IR spectra of the concerned compound.
(iv) Spectral data especially of ^{1}H [37–46] and ^{13}C [47–53] are available in the literature for compounds with a wide range of structural patterns. These data are extremely useful for structure deductions of new compounds from their spectral data [24–31, 37–55, 58] comparison within much less time, and a very quick identification is possible for known compounds. Thus such time-saving data give either the full structure or a part structure.
(v) Stereochemical and conformational information can be derived by judicial analysis of ^{1}H and ^{13}C NMR spectral data (chemical shifts and coupling constants, NOE, and various two-dimensional correlation studies). A number of excellent books [24–31, 37–45, 47–51, 53] on these very important topics have appeared from time to time. For an introduction to 2D NMR spectroscopy references [56, 57] and for details some references quoted under "Further References" may be consulted.
(vi) The mass spectral data [59–66] furnish the correct molecular weight and molecular formula as well as can predict the structural framework on the basis of the fragmentation pattern of the molecule.
(vii) ORD and CD studies [67–74] are capable of yielding information on the absolute stereochemistry of the chiral molecule (vide Chap. 2).
(viii) X-ray crystallographic analysis [75–77] reveals the structure, stereochemistry, and conformation of the chiral molecule and provides clues to the interpretation of NMR spectral data of complex organic molecules. In fact, in such cases X-ray data may complement the NMR data and vice versa.

All these methods collectively may not be necessary to establish the structure. Judicial application of the methods as demanded by the complexity of the molecule should be made. There are remarkable proliferations in the NMR and mass spectral analyses, which are now in extensive use. Carsten Reinhardt in his book [78] states that "Physical instruments threatened to destroy the methodological autonomy of chemistry [now] engineers and physicists appeared on the scene, attempting to displace chemistry by electronics". However, the reviewer of the book, Jerome A. Berson of Yale University commented, "We welcomed with enthusiasm the new power placed at our disposal by instrumental advances" [78].

In this connection we are predisposed to quote Woodward [79], "While it is undeniable that organic chemistry will be deprived of one special and highly satisfying kind of opportunity for the exercise of intellectual *élan* and experimental skill when the tradition of purely chemical structure elucidation declines, it is true too that not infrequent dross of such investigation will also be shed; nor is there any reason to suppose that the challenge for the hand and the intellect must be less, or

fruits less tantalizing, when chemistry *begins* at the advanced vantage point of an established structure."

And to remain competitive in the field of chemical research one has to take advantage of the powerful instrumental analysis. However, some of the classical chemical methodologies, which served earlier as the basic part of chemical research work, especially in the structural studies of natural products, though not much in vogue now, have been discussed in most of the chapters (Chaps. 6–29) in fair detail for compounds on which they were usefully applied.

A general approach for the structural determination of natural products (or any new compound) is now outlined wherein classical chemistry and instrumental analysis play the partnership roles.

(1) *Molecular formula determination can be achieved and confirmed by*

   (a) elemental analysis giving the empirical formula and molecular weight determination
   (b) high-resolution mass spectrometry (MS) giving the correct molecular formula
   (c) ^{13}C NMR (number of carbon atoms) and ^{1}H NMR (number of protons) and identification of the functional groups.

Nowadays the procedure (a), especially the molecular weight determination, requiring time and material is not generally practised. However, it is helpful and convincing to have the elemental analysis data while reporting a new compound. Earlier, before the advent of NMR and MS, much emphasis used to be given on the elemental analyses. Incidentally, it may be mentioned as a relevant example [80] that the Editor of the *J. Am. Chem. Soc.* commented on the elegance of the authors' methodology, but did not accept a paper of Konrad Bloch on the characterization of 14-norlanosterol, an intermediate in cholesterol biosynthesis, for not providing the m.p. and the elemental composition, thus failing to satisfy editorial requirements. Of course, the *Journal of Biological Chemistry* accepted the manuscript as such [80]. The elemental analysis gives the empirical formula; the actual molecular formula = n (empirical formula), where n is an integer like 1, 2, or 3, etc. For an unknown compound the molecular formula may be determined by the analysis of the compound and its suitable derivatives or best by its high-resolution mass spectrometry.

(2) *Detection* of the *chromophore/s* and *functional group/s present* in the molecule *by* UV and IR *spectral measurements*
(3) Number of unsaturation/s and ring/s (if present) together being called unsaturation number (UN)
(4) Classical degradative methods to determine the basic skeleton and the presence of chromophores and functional groups
(5) ^{1}H and ^{13}C NMR spectral analysis.
(6) Mass spectral (MS) analysis.
(7) ORD/CD studies.
(8) X-ray crystallographic analysis

4.2.2 *Unsaturation Number. Degradative Methods. Derivatization*

During prespectroscopic days (before NMR and MS) in the nineteenth century to mid-twentieth century, structural determinations were based on degradative methods and chemical reactions of some functional groups and/or of the structural framework. Some of them are now briefly described.

Determination of unsaturation and the number of rings (Unsaturation Number) Double bond and triple bond, though almost inert to H_2 alone, undergo facile catalytic hydrogenation. One mole of H_2 consumption corresponds to one ethylenic double bond (C=C), while one ethynic triple bond (C≡C) consumes two moles of H_2. In practice, the volume of hydrogen consumed is converted into moles; the number of double bond/s and/or triple bond/s present in the molecule can thus be determined. This is illustrated by a simple example involving hydrogenation of geraniol to give its tetrahydro derivative (Fig. 4.7). Thus, the parent molecule must contain *two* double bonds.

In cases of *sterically hindered, tetrasubstituted*, *and trisubstituted double bonds* which resist catalytic hydrogenation, peracid titration is done. In the conversion of a double bond to an epoxide, one mole equivalent of peracid is consumed, and the number of double bonds can be determined from the number of mole equivalent/s of peracid consumed.

In cases of homocyclic *ring compounds* the number of rings can easily be obtained as follows. The molecule is formally converted into the corresponding saturated hydrocarbon; its hydrogen content is to be compared with the number of hydrogen atoms present in the corresponding saturated open chain hydrocarbon having the formula C_nH_{2n+2}, where n represents the number of carbon atoms present in the parent compound. If the compound contains double bond/s, the number of hydrogen atoms required to saturate the double bond/s should be deducted from the molecular formula of the saturated open chain hydrocarbon. For example, the saturated hydrocarbon caryophyllane, $C_{15}H_{28}$, obtained by catalytic hydrogenation of caryophyllene, $C_{15}H_{24}$, contains four hydrogen atoms more than the parent compound (Fig. 4.8); hence caryophyllene contains two double bonds. Compared to the corresponding theoretical open chain saturated hydrocarbon, $C_{15}H_{32,}$ caryophyllane contains four hydrogen atoms less, which are accountable for being bicyclic.

A general equation determining the number of double bonds and/or triple bond/s and rings, known as unsaturation number **(UN)**, present in any organic compound having C, H, O (or any dicovalent atom like S), N (or any tricovalent atom like P or B), and X (halogen), is given below.

$$\text{For a compound } C_nH_mO_pN_qX_r \text{ the UN} = [(2n+2) - (m - q + r)]/2 \qquad (4.1)$$

The same equation holds good for other bicovalent atoms like S, Se, to be ignored like O, and also for other tricovalent atoms like P, B, As, to be treated within q like N.

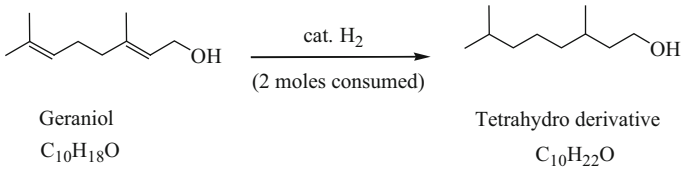


Fig. 4.7 Hydrogenation of geraniol

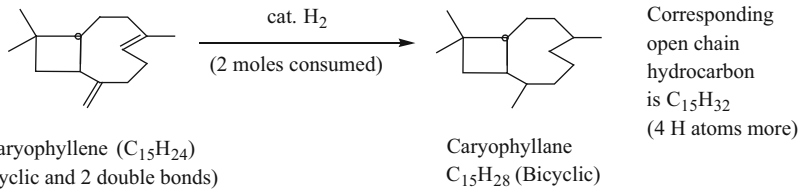


Fig. 4.8 Hydrogenation of caryophyllene

The above equation is derived from the consideration of the following points:

(i) The known molecular formula is compared with the theoretical formula expected for a completely saturated open chain molecule C_nH_{2n+2}.
(ii) Each O atom present as C=O, or S atom present as C=S, will be taken as a double bond.
(iii) Each O atom present as –OH or –OR, standing in place of –H or –R, is ignored.
(iv) Each S atom present as –SH or –SR, standing in place of –H or –R, is ignored.
(v) Each tricovalent atom (N, P, B, etc.) brings one additional H atom. (The simplest example is the H count of CH_3NH_2 compared to CH_4.) >C=NH should be regarded as one double bond and –C≡N as two double bonds, like C≡C.
(vi) Each halogen atom replaces one H atom, so needs addition of one H atom.
(vii) Each unit of unsaturation or each ring removes two H atoms. Hence the UN (unsaturation number) equals half of the shortfall of H atoms from $2n + 2$—the numerator of the general equation stated above, after taking care of the above points (ii) to (vi), whichever are necessary, to deduce the UN for any particular molecule.

A few examples (see Fig. 4.9 for the structures of the compounds) are given below to illustrate the validity of (4.1). Of course, for finding out the UN of an unknown compound one must know the correct molecular formula.

Example 1
For 5-chlorocyclohex-2-ene-1-one (**1**), C_6H_7OCl, *or its enantiomer or any position isomer* $n = 6$, $m = 7$, $r = 1$. Applying (4.1), UN = $[(6 \times 2 + 2) - (7 + 1)]/$

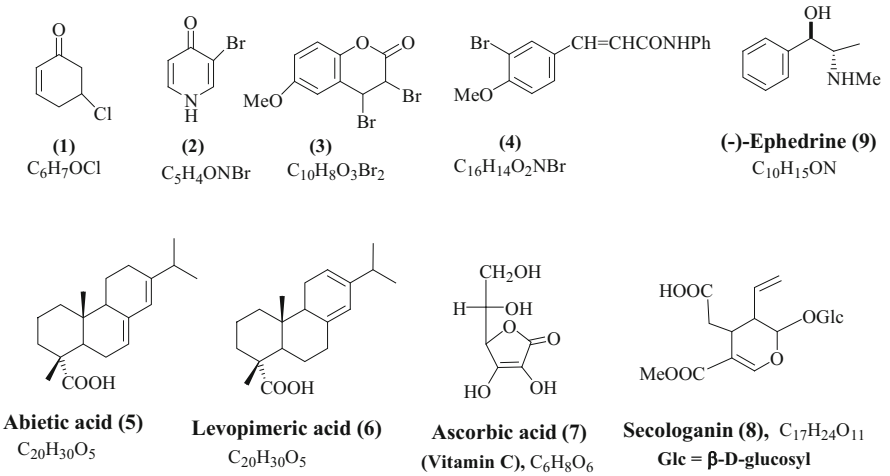


Fig. 4.9 Structures of compounds (**1**)–(**9**)

$2 = (14 - 8)/2 = 3$, which constitutes two double bonds (including one C=O) and one ring.

Example 2
For 3-bromo-1,4-dihydropyridine-4-one (**2**), C_5H_4 ONBr, or its any possible position isomer $n = 5$, $m = 4$, $q = 1$, $r = 1$, so UN $= [(5 \times 2 + 2) - (4 - 1 + 1)]/2 = (12 - 4)/2 = 4$, which constitutes three double bonds (including one C=O) and one ring.

Example 3
For 7-methoxy-3,4-dihydro-3,4-dibromocoumarin (**3**), $C_{10}H_8O_3Br_2$, or its any possible position isomer UN $= [(2 \times 10 + 2) - (8 + 2)]/2 = 6$ (four double bonds and two rings).

Example 4
For the benzanilide of 3-bromo-4-methoxycinnamicacid (**4**), $C_{16}H_{14}O_2NBr$, or its any possible position isomer UN $= [(2 \times 16 + 2) - (14 - 1 + 1)]/2 = (34 - 14)/2 = 10$, constituted of two rings and eight double bonds (including one C=O).

Example 5
For abietic acid (**5**), $C_{20}H_{30}O_2$, or its any possible position isomer [e.g., compound (**6**)] UN $= [(2 \times 20 + 2) - 30]/2 = 6$ [three double bonds (including one C=O) and three rings].

Example 6
For levopimaric acid (**6**), UN = same as abietic acid

Example 7
For ascorbic acid (vitamin C) (**7**), $C_6H_8O_6$ UN = $[(2 \times 6 + 2) - 8]/2 = 3$ [two double bonds (including one C=O) and one ring]

Example 8
For secologanin (**8**), $C_{17}H_{24}O_{11}$, so UN = $(36 - 24)/2 = 6$, of which four are double bonds (including 2C=O's) and two are rings.

Example 9
For (−)-ephedrine (**9**), $C_{10}H_{15}ON$, UN = $[22 - (15 - 1)]/2 = 4$ (three double bonds and one ring)

Degradative methods A major tool in the past has been the structural elucidation by degradative methods. The unknown compounds are chemically degraded, the degraded products are identified, and the structure of the compound is constructed on the basis of its degradation products. This involves lots of chemistry, bench work, and intuition, and unlike the picture puzzles guide no definite structure is available prior to its construction. The structural elucidations on the basis of such degradation in cases of complex alkaloids morphine (Chap. 23) and quinine (Chap. 25) are indeed stunning achievements in the absence of NMR and MS spectral facilities in those days. Of several degradations, a few still survive and the *Hofmann degradation* is the most informative one [coniine, conhydrine, and ψ-conhydrine (Chap. 17) and morphine (Chap. 23)]. *Alkali fusion method*, a favorite reaction of the past, used to give sumptuous structural information of the compound tried for this reaction.

Dehydrogenation with S/Se/Pd, especially of perhydropolynuclear natural products like terpenoids, steroids, alkaloids, etc., furnishes information about their structural skeletons. Such a study was first done by Vesterberg in 1903; he obtained retene from abietic acid on its dehydrogenation with sulfur. However, Ruzicka developed it to be a major method of structure elucidation. The identification of the aromatic compounds produced suggests the number of rings and their orientation, i.e., the major connectivity of the skeletal carbons in the molecule. Some examples are cited in Fig. 4.10. Diels first introduced the use of Se for dehydrogenation of polycyclic compounds (Chap. 31).

Derivatization From the IR and NMR spectral analyses the presence of some functional groups is indicated. Their presence could be confirmed chemically by derivatization (Fig. 4.11). The spectral studies of the derivatives furnish further structural information on the parent compound.

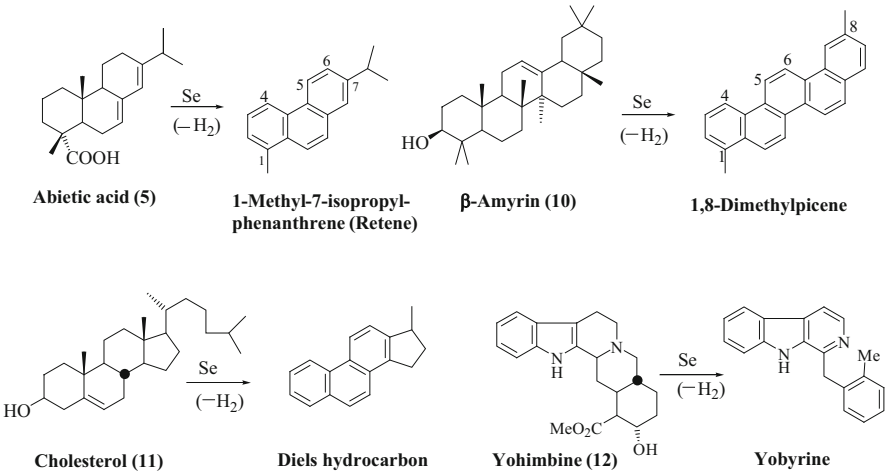


Fig. 4.10 Se dehydrogenation products of some natural products

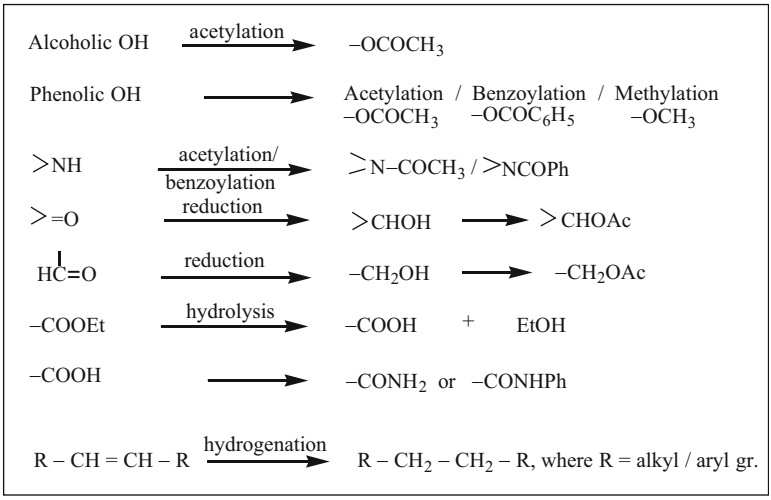


Fig. 4.11 Derivatization of some common functional groups

4.2.3 Spectral Analysis. General Discussion

The general regions of the spectrum of the electromagnetic radiations are shown in Table 4.2, ***although there are no sharp boundaries between regions***. The spectral measurements (except mass spectrometry) are based on the ability of molecules to absorb radiant light at different specific wavelengths (having different

Table 4.2 Regions of the electromagnetic radiation spectrum

	Unit	Gamma (γ) Rays and Cosmic Rays	X-Rays	UV (electronic transition	Visible Blue	Visible Red	IR (bond vibration) Near	IR (bond vibration) Far	Microwave (rotational motion)	Television	Radio
λ	cm	10^{-10}	10^{-8}	10^{-6}	4×10^{-5}	7×10^{-5}	10^{-2}		10^{2}	10^{4}	
	μm	10^{-6}	10^{-4}	10^{-2}	4×10^{-1}	7×10^{-1}	10^{2}		10^{6}	10^{8}	
	nm	10^{-3}	10^{-1}	10	4×10^{2}	7×10^{2}	10^{5}		10^{9}	10^{11}	

		γ-Rays	X-Rays	UV	Visible	IR	Microwave	Television	Radio
Wave number	cm^{-1}	10^{10}	10^{8}	10^{6}	2.5×10^{4}	1.4×10^{4}	10^{2}	10^{-2}	10^{-4}
Frequency	Hz	3×10^{20}	3×10^{18}	3×10^{16}	7.5×10^{14}	4.3×10^{14}	3×10^{12}	3×10^{8}	3×10^{6}
Energy	kcal / mole	2.9×10^{6}	2.9×10^{4}	2.9×10^{2}	72	40	2.9×10^{-1}	2.9×10^{-5}	2.9×10^{-7}

- 1 μm = 10^{-4} cm, μ means "one millionth part of." Thus the unit of length 1 μm (micrometer) = 10^{-6} meter [(or 10,000 angstroms (Å), 1 Å = 10^{-8} cm or, 10^{-10} m] (μ is always used as prefix). However, the unit micron (μ) is often used synonymously with the unit micrometer (μm) by many chemists, especially in case of IR.
- 1 nm = 10^{-9} m or, 10^{-7} cm (n abbreviated for nano, meaning 10^{-9}, is also used as prefix).
- Wave number is defined as the number of waves per cm (i.e., 1/λ) and has the dimension cm^{-1}. **Thus, wave no. = 1 cm / λ (in μ) = 10^{4} μ / λ (in μ); hence λ (in μ) = 10^{4} / wave no. (cm^{-1}).**
- Frequency, ν, refers to the number of waves passing through a point per second. Thus ν = c/λ .

- $E = h\nu$, $\nu = c/\lambda$ — E = Energy associated with the electromagnetic radiation at different wave lengths, and is directly proportional to ν (frequency) and is inversely proportional to wave length λ; the symbol h is the Planck's constant (= 1.57×10^{-34} cal/sec)
- $E = hc/\lambda$ — The symbol c is the velocity of light (= 3×10^{10} cm / sec). The symbols h and c are natural constants. Thus, the lower is the wave length λ, the higher is the associated energy. Energy per mole = Nhc/λ, where N = Avogadro's number, 6.02×10^{23} $mole^{-1}$.

energies) (Table 4.2), which in turn causes different types of excitations. These excitations include electronic transition, bond deformation, rotational and vibrational excitations, and nuclear spin inversion. The excitations are caused by different amounts of energy the molecules absorb and hence are observed at different wavelengths of the electromagnetic spectrum. The energy being quantized, the absorption of energy should be such that they correspond to the energy gap between the two states involved in the transition. The molecule rotates about the axis between its constituent atoms and also vibrates. The quantized energy required for these modes of excitements being less than that required for electron transition, they remain operative and interact with electron transition making the absorption lines of the latter broader as we find in cases of UV spectrum. Ultraviolet (UV) and infrared (IR) spectral data of the compound are initially measured to gain some information about some **chromophores** and **groups**. The bonds the nucleus is held with influence the nucleus from which electrons are excited from the ground state. The atoms and the groups carrying such atoms affecting such absorptions are known as **chromophores**. Several books [22–46] are available dealing with the spectroscopic analysis of organic molecules in fair details.

4.2.4 Ultraviolet Spectroscopy

Ultraviolet spectroscopy was developed first among the various types of spectrometry. But with the advent of IR spectroscopy, especially of NMR—the most sophisticated tool available to the chemists—the usefulness of UV spectroscopy has become relatively less. Yet it is being routinely used to further confirm the presence of different chromophores.

As mentioned, the molecules selectively absorb electromagnetic radiations, sometimes strongly and sometimes weakly. The regions of absorptions are known as bands. UV-active molecules absorb within the span of UV range (UV absorption region 200–400 nm) between X-ray and visible light. The longest wavelength is little shorter than that of the violet light. The bands together represent the UV spectrum of the molecule in which absorbance is plotted against wavelength.

Let I be the intensity of light passing through a sample in solution and I_0 be the intensity of the incident light before it passes through the sample; the ratio I/I_0 is called *transmittance* and is usually expressed as a percentage [$\%T = 100(I/I_0)$]. The *absorbance* A, also called *optical density* (in older literature), is based on the transmittance and, at a specified wavelength λ, is given by $A_\lambda = \log(I_0/I)$.

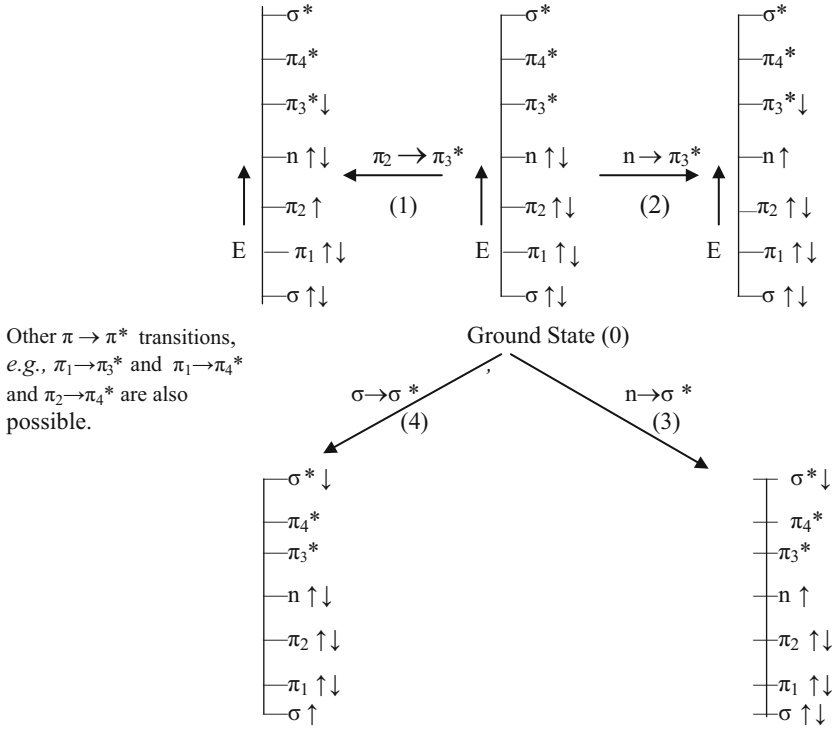


Fig. 4.12 Molecular orbitals involved in electronic transitions (visible, UV, and far UV regions)

According to *Lambert–Beer law*, also known as *Beer–Lambert law*, $A = \varepsilon cl$ for a given wavelength, where ε is the *molar absorptivity*, formerly known as *molar extinction coefficient*, c is the molar concentration of the solute, and l is the length (cm) of the sample cell. So absorbance is linear with c and l and is directly proportional to them. Thus $\varepsilon = A$ at unit length and concentration. Absorptivity is controlled by the nature of the absorbing system and the probability of the electronic transition. Values above 10^4 (up to 10^6) and below 10^3 are called *high-intensity* and *low-intensity* absorptions, respectively. The group of atoms producing an absorption in UV or visible region is called a *chromophore*.

The spectrum involves three major types of ordinarily recognizable electronic transitions involving the valence electrons (Fig. 4.12) from a bonding/nonbonding orbital to an antibonding orbital. The σ–σ* transition requires much energy and is generally observed in the far ultraviolet region below 200 nm.

4.2.4.1 Different Types of Electronic Transitions

Π–π* Transition (*type 1*) is caused by the transition of an electron from a bonding orbital π to an antibonding orbital π*. In a compound containing an isolated double bond, this transition requires much energy and generally occurs at a wavelength below 200 nm; this strong absorption is called *end absorption* indicating the probability of the presence of a double bond. However, if the double bond is present in conjugation, the energy gap becomes less (Fig. 4.12), and absorption takes place at a higher wavelength, which is known as *bathochromic shift (red shift)* of the absorption band. Depending on the region of absorption the extent of conjugation in the molecule could be predicted. Substitutions on the olefinic double bond also cause such bathochromic shifts (shift from lower to higher wavelength). Examples: unconjugated to conjugated acyclic or cyclic olefins. Conjugated double bonds give a strong absorption: *s-cis* (253 nm) and s-*trans* (213 nm) in cases of 1,3-butadiene and substituted cyclic or acyclic 1,3-dienes. Sometimes *hypsochromic shift (blue shift)* (higher to lower wavelength) may also take place. An increase in intensity is termed *hyperchromic effect* and a decrease in intensity—*hypochromic effect*. Substituents which may not give rise to UV absorptions themselves but increase the intensity of absorption of the chromophore are called *auxochromes*; examples are methyl, halogen, hydroxyl, alkoxy, amino groups, etc.

n–π* Transition (*type 2*) involves the excitation of electrons from nonbonding atomic orbital n to antibonding orbital π*. Compounds containing –C≡N, –N=N–, >=O, –N=O, or >C=S group exhibit n–π* transition. However, the absorption is weak when the double/triple bond is isolated.

n–σ* Transition (*type 3*) takes place when an electron is excited from a nonbonding n to an antibonding σ* orbital. It requires higher energy and hence takes place at a shorter wavelength, ~200 nm. Single bonded heteroatom containing groups, e.g., C–S (acyclic or cyclic), R–NH_2, C–Cl, R–X, R–COOH, R–$CONH_2$, $RCOOR_1$, etc., participate in such transition, which are detectable only in vacuum.

σ–σ* Transition (*type 4*) can take place only in case of hydrocarbons (cyclic or acyclic). The σ–σ* transition from a bonding to an antibonding orbital requires much higher energy and hence takes place at a much shorter wavelength in the *far UV region below 200 nm* and may not be detected even in vacuum.

The molecular orbitals of a molecule representing different electronic transitions in visible, UV, and far UV regions are qualitatively shown in Fig. 4.12. The UV spectrum is useful in finding the presence or absence of group/s capable of *type 1* and *2* transitions. The chromophores like substituted conjugated dienes, conjugated enones, etc., often occurring in natural products, are detectable by UV spectra. The frequency or the wavelength of a particular absorption depends on the electronic transition of the molecular orbitals involved. The value of the extinction coefficient ε is increased if the transition is symmetry allowed (e.g., $\pi \rightarrow \pi^*$ transition of conjugated dienes) and is significantly decreased if it is not symmetry allowed (e.g., $n \rightarrow \pi^*$ transition of unconjugated ketones).

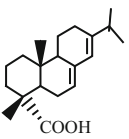
The light absorption maxima positions of dienes were first systematized by Woodward in 1941, based on an extensive study of the UV spectra of terpenoids, steroids, and other molecules. These have been modified by Fieser and by Scott based on the absorptions of a very large number of dienes and trienes. The modified rules [32] are given in Table 4.3. These rules are much helpful in identifying the conjugated diene systems and their environment, present in natural or synthetic molecules. The experimental results (observed λ_{max} values) are in good agreement with those derived from the empirical rules. The light absorption maxima positions of the dienes were first systematized by Woodward in 1941, based on an extensive study of the UV spectra of terpenoids and steroids and other molecules. These have been modified by Fieser and Scott based on the absorption of a very large number of dienes and trienes. These rules are much helpful in identifying the conjugated diene systems and their environment, present in the natural or synthetic molecules.

Table 4.3 Woodward–Fieser–Scott empirical rules for conjugated polyene absorptions

	Parent diene: homoannular (cisoid or *s-cis*)	253 nm
	heteroannular (transoid or *s-trans*)	214 nm
Increments for		
	a) each alkyl substituent or ring residue	5 nm
	b) each double bond of exocyclic nature	5 nm
	c) each double bond extending the conjugation	30 nm
Polar groups:	d) O-Alkyl	6 nm
	e) Cl/Br	5 nm
	f) O-Acyl	0 nm
	g) S-Alkyl	30 nm
	h) $NAlkyl_2$	60 nm
	Solvent correction (compare with **Table 4.4**)	0 nm[Ψ]
	Calc. λ_{max} (EtOH) = Total	

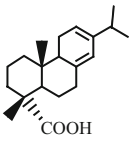
[Ψ]The polyenes being much less polar are insensitive to solvent changes presumably because of the absence of appreciable solvent-solute interaction.

Table 4.4 Calculation of UV absorption maxima (in EtOH) of some conjugated polyenes



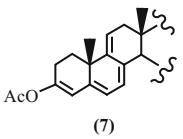
Abietic acid **(5)**

Heteroannular diene	214 nm
1 Alkyl substituent	5 nm
3 Ring residues	15 nm
1 Exocyclic double bond	5 nm
Calc.	239 nm
Obs.	241 nm



Levopimeric acid **(6)**

Homoannular diene	253 nm
1 Alkyl substituent	5 nm
3 Ring residues	15 nm
1 Exocyclic double bond	5 nm
Calc.	278 nm
Obs.	275 nm



(7)

Homoannular diene	253 nm
2 Double bonds extending conjugation	60 nm
5 Ring residues	25 nm
3 Exocyclic double bonds	15 nm
1 OAc substituent	0 nm
Calc.	353 nm
Obs.	355 nm

Examples illustrating the calculation of the UV absorption maxima (in nm) of some polyenes are given in Table 4.4.

Thus a conjugated chromophore strongly absorbs and the band undergoes bathochromic shift to different extents depending on the geometry of the conjugated system and on the substituent/s or ring residues present on the chromophore.

Similarly, Woodward first devised empirical rules for calculating the expected position of the absorption maxima of conjugated ketones and aldehydes from the known absorption maxima of a number of steroids and terpenoids containing such chromophores. Like the diene rules these rules were also modified by Fieser and by Scott and are outlined in Table 4.5. They are similar to but not as much reliable as those for conjugated polyenes. They are still helpful for predicting the absorption maxima of conjugated enones and were useful to determine the correct structure among several alternatives in many cases during the pre-NMR spectroscopic days. *For further details proper books* [22–32] *on spectroscopy dealing with UV spectra of the organic molecules may be consulted.*

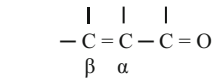
Solvent correction. Some changes in the UV spectra of conjugated enones are observed when the solvent is changed. Decrease in the polarity of the solvent causes decrease in the λ_{max} value (hypsochromic shift) since the energy necessary for the electronic transition is less in the more polar solvent due to the stabilization of the charge separated excited state by increased interaction with a more polar solvent (Table 4.5).

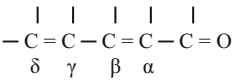
Tables 4.4 and 4.6 allow prediction of the UV absorption maxima, which are found to be in excellent agreement with the experimentally observed ones.

Empirical rules for predicting the absorptions of αβ-unsaturated acids and esters and for the principal band of substituted benzene derivatives have been outlined in tabular forms in the book by Scott [32]. For absorptions of these and many other types of chromophores one can consult other books [24–31].

The two additional absorption maxima in cases of compounds (**9**) and (**10**) are due to the parts of the long chromophoric systems (Table 4.6).

Table 4.5 Woodward–Fieser–Scott empirical rules for conjugated ketone and aldehyde absorptions*





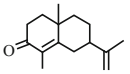
	nm
Parent αβ-unsaturated six-membered ring or acyclic ketone	215
Parent αβ-unsaturated five-membered ring ketone	202
Parent αβ-unsaturated aldehyde	207
Increments for	
a) extended conjugation by one double bond	30
b) homoannular diene moiety	39
c) each alkyl groupor ring residue	10 (α), 12 (β), 18 (γ, and higher)
d) OH	35 (α), 30 (β), 50 (δ)
e) OAc	6 (α, β or δ)
f) OMe	35 (α), 30 (β), 17 (γ), 31 (δ)
g) Cl	15 (α), 12 (β)
h) Br	25 (α), 30 (β)
i) NR_2	95 (β)
j) S-Alkyl	85 (β)
k) each double bond of exocyclic nature	5
Calc. λ_{max} (EtOH) =	Total:

Solvent correction:

Water	+ 8 nm	Chloroform	– 1 nm	Ether	– 7 nm
Methanol	0 nm	Dioxan	– 5nm	Hexane	– 11 nm

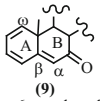
* *ε Values are usually above 10,000 and increase with the length of the conjugated system.*

Table 4.6 Calculation of the UV absorption maxima (in EtOH) of some conjugated ketones



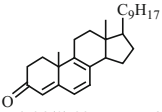
α-Cyperone **(8)**

Parent 6-membered enone	215 nm
1 α-Alkylgroup	10 nm
3 β-ring residues	24 nm
1 Exocyclic double bond	5 nm
Calc.	254 nm
Obs.	252 nm



(9)

Parent 6-membered enone	215 nm
2 Extended conjugations	60 nm
1 Homoannular diene moiety	39 nm
1β-Substitutent	12 nm
1 ω-Substitutent	18 nm
1 Exocyclic double bond (αβ-double bond is exocyclic to ring A)	5 nm
Calc.	349 nm
Obs.	348 nm (ε 11000) 278 nm (ε 3720), 230 nm (ε 18000)



Ergo-4,6,8(9),22-tetraene-3-one **(10)**

Parent 6-membered enone	215 nm
2 Extended conjugations	60 nm
1 Homoannular diene moiety	39 nm
1 β-Substituent	12 nm
3 Substituents at higher than γ	54 nm
1 Exocyclic double bond	5 nm
Calc.	385 nm
Obs.	388 nm (ε 12300), 288nm (ε 2190), 244 nm (ε 17800)

Measurement The UV spectra are measured in dilute solutions prepared in spectral grade solvents having no absorption in the UV range; aldehyde-free ethanol serves as a good solvent. The dilution is further manipulated on the basis of

extinction coefficient (ε), since absorption is measured during the study, and in practice the relative absorbance of light by the solution is measured, while comparing it to the solvent taken in an identical UV cell serving as the control. An idea about the molar extinction coefficient ε of an absorption maximum, having the unit cm^{-3} mol^{-1} (but not usually used), is an important guide for diluting the solution properly for the measurement of the UV or visible spectrum.

4.2.5 Infrared Spectroscopy

Although in Table 4.2 the IR region of the electromagnetic radiation spectrum is shown to be from 0.7 to 100 μm (14,000−100 cm^{-1}), it is conventionally divided into the three regions: the near-, mid-, and far-infrared, named for their relation to the visible spectrum. The *mid-infrared region* from approximately **2.5 to 25 μm (4,000−400 cm^{-1})**, *most commonly referred to as the IR part*, is used to study the *fundamental vibrations* and the associated *rotational–vibrational* structures. The higher energy *near-IR region*, approximately **0.7−2.5 μm (14,000−4,000 cm^{-1})**, can excite *harmonic or overtone vibrations*. The *far-IR region*, **25–1,000 μm (400–10 cm^{-1})** approx., lying adjacent to the microwave region, has low energy and may be used for *rotational spectroscopy*. These classifications are not strict divisions and not based on molecular or electromagnetic properties.

Each bond in a molecule has a vibrational frequency. If IR radiation is passed through a sample, an energy exchange takes place when a bond in a molecule has a vibrational frequency identical to the frequency of the incident radiation leading to its absorption. After absorption, the molecule vibrates at increased amplitude. A molecule can have many vibrational frequencies due to numerous independent vibration modes. For example the molecule having XY_2 moiety (Y=H or any other atom) may undergo various types of vibrations which involve (i) *bond length changes* or ***stretching*** [(a) ***symmetric***—when both bonds simultaneously become longer/shorter, and (b) ***asymmetric***— when the bonds alternately become longer/shorter], and (ii) *bond angle changes* or *bending/deformation* [(a) ***in-plane movements—scissoring*** when bond angle increases or decreases, and ***rocking*** when bonds move in the same direction, and (b) ***out-of-plane movements—wagging*** when both bonds simultaneously move toward/away from the viewer, and ***twisting***—when each bond alternately makes such movements]. These vibrations have different energies and hence cause IR absorptions at different frequencies. Such an analysis can be extended to a structural unit of any type. In general, asymmetric stretching vibrations occur at higher frequencies, and stretching vibrations occur at higher frequencies than bending vibrations: the order of decreasing frequencies is *scissoring* > *wagging* ~ *twisting* > *rocking* which is also the expected decreasing order of energy. The frequencies of vibrations depend on the masses of its atoms, strength of the connecting bonds, and the geometry of the molecule. Hence each pure compound is characterized by its own unique IR absorption spectrum containing a great number of peaks. Because of the presence

of identical groups, sometimes certain parts of the IR spectra of two different compounds appear to be similar. However, the region **~1,350–900 cm^{-1} (7.4–11 μm)** containing large number of peaks is difficult to interpret fully. The peaks in this region are characteristic of the particular compound and can be used, just as a *fingerprint* used for a human. This region is known as the ***fingerprint region***. When two compounds obtained from two different sources are suspected to be identical with each other, the superimposable IR spectra serve as the sure evidence of their identity. Superimposibility of the IR spectrum (peak for peak along with relative absorption) of a natural or synthetic compound with that of an authentic sample establishes the identity of the former with certainty.

IR-inactive molecules If the resultant dipole moment of the bonds of a molecule does not change as a function of time when subjected to IR radiation, the transfer of energy cannot take place, and the IR radiations cannot be absorbed, and the molecule becomes **IR inactive**. Such molecules will have symmetrical stretching vibrations, e.g., H_2, CH_4, O_2, N_2, C_6H_6, symmetric, and pseudosymmetric alkenes and alkynes, e.g., $CH_2{=}CH_2$, MeCH=CHMe, $(Me)_2C{=}C(Me)_2$, and MeCH=CHEt, CH≡CH, MeC≡CMe, and MeC≡CEt, etc.

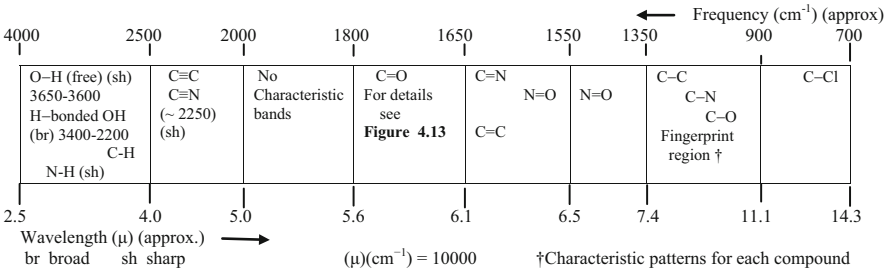
The IR spectra exhibit a number of absorption bands associated with various structural units of organic compounds. We examine the IR spectrum of a compound to search for the presence of –N–H, –O–H, C–H, >C=O, C≡C, C≡N, –COOH, –CONH-, C=N, C=C, N=O, C–C, C–N, C–O, C–Cl, etc. vibrations; the approximate regions of their stretching vibrations are given in Table 4.7. Bending, twisting, and other types of bond vibrations have been omitted for clarity [23]. One should record the broad visual patterns of this table quite firmly in mind. From the base value of any particular stretching vibration one can find out the kind of absorption from the pertinent correlation charts of various group frequencies in a number of the available books [22–31, 33–36]. An overwhelming number of compounds possessing these groups variously associated with other bonds or groups have been studied and the positions of the bands have been generalized. These bands are useful in identifying the presence of such group/s in the compounds under investigation. Here, we will briefly talk about O–H and C=O groups often encountered.

Wave numbers are expressed as reciprocal of the wavelength expressed in cm. A higher wave number corresponds to higher energy. Wavelength values are encountered more in older literature. Wavelengths (μ or μm) are converted to wave numbers by using the following relationship.

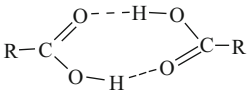
$$\text{wave no.} = 1\ \text{cm}/\lambda\ (\text{in}\ \mu) = 10^4\ \mu/\lambda\ (\text{in}\ \mu);\ \text{hence}\ \lambda\ (\text{in}\ \mu)$$
$$= 10^4/\text{wave no.}\left(\text{cm}^{-1}\right)\ \text{or frequency}$$

Hydroxyl groups The –OH group may be alcoholic, phenolic, or carboxylic. Sometimes –OH is present in the free state when the peak appears to be sharp. In case of hydrogen bonding, the bond appears to be broad and shifted (bathochromic) from the normal frequency of O–H stretching. The IR spectra of intermolecularly hydrogen-bonded compounds are concentration dependent. In

Table 4.7 The approximate regions of the absorptions (stretching vibrations only) of various common types of bonds



very dilute solution the hydrogen bonding is destroyed. However, in cases of intramolecularly H-bonded compounds, their IR spectra are independent of concentration. In cases of carboxylic acids, the hydrogen bonding is so strong that they remain in the dimeric form in the pure liquid. The entire range of bands due to hydroxyl group lies between 3,650 and 2,200 cm^{-1}. The free hydroxyl group appears at 3,650–3,600 cm^{-1} (sharp), hydrogen-bonded OH (3,400–3,200 cm^{-1}), and very strongly bonded hydroxyl group, as in a carboxylic acid (**11**), appears at 3,400–2,200 cm^{-1} as a very broad band. A cyanide (C≡N) group appears at ~2,250 cm^{-1} as a very sharp band.

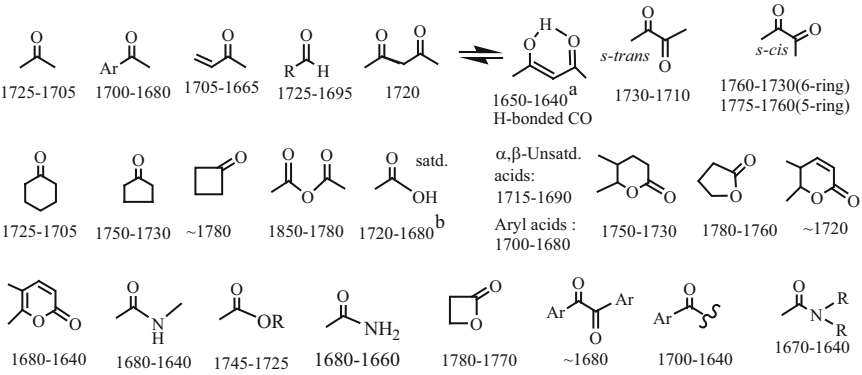


(11)

Carbonyl groups They may be present as free carbonyl, conjugated carbonyl, carboxylic carbonyl, ester carbonyl, cyclic carbonyl (4,5,6,7 ... membered), conjugated cyclic carbonyls and lactones of different ring sizes, amide carbonyl, etc., appear within the normal range of carbonyl ~1,830–1,640 cm^{-1}, and exhibit peaks at specific frequencies. General frequency ranges of the vibrational modes of various types of carbonyl groups are given in Fig. 4.13. In case of ring compounds, ring strain plays an important role. Additionally, the presence of various other groups could also be detected [26–32]. If the IR spectrum of a natural or synthetic product shows one or more than one such peaks, the compound is likely to contain such a group/s.

Sample preparation The samples for IR spectral studies may be prepared *in one of the following ways*. The choice depends on the nature of the compound.

(i) ***As a KBr pellet***. The compound/sample, if solid, is finely powdered with a non-absorbing metal halide, usually KBr (IR-grade), and compressed under



[a] Hydrogen bonded carbonyl band moves to a lower frequency. α,β-unsaturated CO 1715-1690, Aryl CO 1700-1680 cm^{-1}.

[b] A lower frequency CO (1720-1680) along with a broad H-bonded OH (3400-3200) suggest the presence of a COOH group in the molecule.

Fig. 4.13 The absorption maxima (cm^{-1}) of C=O groups (vibrational modes) of different types and in different environments

high pressure into a thin transparent film of KBr carrying the compound (a matrix). The resulting pellet is inserted in a holder and is placed in the spectrometer. No absorption is added to the spectrum due to KBr.

(ii) ***In the form of a mull***. The compound/sample, if solid, is finely ground in an agate mortar (using a pestle) with liquid paraffin, a high molecular weight hydrocarbon (usually Nujol), and the dispersion (mull) is pressed between two IR transparent salt plates. The peaks due to Nujol are sometimes superimposed. Strong peaks due to Nujol appearing at 2,924 (sp^3 C–H stretching), 1,462 (CH_2 bending), 1,377 (CH_3 bending) cm^{-1} (approx.), and a weak peak at 760 cm^{-1} (long chain band) are to be ignored. Thus the main disadvantage of using this method is to obscure the bands of the analyzed compound, if present in these regions. However, if the Nujol bands are stronger than the sample peaks appearing in those regions, more sample and less Nujol should be used.

(iii) ***As a pure liquid***. A drop of liquid completely free from water is placed between a pair of finely polished NaCl or KBr plates (*salt plates*) and squeezed carefully when a thin film of the liquid is formed in between. The thin plate is then placed in the spectrophotometer. An IR transparent cell of appropriate length may also be used for this purpose. Since no solvent is used, the spectrum thus obtained is referred to as a ***neat*** spectrum.

(iv) ***As a solution***. A solution of the sample, if solid or liquid, is made in a suitable solvent (CCl_4—most commonly used, $CHCl_3$, or CS_2, etc.) and its spectrum is taken in a suitable IR transparent cell. Some regions of the spectrum are obscured by the absorption bands of the solvents: (CCl_4, 850–700 cm^{-1}; $CHCl_3$, 1,250–1,175 and below 850 cm^{-1}; CS_2, 2,200–2,100 and 1,600–1,400 cm^{-1}); these are to be taken into account while interpreting the spectra.

Calibration of the spectrum The IR spectrophotometer should be calibrated for accuracy of the bands appearing in a spectrum by comparison with the bands of a standard. Usually a thin film of polystyrene is used for this purpose. Polystyrene shows a number of sharp absorption bands of which the band at 1,601 cm^{-1} (6.246 μm) or 1,944 cm^{-1} (5.144 μm) is recorded on the chart paper for calibration, after running the spectrum of the compound being measured.

Fourier Transform Infrared (FTIR) Spectrometers The design of the optical pathway of such spectrometers produces a pattern called an interferogram. Its wavelike pattern contains all the frequencies which make up the IR spectrum. The FTIR instrument acquires the interferogram in less than a second, and thus it can collect dozens of interferogram of the sample within a minute and accumulate them in the memory of a computer. The sum of the interferograms is subjected to a mathematical operation known as ***Fourier Transform (FT)*** which separates the accumulated individual frequencies, and a spectrum with a better signal-to-noise ratio can be plotted. Thus FTIR instrument has a greater speed and a greater sensitivity than a traditional dispersion instrument and needs a much less amount (<0.1 mg) of the material. Moreover, the computer software automatically subtracts the spectrum first taken of the background [containing IR-active gases like CO_2 and H_2O vapor (O_2 and N_2 are not IR-active)] and yields the spectrum of the compound only, being analyzed.

4.2.6 ^{1}H and ^{13}C NMR Spectroscopy [54–58]

We will now concentrate on Nuclear Magnetic Resonance (NMR) spectral analysis, the most rewarding of all spectroscopic measurements. It is capable of revealing information about the number of magnetically distinct atoms present in the compound under investigation and about its immediate as well as distal chemical environments. Because of remarkable evolution of instrumentation NMR has become the most sophisticated useful tool to organic chemists. Various types of 2D-NMR and correlation diagrams indicate/confirm the complete structure and stereochemistry of the compounds in most cases. A discussion of these tools developed during the last few decades is beyond the scope of this chapter, and the reader is referred to the available excellent books [22, 23, 27, 29, 38–44]. A simple basic treatment and application of some pertinent data will be briefly presented. There are excellent books [37–52] which may lead to thorough understanding of the theoretical basis of the nuclear resonance phenomenon and adequate interpretation of results.

All nuclei carry a charge which spins around nuclear axis. Circulation of nuclear charge (of nuclei with spin number not zero) generates magnetic dipole and angular momentum along the axis of spin. The nuclei of all atoms may be characterized by a nuclear spin quantum number ***I***, which may have values *equal to zero or multiples*

of ½. The spin number ***I*** is related to the mass number and atomic number as follows:

Mass number	Atomic number	Spin number ***I*** (multiples of ½)
(i) Odd	Even or odd	1/2, 3/2, 5/2, ...
(ii) Even	Odd	1, 2, 3, ...
(iii) Even	Even	0 (no nuclear spin)

The nuclei having atomic mass and atomic number both even, e.g., $^{12}C_6$, $^{16}O_8$, have zero spin ($\boldsymbol{I} = 0$); such nuclei cannot exhibit nuclear magnetic resonance and are termed "*NMR silent*." However, many elements have at least (i) one isotope having nuclear spin of magnetic quantum number $\boldsymbol{I} = 1/2$ (e.g., $^{1}H_1$, $^{13}C_6$, $^{15}N_7$, $^{19}F_9$, $^{29}Si_{14}$, $^{31}P_{15}$) and spherical charge distribution, or (ii) one isotope having nuclear spin $\boldsymbol{I} = 1$ (e.g., $^{2}H_1$ or D, $^{14}N_7$) and nonspherical charge distribution; such isotopic nuclei are observable by NMR. *In an external static (constant) magnetic field denoted by* B_0, the microscopic magnetic moments align themselves relative to the field in a discrete number of orientations because of quantized energy states involved. *For a spin number* ***I*** *the number of possible orientations with spin states of different energies is given by* $2\boldsymbol{I} + 1$. Thus for a proton there are two possible orientations: a low-energy orientation aligned with the applied static field and denoted by +1/2 (α-state, parallel) and a high-energy orientation opposed to the applied field and denoted by −1/2 (β-state, antiparallel). These are two energy levels, and in accordance with Boltzman distribution, there is a slight excess of proton population N_α at lower energy over proton population N_β at higher energy. The nuclei possessing angular momentum behave like a bar magnet and experience a precessional motion at right angle to the direction of the static magnetic field. The energy difference $\boldsymbol{\Delta}E = h\gamma\ B_0/2\pi$, where h is Planck's constant, and γ is the *magnetogyric ratio* (also known as *gyromagnetic ratio*) which is a measure of the strength of the nuclear magnet and is constant for a given nucleus having spin. Thus the energy difference is proportional to B_0.

The transition from one orientation to another orientation occurs when the frequency of absorption or emission of electromagnetic radiation is equal to the precessional frequency of the nuclei. NMR occurs in the radiofrequency region ($\lambda > 10^4$ cm) of the electromagnetic spectrum (see Table 4.2). The transition from the lower energy level to the higher one is induced by introducing another magnetic field at right angle to the applied external magnetic field. For the aforesaid transition the proton has to be irradiated with an electromagnetic radiation of frequency ν so that $\Delta E = h\nu$. Thus $\nu = \gamma\ B_0/2\pi$ and this will vary with the structural environment.

Chemical shift Proton environment is affected by the orbital electrons creating a small magnetic field. The net effect is the *decrease in the magnetic field (H) felt by the nucleus* ($H_{\text{at nucleus}} = H_{\text{ext.}} - H_{\text{shielding}}$), where $H_{\text{ext.}} = B_0$. Thus the proton is shielded. For a slight variation in the structural environment of a proton, variation of radiation frequency is needed to cause the resonance of the proton. This variation is known as ***chemical shift***, which thus represents the separation of the resonances

of a type of nuclei in a different chemical environment with respect to a standard reference.

The most used ***standard reference*** is tetramethylsilane (TMS), all the methyl protons of which are more shielded compared to the protons of most of the organic compounds. Further, it gives a strong singlet peak of 12 equivalent protons at a very low concentration and the compound is chemically inert. The scale starts from this peak and is taken as zero (δ), so that positive values appear for less shielded protons. An alternative unit τ has been used to express chemical shift. It holds a relationship with δ as follows: $\tau = 10 - \delta$. Thus, a proton with less magnetic shielding will have a smaller value of τ (or a larger value of δ) and vice versa.

The NMR spectrum, like UV and IR spectra, represents a plot of energy of absorbance versus frequency of electromagnetic radiation. *The chemical shifts are dimensionless and are expressed for the required accuracy, as parts per million (ppm), and the displacement is measured from the reference compound TMS (*Fig. 4.14*), added in very minute amount in the solution of the sample prepared in a thin-walled specially made glass tube, called an NMR tube*.

This is the reason that the chemical shifts of the protons of a particular compound when measured in different instruments with different magnetic field (external) strength will feel the same fraction of external magnetic field and hence the chemical shifts are independent of the external applied field, but is very much dependent on the electron density around the nucleus under study and the associated atoms with which it is bonded. The decrease in the electron density decreases the shielding effect and the resonance takes place in a lower field. The situation is reversed with increase of electron density around the nucleus. The relationship between δ (chemical shift), τ, and R_f frequency of the ^{1}H NMR instrument is depicted in Fig. 4.14.

A proton which shows observed shift 120 Hz in a 60 MHz instrument will show 1,000 Hz shift in a 500 MHz instrument, and the chemical shift δ will be 2 ppm in each case (Fig. 4.14). Since the difference of the two energy levels depends upon the magnetic field felt by the nucleus, the frequency for transition will depend on the strength of the magnetic field. Chemical shift values of protons of different environments are available from standard books.

Integration The intensity of the absorption peak/s appearing in the ^{1}H NMR spectrum is directly proportional to the number of nuclei responsible for the peak/s. The area is electronically measured in a separate operation with the NMR machine. In cases of ^{1}H NMR, integration is easy and reliable. Integration for each resonance is recorded as vertical lines and the relative height represents the number of protons. In current machines the digital number appearing at the base line under the peak dictates the relative intensity of the peak. In case of ^{13}C NMR spectra, the integration of the signals depends on the relaxation rate of the nucleus concerned and its scalar and dipolar coupling constants. Very often these factors are poorly understood— therefore, the integration of the ^{13}C NMR signal is very difficult to interpret.

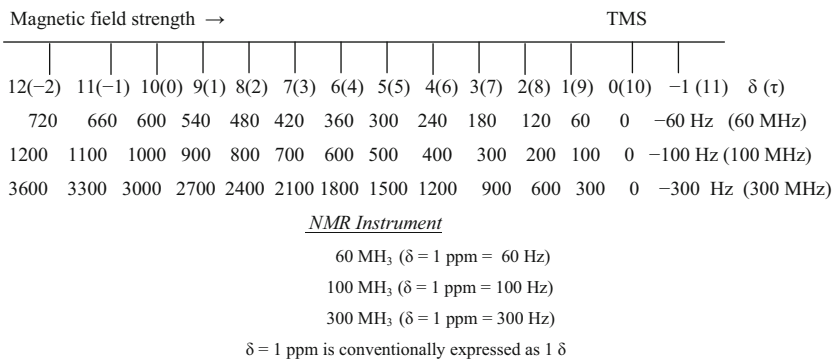


Fig. 4.14 Relationship between chemical shift in Hz, δ, τ, and R_f frequency of the NMR instrument

Splitting Patterns and Coupling Constants: The splitting patterns and the coupling constants (***J-coupling or scalar coupling—a special case of spin–spin coupling***) between NMR-active nuclei give some of the most useful information for structural elucidation and sometimes reveal the stereochemical features of the molecule. The nuclear spin–spin interaction is responsible for the origin of splitting of the resonance lines (more than one line). A nucleus with spin quantum number I (>0) is equally separated into the spin states represented by $(2I + 1)$ when placed in a magnetic field. By spin angular momentum polarization, the effects of the individual spin states are transferred to the adjacent nuclei (coupling nucleus). Chemically equivalent nuclei either by symmetry (homotopic or enantiotopic) (Chap. 2) or by averaging due to free rotation are *isochronous* showing same chemical shift and appear as a singlet. However, some nuclei become isochronous accidentally.

The coupling arises from the interaction of different spin states of anisochronous nuclei through the chemical bonds of a molecule and results in the splitting of NMR signals. These splitting patterns can be complex or simple and, likewise, can be straightforwardly interpretable or deceptive. The coupling provides detailed insight into the connectivity of atoms in a molecule. Coupling to n equivalent (spin ½) nuclei, three bonds apart, splits the signal into a $n + 1$ *multiplet* with intensity ratios following ***Pascal's triangle*** as given below. The intensity ratio in case of ^{1}H NMR is also same as the coefficients of the terms in the expansion of $(a + b)^n$. For example, the intensity ratio of the septet, when $n = 6$, may be derived from the expression $(a + b)^6 = a^6 + 6a^5\, b + 15a^4\, b^2 + 20a^3b^3 + 15a^2b^4 + 6ab^5 + b^6$. The

intensities of the peaks of such a multiplet are thus symmetric about the midpoint of the band.

Pascal's triangle

Multiplicity	Intensity ratio
Singlet (s)	1
Doublet (d)	1:1
Triplet (t)	1:2:1
Quartet (q)	1:3:3:1
Quintet	1:4:6:4:1
Sextet	1:5:10:10:5:1
Septet	1:6:15:20:15:6:1

A simple example is given. In the proton spectrum for ethanol described above, the CH_3 group is split into a *triplet* with an intensity ratio of 1:2:1 by the two neighboring CH_2 protons. Similarly, the CH_2 is split into a *quartet* with an intensity ratio of 1:3:3:1 by the three neighboring CH_3 protons. In principle, the two CH_2 protons would also be split again into a *doublet* to form a *doublet of quartets* by the hydroxyl proton, but intermolecular exchange of the acidic hydroxyl proton often results in a loss of coupling information. Coupling to additional spins will lead to additional splittings of each component of the multiplet, e.g., coupling to two different spin ½ nuclei with significantly different coupling constants will lead to a *doublet of doublets* (abbreviation: dd). No coupling occurs between nuclei that are chemically equivalent (that is, have the same chemical shift). Couplings between nuclei that are distant (usually more than three bonds apart for protons in flexible molecules) are usually too small to cause observable splittings. *Long-range* couplings over more than three bonds are often observed in cyclic and aromatic compounds, leading to more complex splitting patterns.

Chemical shift (and the integration for protons) combined with multiplicity and coupling constant/s gives information not only about the chemical environment of the nuclei, but also the number of *neighboring* NMR-active nuclei within the molecule. Coupling constants (J-values in Hz) of interacting anisochronous protons of different environments and hence of different chemical shifts commonly encountered in natural and synthetic organic compounds are shown in Fig. 4.15. One can differentiate and characterize the different dimethoxycoumarins on the basis of the chemical shifts and the coupling constants of the aromatic and olefinic protons. The ^3J-value suggests the strength of interaction between the nuclei concerned. Martin Karplus first found out that the coupling constant (J) between protons attached to vicinal carbons depends on the dihedral angle α between them, and he developed an expression known as **Karplus equation** as follows:

$$^3J_{HH} = A + B\cos\ \alpha + C\cos\ 2\alpha$$
$$A = 7 \quad B = -1 \quad \text{and} \quad C = 5$$

These values of the constants A, B, and C are accepted as those that predict the J-values best. The curve plotting ^3J-values obtained by application of Karplus

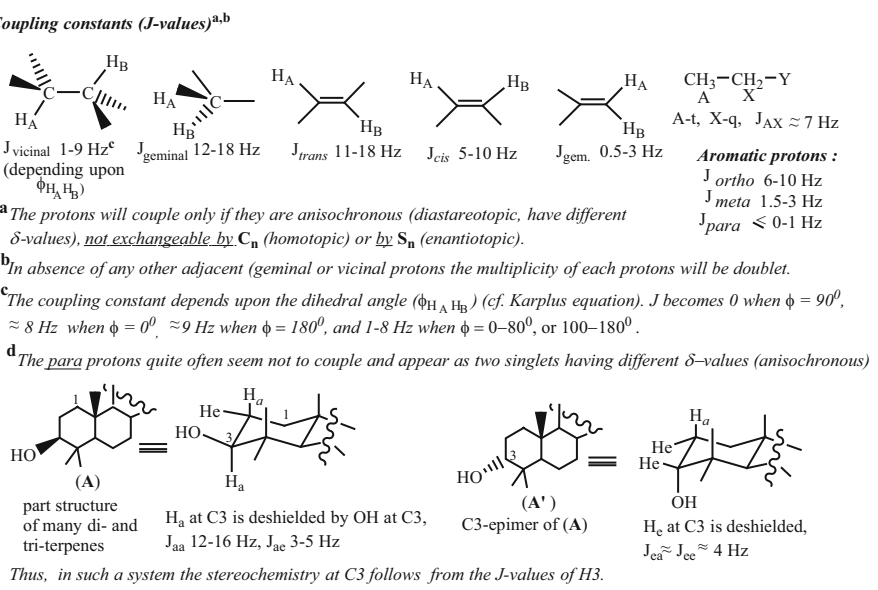


Fig. 4.15 The coupling constants (*J*-values) of interacting nonequivalent protons—common in natural and synthetic products

equation against α (different dihedral angles) is called the **Karplus curve**. But actual experimental data display a wide range of variation for different types of compounds based on the minimum energy conformations, if any, and other factors influencing the interactions. The *J*-value becomes **zero** when α is ~90°, since the side-to-side overlap of the two *perpendicular* C−H bond sp^3 orbitals (and hence their interaction) is at a minimum, whereas *J*-value becomes maximum (8.4–12.2) or (9.6–13) when α is ~0° or ~180°, respectively, since the side-to-side overlap of the two *parallel* C−H bond orbitals (and hence their interaction) is maximum. Overlap of the front lobes occurs at $\alpha = 0°$, whereas that of the back lobes and front lobes of the sp^3 orbitals occurs at $\alpha = 180°$ [23].

In more complex spectra with multiple peaks at similar chemical shifts or in spectra of nuclei other than hydrogen, coupling is often the only way to distinguish different nuclei.

Second-order (or strong) coupling The above description assumes that the coupling constant is small in comparison with the difference in NMR frequencies between the nonequivalent spins. If the shift separation decreases (or the coupling strength increases), the multiplet intensity patterns are first distorted, and then become more complex and less easily analyzed (especially if more than two spins are involved). Intensification of some peaks in a multiplet is achieved at the expense of the remainder, which sometimes almost disappear in the background noise, although the integrated area under the peaks remains constant. In most high-field NMR, however, the distortions are usually modest and the characteristic distortions

(*roofing*) can in fact help to identify related peaks. Second-order effects decrease as the frequency difference between multiplets increases, so that high-field (i.e., high-frequency) NMR spectra display less distortion than lower frequency spectra. Early spectra at 60 MHz were more prone to distortion than spectra from later machines typically operating at frequencies at 200 MHz, 300 MHz, 400 MHz, or above.

Magnetic nonequivalence More subtle effects can occur if chemically equivalent spins (i.e., nuclei related by symmetry and so having the same NMR frequency) have different coupling relationships to external spins. Spins that are chemically equivalent but are distinguishable (based on their coupling relationships) are termed magnetically nonequivalent. For example, the four H sites of *1,2-dichlorobenzene* divide into two chemically equivalent pairs (H3,H6) and (H4,H5) by symmetry (C_2 operation), but each pair is magnetically nonequivalent, since an individual member of one of the pairs has different couplings to the spins making up the other pair; thus here, $J_{3,4} = J_{\text{ortho}}$, $J_{3,5} = J_{\text{meta}}$, whereas, $J_{6,4} = J_{\text{meta}}$, $J_{6,5} = J_{\text{ortho}}$. Another example is 4-nitrochlorobenzene; here the pairs (H2,H6) and (H3,H5) are chemically equivalent (homotopic), but magnetically nonequivalent. Magnetic nonequivalence can lead to highly complex spectra which can only be analyzed by computational modeling. Such effects are more common in NMR spectra of aromatic and other non-flexible systems, while conformational averaging about C–C bonds in flexible molecules tends to equalize the couplings between protons on adjacent carbons, reducing problems with magnetic nonequivalence.

Coupling to any spin ½ nuclei such as ^{31}P or ^{19}F works in this fashion (although the magnitudes of the coupling constants are very different). But the splitting patterns differ from those described above for nuclei with spin greater than ½ because the *spin quantum number* has more than two possible values. For instance, coupling to deuterium (a spin 1 nucleus) splits the signal into a 1:1:1 *triplet* because the spin 1 has three spin states. Similarly, a spin 3/2 nucleus splits a signal into a 1:1:1:1 *quartet* and so on.

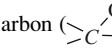
Correlation spectroscopy It is one of several types of *two-dimensional nuclear magnetic resonance (2D NMR) spectroscopy*. This type of NMR experiment is best known by its *acronym*, COSY. Other types of 2D NMR include Nuclear Overhauser effect spectroscopy (NOESY), J-spectroscopy, exchange spectroscopy (EXSY), total correlation spectroscopy (TOCSY), and heteronuclear correlation experiments, such as HSQC, HMQC, and HMBC. Two-dimensional NMR spectra provide more information about a molecule than one-dimensional NMR spectra and are especially useful in determining the structure of a molecule, particularly for molecules that are too complicated to work with using one-dimensional NMR. The first two-dimensional experiment, COSY, was proposed in 1971 and was later implemented in 1976 [44]. Limitation of space does not allow us to discuss even briefly different types of 2D NMR experiments, for which the readers may consult refs. [43, 44, 47–49, 52, 53, 57, etc.].

Sample preparation Samples are generally prepared in $CDCl_3$ taken in an NMR tube. Depending on the solubility. Sometimes d_6-DMSO, C_6D_6, CF_3COOD,

Table 4.8 Corresponding approximate field strengths of ^{1}H NMR and ^{13}C NMR spectra

^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
60 MHz	15 MHz	200 MHz	50 MHz	400 MHz	100 MHz
100 MHz	25 MHz	300 MHz	75 MHz	500 MHz	125 MHz

Table 4.9 Common ranges for ^{13}C NMR chemical shifts (δ-values in ppm[a])[b]

sp^3 Carbon (when associated only with H or C)	4–32	Methylenedioxy (–O–*C*H_2–O)	90–100
sp^3 Methyl (–*C*H_3)	4–24	Olefinic carbon (>*C*=*C*<)	100–145
sp^3 Methylene (–*C*H_2–)	14–50	Acetylenic carbon (–C≡C–)	105–130/150
sp^3 Methine (>*C*H–)	22–54	Aromatic carbon (e.g., )	110–150 (105–130)
sp^3 quaternary C (>*C*<)	30–56	Amidecarbonyl (–NH–*C*O–)	165–175
Aromatic methyl (Ar–*C*H_3)	12–35	Acid carbonyl (–*C*OOH)	160–175
Olefinic methyl (=CH–*C*H_3)	15–35	Aromatic Aldehyde (Ar–*C*HO)	170–200
Aromatic methoxy (Ar–O*C*H_3)	55–62	Aldehyde carbonyl (–*C*HO)	185–200
Oxymethylene (–O–*C*H_2–)	60–65	Aromatic ketone (Ar–*C*O–)	185–210
Epoxy carbon ()	50–80	Aliphatic ketone carbonyl (–*C*O–)	200–220
Oxymethine (O–*C*H<)	65–75	Nitrile carbon (–*C*N)	112–126

[a]For substitution effects and nature of substituents the δ-values are different from the normal ones
[b]In SFORD spectra –CH_3 (primary), –CH_2 (secondary), >CH– (tertiary) and >C< (quaternary) carbons appear as quartet (q), triplet (t), doublet (d), and singlet (s), respectively

CD_3CN, etc., solvents are also used as the solvent. A minute drop of TMS is added acting as internal reference.

^{13}C NMR spectroscopy It is a boon to the natural product chemistry. The natural abundance of ^{13}C is low (~1.1 %); hence compared to the ^{1}H NMR spectrum the ^{13}C NMR spectrum requires more material. The gyro magnetic ratio of ^{13}C being ¼ of that of proton, the field strength for ^{1}H NMR spectrum will be approximately four times that for ^{13}C NMR spectrum, as shown in Table 4.8.

The ^{13}C resonance is much weaker (~6,000 times) than proton and is ordinarily difficult to observe for mainly two reasons: (i) the natural isotope ^{13}C abundance is low (~1.1 %) and (ii) its gyromagnetic ratio is ¼ of that of proton; hence it resonates at a lower frequency than that of proton. For a given magnetic strength, when the protons are observed at 300 MHz, the carbon will be observed at 75 MHz. Like proton ^{13}C also couples with the neighboring nuclei. Thus, in the single frequency off-resonance decoupled (SFORD) spectrum, CH_3 carbon appears as a quartet, CH_2 carbon as a triplet, CH carbon as a doublet, and a quaternary carbon as a singlet. A noise decoupled (ND) ^{13}C NMR spectrum results when the whole range of ^{1}H NMR spectrum is decoupled, and each carbon of any molecule appears as a singlet. For example, a C_{30} molecule (e.g., a triterpene) usually shows 30 singlet peaks in its ND ^{13}C NMR spectrum. For identification of protonated (primary, secondary, and tertiary) and nonprotonated (quaternary) carbons APT and DEPT spectra are

routinely measured. Some common ranges for ^{13}C NMR chemical shifts of different carbons are given in Table 4.9.

Scopes of the discussions on the various types of NMR spectroscopic analysis being much less, the readers are advised to consult some helpful books (names given at the end) for such spectral analysis. The Chemical Shifts **(for both H and C) for commonly used solvents are given in tabular form I Appendix C**.

4.2.7 Mass Spectral Analysis [59–66]

The mass spectrometric analysis in principle differs from the electromagnetic spectral analysis. The molecule does not absorb any part of the electromagnetic radiation frequency; rather it involves the formation of the molecular ion and its subsequent fragmentation to other ions. These ions are separated according to their mass to charge ratios (m/z) when the ion beam enters a magnetic field. The mass spectrum is a record of the relative abundance of the ions and is measured relative to the strongest ion peak termed as **base peak.** The highest m/z peak is the molecular ion peak in EI mass spectrum. There are various ways of ionization. The most used one is the bombardment by energized electron to the molecule in the gaseous phase. Ionization of the compound takes place when bombarded with electron emitted from the hot filament, accelerated by an electric field, and having kinetic energy higher or equal to the ionization potential of the compound. The rate of ionization is extremely fast, and being faster than the bond vibration, the molecular ion thus formed will have the same molecular configuration of the parent molecule and is known as molecular ion peak. Electron with 50–70 eV is used for electron-impact mass spectrometric studies. The molecular ion peak then undergoes fragmentation by breaking of the susceptible bonds to yield ions. On the way to fragmentation some neutral molecules are eliminated.

If the electron energy is raised above the ionization potential of the compound, the molecular ion will undergo fragmentation. When the molecular ion is very unstable it undergoes instant fragmentation prior to the recording of its molecular ion peak. In such cases special techniques are applied to obtain the molecular ion peak and sometimes to limit the fragmentation to fewer ions. Because of the presence of isotopes in high proportion in some atoms the isotopic peaks also appear in the spectrum along with the true molecular ion peak. Depending on the percentage of isotope, the relative intensity of the isotopic peak (e.g., in case of bromo or chloro compound) is observed.

A few important ***methods of ionization*** are now described briefly.

1. ***Electron Impact (EI)***. The most commonly used method is the EI ionization. In this method the molecular ion formation (M^+) (by loss of an electron) and the subsequent structurally significant fragmentations are initiated by bombarding the molecules: in the gaseous phase with energized electrons (50–70 eV) at a low pressure in the ionization chamber. The method is applicable to molecules

capable of vaporization and not prone to thermal decomposition prior to the recording of the molecular ion.

2. ***Chemical Ionization (CI)***. In this method the ionization of the sample is effected by gas phase ion or molecule interactions. Different reagent ions can be used, and the molecular ion remains associated with the reagent. When the reagent ions are NH_4^+, H_3^+, CH_5^+, the peaks are $(M+NH_4)^+$, $(M+H)^+$ etc. However, whatever may be the reagent ion the protonated molecular ion peak is produced in relatively high abundance. Ions produced are of low energy and the fragmentations are limited. Because of the low-energy ions produced in the process, the method could be utilized in identifying isomers including stereoisomers which is not possible with EI mass spectral study. Sometimes negative ion CI are also studied, but only relatively used in natural product chemistry but more applicable in environmental chemistry.
3. ***Field Desorption (FD)***. In cases of compounds which fail to vaporize or suffer decomposition during vaporization FDMS is applicable. A solution of the material is applied between two plates or emitters and evaporated to grow small whiskers and are placed in the strong electrostatic field (10^7–10^8 ν cm^{-1}) potential difference is maintained when a molecule loses an electron to form positively charges ions. Mostly molecular ion or protonated molecular ion is formed depending on the nature of the molecule.
4. ***Fast Atom Bombardment Mass Spectrometry (FABMS)***. In this method ions are produced by bombarding the compound under investigation with a beam of fast atoms. Nowadays solution in a liquid matrix-coated probe surface is used which allows interaction between fast atom beam with fresh solute molecules. Glycerol has been found to a useful liquid matrix since it is less volatile and its solvent capacity for polar compound is appreciative.

Tandem Mass Spectrometric System This technique has been found to be useful in identifying followed by collection of the fractions of interest ***when coupled with chromatographic separation techniques*** like GC/MS and LC/MS. This technique serves as the guide for the collection of biologically active fractions previously identified by bioassay. It can also act as the detector especially in GC/MS.

It is possible to measure precisely the molecular weight with high-resolution mass spectrometer, suggesting the probable elemental composition of the molecule.

The Major Modes of Fragmentation In cases of polyheteroatom containing natural products, the predictability of fragmentation pattern is low. However, the fragmentation patterns of the major classes of natural products have been rationalized and advantageously utilized in settling the structures of many new compounds. Mass fragmentations of some natural products have been rationalized in relevant chapters.

4.2.8 Electronspray Ionization Mass Spectrometry

Electronspray Ionization Mass Spectrometry (ESI-MS), less commonly Electronspray Mass Spectrometry (ES-MS), is a technique used in mass

spectrometry to produce ions. It is especially useful in producing ions from macromolecules without fragmenting them and can be analyzed intact. The development of ESI for the analysis of biological macromolecules [81] was rewarded with the award of Nobel Prize in Chemistry to John Bennet Fenn in 2002. One of the original instruments used by Fenn is on display at the Chemical Heritage Foundation in Philadelphia, Pennsylvania.

In an ESI-MS the molecules to be analyzed pass into the mass spectrometer in a fine spray, typically following chromatographic separation, whether traditional liquid chromatography, HPLC, or nano-LC. As the spray emerges from the ESI source, the molecules are ionized by the nozzle's eclectically charged tip. As the mist travels and evaporates, electrostatic repulsion between like charged ions ultimately forces the molecules apart which may then be analyzed by a wide variety of mass analyzers.

Small organic molecules or pharmaceutical drugs (MW 200–500) in most instances ionize quite well to form quasimolecular ions created by the addition of proton and denoted by $(M+H^+)$. Quasimolecular ions created by the addition of another cation such as sodium ion, $[M+Na]^+$, or by the removal of proton, $[M-H]^-$, may also be observed. Thus interpretation becomes fairly straightforward. The mass spectrometers use so-called soft ionization technique that enables the ionization of the large biological molecules such as proteins.

ESI-MS has become an increasingly important technique in the chemical laboratory for structural study and also for quantitative measurement of metabolites in a complex biological system.

Liquid Chromatography-Mass Spectrometry (LC-MS) Electronspray ionization is the ion source of choice to couple liquid chromatography with MS. The analysis can be performed on line, by feeding the liquid eluting from the LC column directly to an electronspray, or off line, by collecting fractions to be analyzed later in a classical nanoelectronspray-mass spectrometry set up.

4.2.9 X-Ray Crystallography: Relative and Absolute Configuration. Conformation

The basic concepts of X-ray crystallographic analysis for full structural information of compounds have been dealt in fair details in various books [75–77].

X-ray crystallographic analysis requires single crystals characterized by parallelepiped unit cells which occur in a repetitive manner in the lattice. Connecting identical regular arbitrary points in the microscopic structure of the crystals forms the lattice. For structural determination excellent results are obtained with a good single crystal, which is roughly equidimensional with edges. X-ray of suitable wavelength is allowed to fall on the suitably mounted single crystal. The intensity of the diffracted beam is dependent on the distances between the atoms and independent of the orientations. Thus enantiomers will have the same X-ray

diagram. The phase changes due to scattering of incident radiation for atoms like C, H, N, and O are nearly same and X-ray diffraction is centrosymmetric. However, if a heavy atom like Br or any atom having mass ~30 more than the above atoms could be introduced into the molecule by chemical means, the work will be easier as these atoms increase the scattering power of the crystal. The phase change increases with atomic mass and the refraction pattern is no longer centrosymmetric. The heavy atom in the unit cell is located by diffraction pattern, which serves as the reference, and the structure factors are calculated from atomic coordinates, electron density map, and the phase number scattering amplitudes. In cases of chiral compounds both relative and absolute configuration can be determined. In the latter case X-ray wavelength should be close to the absorption of wavelength of the inner shell electrons of one of the atoms in the crystal. If the relative stereochemistry of all the chiral centers of a compound is known by chemical methods and if the absolute configuration of its one chiral center is determined by chemical correlation with a compound of known absolute configuration (by X-ray), the absolute configurations of all other chiral centers of the former follow. Before the advent of X-ray crystallography, the absolute configuration of some natural products having known relative stereochemistry was determined by application of Prelog's method (Chap. 2) on the secondary alcoholic function. The conformation of the molecule may also be known from X-ray crystallographic studies.

4.2.10 ORD and CD: Absolute Stereochemistry. Conformation

Optical Rotatory Dispersion (**ORD**) and Circular Dichroism (**CD**) studies become useful for determining the absolute stereochemistry of rigid molecules and the preferred conformation of flexible molecules, when applicable. Some rules with examples have been briefly discussed in Sect. 2.19.

4.2.11 Synthesis. Retrosynthesis. Green Chemistry. Atom Economy

Synthesis is an integral part of organic chemistry. In the field of natural product chemistry, synthesis confirms the structure assigned to a natural product on the basis of spectral and chemical methods and also establishes the stereochemistry of a chiral natural molecule by carrying out its asymmetric synthesis.

For designing a synthetic plan for a natural product and for its subsequent implementation one needs to have knowledge of reactions and their scopes and limitations. The target molecule (TM) may be formally fragmented or reasonably chemically disconnected to arrive at the much simpler molecular entities or ionic

components (synthons/synthon equivalents) from which the original bonds or connections, as present in the TM, could be chemically recreated in steps leading finally to its formation [82, 83]. To achieve this, one has to proceed in a backward way and hence the name ***retrosynthetic approach*** for this organizational chart. The path for such search is shown by hollow arrow ==>. Sometimes the target molecule may be disconnected in more than one way leading to different synthesis of a single natural product. It is also true that even though several possibilities are there, in reality, all of them may not be chemically viable.

Synthesis offers a challenge to the organic chemists, provides an intellectual exercise, and causes the development of new methodologies. Three excellent volumes in the Series "Classics in Total Synthesis" [84–86] have appeared in 1996, 2003, and 2011, respectively. The first two volumes contained much-acclaimed accounts of the total synthesis of some 60 complex natural molecules. The third volume features 42 most impressive total syntheses of 25 challenging natural products of the most recent era (i.e., 2003–2010). Each synthesis in these volumes features important strategies and tools employed and explains the key steps of the synthetic pathway. Each synthesis represents a small world of chemistry, logic, and thoughts of great masters. ***Retrosynthetic approaches*** on most natural product syntheses have been briefly discussed. In course of the syntheses new concepts and methodologies have generated which are generalized to enrich the chemistry in general and the area of application in particular. On the whole, such total syntheses contribute to the overall development of organic chemistry. Corey wrote in the *Foreword* of the Classics III [86], "In aggregate, Classics I–III elegantly and effectively chronicle monumental scientific achievements across a broad front." The reader will find the contents of all the volumes "equally enjoyable, instructive, and inspirational." A critical review [87] contains the structures and the colorful highlights of >75 selected natural products synthesized in the lab of Nicolaou since 1980, and the structures of >100 natural products synthesized in other labs since 2000, including many complex ones and their discussions, are a very useful archive of this field. The other reviews on *Trilogy* [88] and on the structure of the molecule and the art of its synthesis [89], appearing in 2012 and 2013, respectively, are also highly instructive and demands thorough reading. Two informative and instructive books entitled "Classics in Stereoselective Synthesis" [90] and "Elements of Synthesis Planning" [91] have appeared in 2009.

From extensive literature survey one can have the advantage of suitable methodologies, specially the current ones to be applicable or to be attempted for the synthesis of TM. In cases of complex molecules, researcher's insight and intuition play a vital role in the sequential organization of the synthetic planning. Only one enantiomer of optically active natural products is biosynthesized by Nature, with very rare exceptions. Further, it has been observed that the enantiomers of an asymmetric compound, natural or synthetic, possess different biological activities (see Chap. 32). So the synthesis of the natural or synthetic compound with desired biological activity is generally aimed at. *Firstly*, the (±) racemic product is synthesized, followed by resolution through the formation of diastereomeric salts or esters to get the desired enantiomer. The overall yield of the desired enantiomer is,

however, always less than 50 %. The rest being the undesired enantiomer is wasted. *Secondly,* by asymmetric synthesis one enantiomer/diastereomer is synthesized in a satisfactory excess of the other (enantiomeric/diastereomeric excess, ee/de) in a particular step and in a good yield. *Thirdly*, one can start with a chiral synthon for the synthesis of the desired molecules having multichiral centers. Suitable carbohydrates and amino acids may serve as good chiral synthons. In the second/third case diastereomers may be obtained in one or more step/s on the way, and the desired diastereomer is needed to be separated by suitable chromatographic technique/s (see Sect. 4.1). Many syntheses including stereoselective syntheses of natural products of different skeletal patterns have been discussed in various chapters.

Though the total synthesis of a natural product offers a great challenge to the organic chemists, its commercial use is not economically viable, especially in cases of complicated molecules. However, in some cases semisynthesis becomes effective, as in the case of (−)-taxol (Sect. 8.4.6.1).

Green Chemistry. Atom Economy [92–94]. Basically, three important interdependent aspects are to be considered in the planning of an efficient synthesis:

(a) Environmentally friendly experimental condition (green chemistry)
(b) Atom economy for each step of a multistep synthesis
(c) Economic viability, especially in cases of large/industrial scale synthesis and the overall yield.

Environmentally friendly experimental conditions refer to the cleaner conditions in which nontoxic and nonhazardous chemicals (synthons, reagents, solvents) should be used, and the waste products are biodegradable and either noninjurious or less injurious to the environment. On the whole, the pollution of the environment should not be caused, or the balance of the ecosystem should not be disturbed by such synthesis, especially in case of an industrial scale synthesis which includes reactions, conversions, etc., in large scales.

Chemical studies performed under such benign experimental conditions are referred to as green chemistry, a term coined only in early nineties. A number of journals, e.g., *Green Chemistry*, have been launched and ***dedicated to green chemistry***.

In shifting from traditional chemistry to green chemistry the atom economy [92, 93] and the improvement of yield are the two vital points to be taken care of. The atom economy or atom efficiency [94] for any reaction is given by the following expression:

$$\text{Atom economy} = \frac{\text{Molecular weight of the desired product}}{\text{Sum of the mol. wts. of all products in the stoichiometric equation of the reaction}}$$

Thus efficient synthetic methods include reactions that are both selective and economical in atom count so that maximum number of atoms of reactants appears

in the products. Methods that involve combining two or more building blocks with any other reactant, which serves as a catalyst, constitute the highest degree of atom economy [94]. *Enzymes are the most benign and efficient catalysts known, and enzyme-catalyzed reactions have the highest atom economy.*

Firstly, enzymes are chemo-, regio-, diastero-, and enantioselective in their behavior. The step of blocking the undesirably located similar group/s followed by their deblocking is not necessary for enzyme-catalyzed reactions. Such steps are thus eliminated leading to the improvement of the yield. *Transition metal-catalyzed methods* being selective and economical are now being increasingly used for synthetic efficiency. Barry M. Trost has published two elegant review articles [92, 93] on atom economy—a search for synthetic efficiency, and a challenge for organic synthesis using homogeneous catalysis—citing many examples.

Secondly, in the enzyme-catalyzed reactions the first chiral center is generated in a highly enantiomeric excess; enzyme-catalyzed generation of more chiral centers in subsequent steps give rise to highly diastereomeric excess (~100 %).

On the other hand, *an achiral synthesis starting with a symmetric/achiral substrate and using achiral reagents leads to racemic products in each step from the very first one, generating a chiral center, and in subsequent steps generating racemate diastereomers without much desired diastereoselectivity*. In the final step, the desired enantiomeric product may be obtained only after resolution, reducing its yield by 50 %. In chiral syntheses also, starting from a suitable chiral synthon the problem of the diastereoselectivity of the desired product still remains. At present, attempts are usually made to employ proper chiral reagents or catalysts to obviate the formation of undesired diastereomers, thus increasing the diastereoselectivity. Of course, attempts should always be made to use the undesired diastereomers in one way or other.

Thirdly, in green chemistry the ***waste*** should be minimum. The ***waste*** is defined as the entire chemical component/s produced in the process other than the desired product, and the ***E factor*** gives the waste per kg product [94]. Thus, the more the waste, the less is the atom economy. The waste is minimized in fine chemicals manufacture by widespread substitution of classical organic syntheses using stoichiometric amounts of inorganic reagents with cleaner catalytic alternatives. The E factors of chemical processes increase dramatically on going from bulk to pharmaceuticals and fine chemicals mainly due to the use of stoichiometric methods. The atom-efficient catalytic processes have been illustrated by Sheldon [94] with industrially relevant examples.

The Role of Catalysis A primary cause of *waste* generation is the use of stoichiometric inorganic reagents [94]. For example, stoichiometric reductions with metals (Na, Mg, Fe, Zn) and metal hydrides ($LiAlH_4$, $NaBH_4$) and oxidations with permanganate or chromium (VI) reagents are rampant for manufacture of fine chemicals. Replacing stoichiometric methodologies with cleaner catalytic alternatives may solve this. The atom economies of the stoichiometric oxidation of a secondary alcohol to the corresponding ketone with chromium trioxide in sulfuric acid and its catalytic oxidation with oxygen may be compared, e.g., in case of

Stoichiometric method:

$$3\ PhCH(OH)CH_3 + 2\ CrO_3 + 3\ H_2SO_4 \longrightarrow 3\ PhCOCH_3 + Cr_2(SO_4)_3 + 6\ H_2O$$

Atom economy = 3 x 120 / (3 x 120 + 392 + 6 x 18) = 360 / 860 = 42 % (approx.)

Catalytic method:

$$PhCH(OH)CH_3 + \frac{1}{2}\ O_2 \xrightarrow{\text{catalyst}} PhCOCH_3 + H_2O$$

Atom economy = 120 / (120 + 18) = 120 / 138 = 87% (approx.)

Fig. 4.16 Atom economy of stoichiometric oxidation versus catalytic oxidation

oxidation of α-phenylethanol to acetophenone, as shown in Fig. 4.16 [94]. Naturally, atom economy or atom efficiency dictates the adoption of the catalytic method, and a suitable catalyst is to be employed for this purpose.

Biocatalysis Biocatalysis has the potential to deliver *greener* chemical syntheses. Some of these opportunities and outstanding challenges are presented in a review article by J. M. Woodley [95].

4.2.12 Biosynthetic Compatibility of the Proposed Structure

The structure is expected to be biosynthetically compatible. The formation should be conceived following the discipline of the asymmetric biosynthesis in the cell. The precursors are to be selected retroasymmetric-biosynthetically to fit preferably into the identified biosynthetic pathways. In fact, structures of many natural products have been proposed, and in some cases corrected, based on biosynthetic compatibility. In quite a number of cases the proposed structures have been confirmed by X-ray studies and unambiguous syntheses.

4.2.13 Conclusions

In summary we can conclude that

- **UV** spectra show the presence of chromophore/s.
- **IR** spectra reveal the type of functional group/s present.
- **NMR** spectra provide information about the magnetically distinct atoms (mostly protons and carbons in organic molecules). Carbon connectivity studies by various correlation NMR spectroscopy give us the structural pattern of the molecule under investigation. The appropriate correlation diagrams are quite often equivalent to X-ray analysis, being capable of delivering complete structural information.

- **X-ray** analysis provides complete structural information of the molecules especially carrying heavy atom (absolute stereochemistry).
- **Optical Rotatory Dispersion (ORD)** and Circular **Dichroism (CD**) studies, when applicable, furnish absolute stereochemistry of the molecule.
- **Chemical Correlation** with a compound of known structure and absolute stereochemistry, if possible, reveals the absolute stereochemistry of the chiral natural molecule.
- **Synthesis** gives definitive support to the structure and stereochemistry.

All the above analyses may not be necessary for establishing the structure of a new natural product. Selection of the above analytical tools depends on the nature as well as complexity of the molecule.

4.2.14 Naming of Natural Products

To put a name to a new natural product is essential for its identity. The associated properties and other chemical details of the compound can be obtained from the published literature on the compound, or from the laboratory of its isolation—using its name. Hence, once the structure of a new natural product is established and its various physical constants (e.g., mp/bp, specific rotation, refractive index, etc.) are measured, it should be named. Earlier, prior to their structural assignment they used to be named for the sake of convenience. In most cases the structures of natural products being complex in nature, the IUPAC nomenclature in terms of homocyclic and heterocyclic systems present in many natural products poses difficulty to the readers for getting the name translated into structure and vice versa. Artemisinin (qinghaosu) (**1**), an important antimalarial drug, was isolated from *Artemisia annua* (fam. Compositae) [for structures (**1**)–(**41**) see Fig. 4.17] (*the structure numbers of the natural products mentioned here refer to the present subsection only).* The name qinghaosu is derived from a Chinese drug *qinghao*, well known for its medicinal use, especially against fever, dating from 168 BC. According to the IUPAC nomenclature it is octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10(3H)-one.

Echinulin, a fungal metabolite of *Aspergillus echinulatus,* later isolated from the peels of the green fruits named *Parwal* (Hindi) and *Potol (*Bengali) of a higher plant *Trichosanthes dioica* (Cucurbitaceae), is established to have structure (**2**). Its IUPAC nomenclature is 3*S*,6*S*,3-[{2-(1,1-dimethylallyl)-5,7-bis(3,3-dimethylally1*H*-inda-3-yl)}methyl]-6-methyl-2,5-piperazinedione. Longifolene, a sesquiterpene from *Pinus longifolia* (Pinaceae), possesses structure (**3**). Its IUPAC nomenclature is decahydro-4,8,8-trimethyl-9(15)ene-1,4-methanoazulene. To avoid the complexity of the IUPAC nomenclatures trivial names are always used.

It may be mentioned that another way of numbering the skeleton is the biogenetic numbering, e.g., in cases of sesquiterpenes, farnesol numbering system (**5**) is

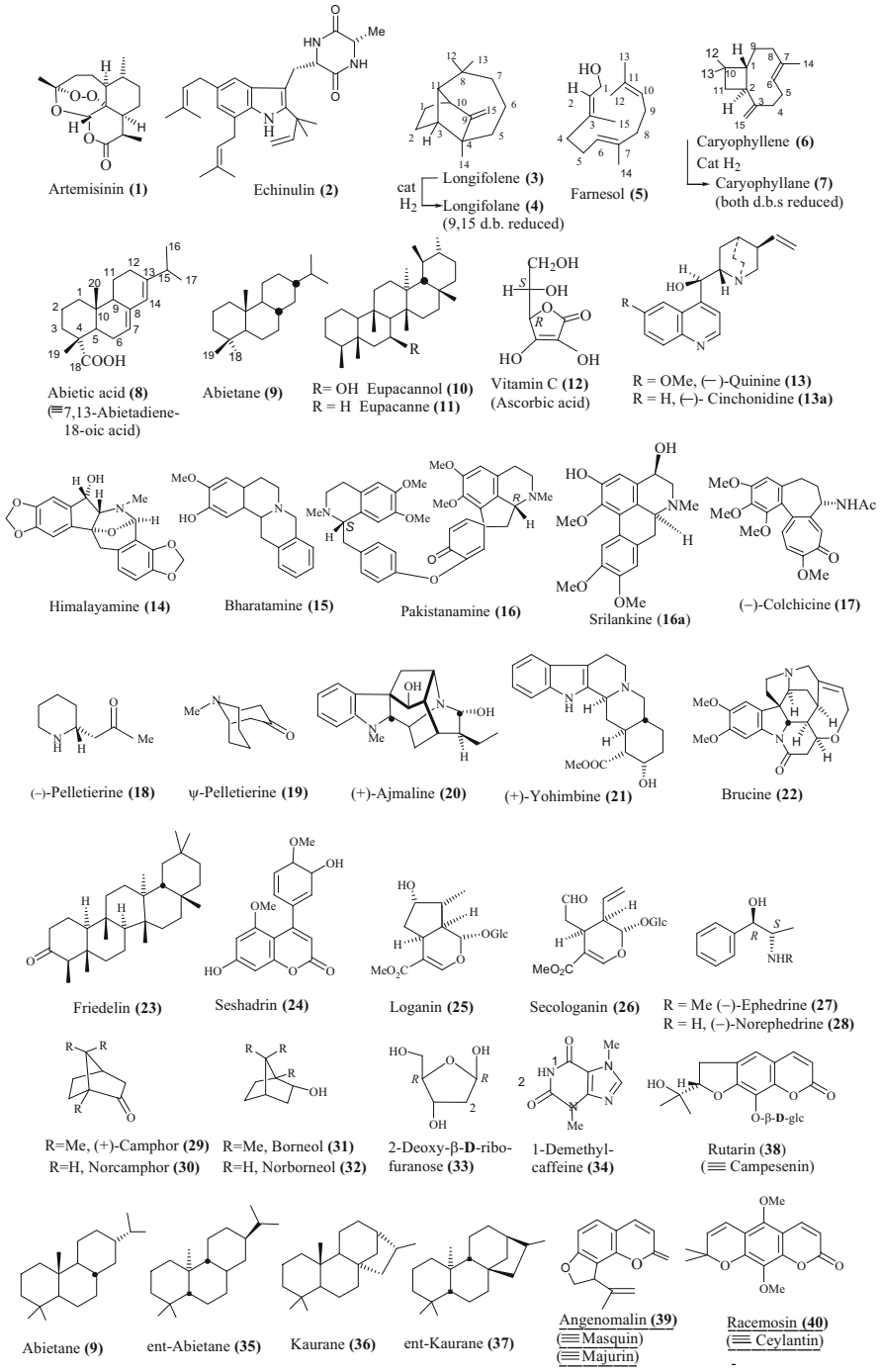


Fig. 4.17 Structures of the natural products (**1**)–(**40**) of plant origin

used. Here the folding of farnesol corresponds to the longifolene skeleton (**4**) having the same numbering system as in (**5**).

For convenience, complex natural products, especially terpenoids and steroids, are named based on the trivial names of the corresponding basic saturated hydrocarbons. Likewise, sometimes the complex alkaloids are named on the basis of the trivial names of the corresponding basic heterocycles. The individual compounds having the same basic stereostructural skeleton can be given IUPAC names based on the latter, mentioning the substitution locations. Caryophyllene, a sesquiterpene from mentha oil, possesses structure (**6**); the corresponding saturated hydrocarbon has been named caryophyllane (**7**). In terms of this basic hydrocarbon caryophyllene will have the IUPAC nomenclature, caryophylla-3(15),6-diene. Similarly longifolene will be longifola-9(15)-ene. Another example is abietic acid (**8**); the corresponding saturated hydrocarbon skeleton is named abietane (**9**). Hence abietic acid may be named abieta-7,13-diene-18-oic acid. A pentacyclic triterpene eupacannol (**10**), possessing a new skeleton, is named eupacann-7β-ol and the corresponding ketone is named eupacann-7-one, based on the corresponding saturated hydrocarbon eupacannane (**11**). The IUPAC nomenclatures of the compounds discussed in different chapters (Chaps. 6–15) have been mentioned along with their trivial names, wherever possible.

Thus, to avoid complications, the natural products are named mostly after the sources from which they are first isolated, as has been the practice since the early days of research on natural product chemistry. The new compounds of plant origin are named after the botanical names of the plants, barring few exceptions to be mentioned later. Some letters of the name/s of the genus and/or species are used in various pronounceable combinations to name them. In some cases the plant constituents have been named based on their biological activity. Several examples (Table 4.10) will illustrate these conventions.

Vitamins are named by letters as vitamins A, B, C, D, E, K, etc.; perhaps this nomenclature followed the chronology of their discovery, since the structures were not known at that time. They are not structurally related. The name vitamin was coined by C. Funk in 1913. In Latin *vita* means life. The compounds were erroneously thought to be amines and hence the name *vitamin*. Some vitamins belong to B, D, and K groups having numeral subscripts, e.g., B_1, B_2, B_6, B_{12}, D_1, D_2, D_3, K, K_1, K_2, K_3, etc. These compounds are essential for our well-being with disease-healing and health-giving properties, when taken in optimum amounts in the form of foods or medicines.

Quinine was named from the cutoff part of *Cinchona* species whose local name (South Africa) is *quina quina*. The name was given by Pelletier and Caventau. The suffix *ine* is always added in naming the alkaloids with few exceptions (e.g., camptothecin, taxol when considered as alkaloid). However, in German spelling the last letter *e* is dropped.

Sometimes compounds are named after the place/country of collection of the plant material, e.g., himalayamine from *Meconopsis villosa*, the plant being collected from the alpine region of Himalayas, bharatamine, isolated from an Indian (Bharat) plant, and pakistanine, isolated from a plant of Pakistan.

Table 4.10 Plant constituents derived from their sources[a]

Source (plant)	Name	Class of compound
Abies grandis	Abietic acid	Diterpene acid
Aegle mormelos Correâ	Marmesin, aegelenine	Coumarin, alkaloidal amide
Ailanthus malabaricus	Malabaricol	Triterpene
Anhalonium lewinii	Mescalin[b]	Alkaloid (protoalkaloid)
Artemesia annua	Artemisin	Sesquiterpene peroxide
Atropa belladonna	Atropine	Alkaloid
Capsicum annuum	Capsaicin	Alkaloidal amide
Chloroxylon swietenia	Xylotenin	Coumarin
Citrus fruits and plants	Ascorbic acid[c]	Sugar
Cinchona species	Cinchonine, quinine	Alkaloids
Coffea arabica	Caffeine	Xanthine derivative
Dendrobium gibsonii	Dengibsin, dengibsinin	Fluorenones
Dendrobium nobilie	Denbinobin	1,4-Phenanthraquinone
Ephedra sinica	Ephedrine	Alkaloid
Eupatorium cannabinum	Eupacacannol	Triterpene
Gelonium multiflorum	Multiflorinol, gelomulides A-K[d]	Triterpene, diterpene lactones
Gibberella fujikuroi	Gibberellins[e]	19-Diterpene acids with skeletal oxygenation sites
Ginkgo biloba	Ginkgolides A-C[d]	Diterpene lactones
	Bilobanone, Bilobalone	Sesquiterpenes
Nicotiana tobaccum	Nicotine	Alkaloid
Papaver somniferum	Papaverine, morphine[c]	Alkaloids
Rauwolfia serpentina	Reserpine, serpentine	Alkaloids
Seseli sibiricum	Sesebiricol	Coumarin
Taxus brevifolia	Taxol	Diterpene

[a]Literature references of most of the natural products may be found in the relevant chapters

[b]The flowering heads of this plant used to be imported to Europe under the name "*mescal buttons*"; hence the name

[c]*Compounds are named on the basis of their biological activity*: hexuronic acid (vitamin C) was trivially named ascorbic acid (**11**) [Greek *a* (without) and Latin *scorbutus* (scurvy)], since its deficiency causes a disease called scurvy, first observed in sailors, whose diet during their long voyage was found to be devoid of vitamin C. The name was given by Albert Szent-Gyorgyi (NL 1937). Its nomenclature is threo-hex-2-enoic acid-γ-lactone (threo-hexulosono-1,4-lactone-2,3-enediol). Morphine was named after the Greek God of dream and son of God of sleep *Morpheus*, because of its profound sedative and pain killing properties. The name was given by Gay-Lussac

[d]When more than one compound with the same but differently substituted skeletal pattern are obtained from the same plant, or from different species of the same genus, they may be named as A, B, C, preceded by a general name (based on the genus) given to the congeners possessing that skeletal pattern

[e]Gibberellins are named as Gibberellic acid (GA) with suffixes 1, 2, 3, 4, e.g., GA_1, GA_2, GA_3, GA_4. . .

Some compounds have been named after some famous persons who are related directly or indirectly with the work. The alkaloids pelletierine and ψ-pelletierine were named after Pelletier, regarded as the father of alkaloids. The alkaloid nicotine as well as the genus *Nicotiana* of the plant was named after a French diplomat Jean Nicot de Villemain who introduced tobacco in France. S. Siddiqui during 1930s isolated nine alkaloids from *Rauwolfia serpentine*; he named the main alkaloid "ajamaline" after the name of Hakim Ajmal Khan who had used *R. serpentina* for treatment of mental ailments for nearly two decades. Again, the alkaloid brucine (**12**), isolated from *Strychnos nuxvomica* and many other *Strychnos* species, derived its name from the plant source *Brucea antidysenterica from which it was first isolated*. Incidentally it may be mentioned that the genus of the plant was named after the explorer James Bruce (1730–1794), and the species, probably, was named in view of the plant's physiological property.

In 1899 Constantin Istrati and A. Ostrogovitch of the University of Bucharest isolated a compound from cork and named it fridelin after the famous nineteenth century French chemist, Charles Friedel. The compound later turned out to be a pentacyclic triterpene and has been isolated from many plants. A neoflavone derivative has been named seshadrin after the name of an Indian natural product chemist T. R. Seshadri. These ways of naming the compounds reflect the personal fascination of the researchers to remember the place, honor the man, and also highlight their biological properties and do not fall under the normal purview of the convention.

None of the methods of naming, discussed so far, reflects the nature/structure of the compounds. However, the suffix "ine" after the name points to its being an alkaloid (in German spelling e is dropped), the suffixes "ol" and "one" point to alcohol and carbonyl compounds respectively and the suffix "lide" points to terpene (sesqui-, di-) lactones. The following prefixes before the name of the parent compounds bear some significance.

"Seco" means the presence of a cleaved ring, e.g., loganine (13) and secologanin (14). The prefix "nor" indicates the removal of one or more carbon atoms from the skeletal framework or from a group containing a heteroatom, e.g., ephedrine (15) and norephedrine (16). The lack of two, three, or four, etc., skeletal atoms of the parent compound is indicated by the numerical prefixes "dinor," "trinor" or "tetranor," etc. In certain cases "nor" is used to denote lack of even three skeletal atoms with respect to the parent compounds, e.g., camphor (17) and norcamphor (18) and borneol (19) and norborneol (20). Tetranortriterpenoids (C_{26} compounds) form an important class of natural products. From the name it is clear that they have four carbon atoms less, compared to the skeletal carbon content of triterpenes (C_{30}). The prefix "des" means one unit less. Compounds containing $–OCH_3$ (either a natural product or a reaction product) when demethylated results in a desmethyl compound. The prefix "de" also represents absence of some group or atoms, e.g., deoxy, deactoxy, deacetyl, etc., compounds like 2-deoxy-D-ribofuranose (**21**) and 1-demethylcaffeine (**22**). The prefix "*ent*-" (standing for *enantio*-) indicates stereochemical inversions at all chiral centers compared to the parent compound, e.g., abietane (**23**) and *ent*-abietane (**24**) and kaurane (**25**) and *ent*-kaurane (**26**).

Literature survey shows that a single compound isolated from different sources, sometimes, has been given two or more names. This happens due to its simultaneous independent publications, or may be due to the absence of knowledge of the previous work by the later workers. A few examples are given: the coumarin (**27**) named rutarin (isolated from *Ruta graveoleus*, 1967) and campesinin (from *Seseli campestre*, 1970); the coumarin (**28**) named angenomalin (from *Angelica anomala*, 1967), masquin (from *Pimpene cola*, 1967), and majurin (from *Amni majus*, 1971); and the coumarin (**29**) named racemosin (from *Atalantia racemosa*, 1978) and ceylantin (from *Atalantia ceylanica*, 1984). However, nowadays in view of the electronic library facilities available such a probability may be avoided.

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Chapter 5
Biosynthesis of Terpenoids: The Oldest Natural Products

5.1 Biochemical History

Terpenoids constitute the largest and structurally vastly diverse class of natural products with spectacular abundance (~30,000 known compounds) and have an extremely rich and long biochemical history. They are the oldest natural products known, as they are found to occur in fossils and sediments of different ages [1–4]. Terpenoids in various forms play an essential role in the biomembrane reinforcement [3].

5.1.1 Terpenoids as the Precursor of Cholesterol

Cholesterol is biosynthesized from lanosterol in animals and fungi and from cycloartenol in plants (vide Sect. 11.2.5). It is an important component of biomembrane (eukaryotic phospholipid membrane), and it participates in the reinforcement of membrane architecture by forming hydrogen bonds with its OH group and the head group of the phospholipids [3]. Both lanosterol and cycloartenol are formed from squalene 2,3*S*-oxide (vide Sect. 11.2.5).

5.1.2 Terpenoid Derived Diagenetic Entities [1–4]

An understanding of geochronology along with organic geochemical knowledge would certainly reveal many mysteries of evolution. The occurrence of terpenoid-derived diagenetic entities in the informative fossils, and ancient and recent sediments serve as the pointer to estimate to some extent the degree of maturation of the sediment, and to reconstruct the paleo-environment. It may be mentioned that the diterpenoid phytane (C_{20}-hydrocarbon) having the same carbon skeleton of phytol

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moiety of chlorophyll, and supposed to be generated from chlorophyll in a very early stage of degradation, has been found to be present in some fossils. Many other diagenetic entities from terpenoids belonging to higher terrestrial plants, in which they are biosynthesized, have been isolated from fossils and sediments [1–4] (vide Sects. 8.3.9 and 10.3). Their isolation and identification are the indicators of the presence of photosynthetic organisms during that contemporary time. Fossil records suggest that the precursor organisms in many cases were plants [3].

5.1.3 *Ruzicka's Isoprene Hypothesis [5–7]*

The carbon contents of terpenoids show that the skeletal carbon numbers are multiples of 5, *i.e.*, $5x$, where $x = 1, 2, 3, 4, 5, 6$, etc.; hence, C_5 is the building block of terpenoids. Their known molecular structures, when formally dissected, appear to have a synchronized combination of C–C(C)–C–C units. Of the several C_5 potential candidates like isovaleric acid, dimethylacrylic acid, and isoprene, the last one has been found to be the obvious choice, as was initially surmised by Otto Wallach (NL 1910). Ruzicka (NL 1939) meaningfully hypothesized [5–7] the compositional regularity involving the isoprene unit in the structures of terpenoids. This hypothesis, termed as biogenetic *isoprene rule* [5, 6], is inspirational; it serves as a very useful guide and forms the basis for the biosynthetic and structural speculations of terpenoids.

5.1.4 *Discovery of Isopentenyl Pyrophosphate (IPP): The Biological Isoprene Unit*

Isoprene itself has been found to be biologically inactive. Search for biological equivalent of isoprene led to the isolation and identification of isopentenyl pyrophosphate (IPP)—"a long sought biological *isoprene unit*" [8] by Lynen (NL 1964) when he was studying the conversion of (*R*)-(+)-mevalonic acid into squalene [9]. Bloch (NL 1964) also made similar observations independently [10]. The identification of the true biologically active isoprene, IPP, paved the way for detailed biosynthetic studies of terpenoids at the enzymological level. The skeletal carbons of (*R*)-(+)-MVA pyrophosphate, the biogenetic precursor of IPP in the MVA pathway, have been obtained from three molecules of acetyl coenzyme A (Sect. 5.2.1).

5.1.5 Concept of Biogenesis and Biosynthesis

Prior to the discussion of the biosynthetic pathways, the terms *biosynthesis* and *biogenesis* need clarification. Plants synthesize natural products within the cells—the process is called *biosynthesis*, because the syntheses are carried out in the biosystem. But when we formulate the biosynthetic pathway of a structurally known natural product from the assumed precursor/s—simple primary metabolite/s (equivalent to synthon/s in a laboratory synthesis), following the mechanistic discipline and logic of organic chemistry, we call this assumed pathway as the *biogenetic pathway* of formation of that natural product or of the class it belongs to. *Biogenesis* may also be looked upon as the *retrobiosynthetic process*, the precursor being selected retrobiosynthetically, just as we select the synthon retrosynthetically for the laboratory synthesis of an organic compound of known structure. However, in case of retrobiosynthesis, the selection of the precursor is to be restricted within the repository of the known simple metabolites or some metabolites derived from them.

The assumed precursor is selectively labeled by an isotope or isotopes, and feeding experiments are done with it. If the isotope/s gets/get incorporated in the product/s at the expected location/s, it will be established that the synthesis has taken place in the biosystem (plant, in vivo) following the designed pathway. This *experimentally supported biogenesis* is also called *biosynthesis*.

However, unexpected incorporation at a location or locations in some cases compels the investigators to rationalize the biosynthesis following a different pathway, as is the case of non-MVA pathway (Sect. 5.3) for terpene biosynthesis, discussed later.

In summary, it may be said that all natural products of plant origin are biosynthesized in plant cells, and that a hypothetical biosynthetic pathway for the formation of a natural product or its class is referred to as *biogenesis*. When the hypothetical pathway is backed by experiment, it is also known as *biosynthesis*.

5.2 Mevalonic Acid Pathway

5.2.1 Acetyl Coenzyme A to Isopentenyl Pyrophosphate (IPP): Stereochemical Implications

Acetyl coenzyme A ($CH_3COSCoA$) is thought to be involved in its initial nucleophilic addition to biotin bound CO_2 to yield malonyl coenzyme A, generating a better activated nucleophilic methylene group, flanked between two electron withdrawing groups (Fig. 5.1). Malonyl coenzyme A then reacts with a second molecule of acetyl coenzyme A in a Claisen condensation fashion, followed by

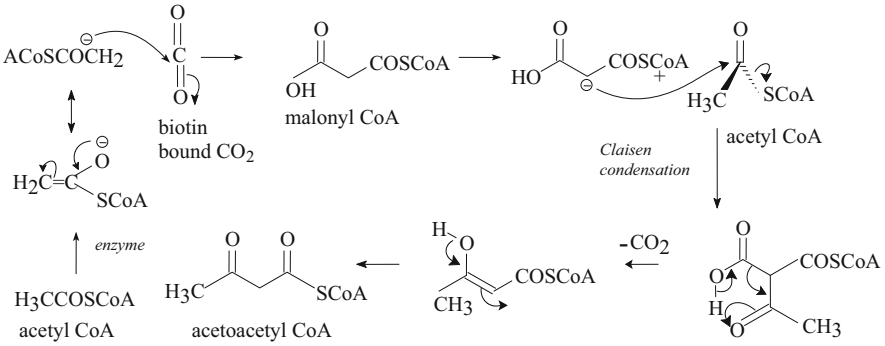


Fig. 5.1 Biosynthesis of acetoacetyl CoA *via* malonyl CoA

decarboxylation to yield acetoacetyl coenzyme A. However, it has been shown that malonyl coenzyme A, though involved in fatty acid and polyketide biosynthesis, is not accepted in the mevalonic acid pathway.

5.2.2 *Bioformation of* (R)-(+)-*Mevalonic Acid*

The ***mevalonic acid pathway*** uses three molecules of acetyl coenzyme A in two steps (Fig. 5.2). The first two molecules undergo Claisen condensation, mediated by acetoacetyl coenzyme A thiolase (AACT), to yield acetoacetyl coenzyme A. The third molecule of acetyl coenzyme A, prior to its participation in aldol condensation with acetoacetyl coenzyme A, gets attached to the catalyzing enzyme to form an acetylated enzyme [11, 12]. The latter then reacts with acetoacetyl coenzyme A to yield a chiral molecule. The enzyme-bound thioester group is then hydrolyzed to yield (*S*)-β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA). The thioester group of HMG-CoA is reduced to a hydroxymethyl group in two reductive steps (−COSCoA → –CHO → $–CH_2OH$) using two molecules of NADPH to yield (*R*)-(+)-mevalonic acid, [via (*R*)-mevalonic acid], being catalyzed by HMG-CoA reductase.[1]

The name mevalonic acid is derived from its systematic name β,-δ-dihydroxy-β-methylvaleric acid. It was discovered serendipitously by Karl Folkers and his associates. “The discovery of 3*R*-mevalonic acid was most

[1] This reaction is irreversible and is a committed key step in the biosynthesis of cholesterol (*vide* Sect. 11.2.5) in eukaryotes. Thus cholesterol formation can be regulated by synthesizing drugs which can act as the inhibitor in this enzymic reaction. Incidentally, it may be mentioned that in 2001 Bayer’s cerivastatin, one of the cholesterol reducing statins that inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase involved in cholesterol synthesis, is withdrawn because of the increasing reports of its side effects.

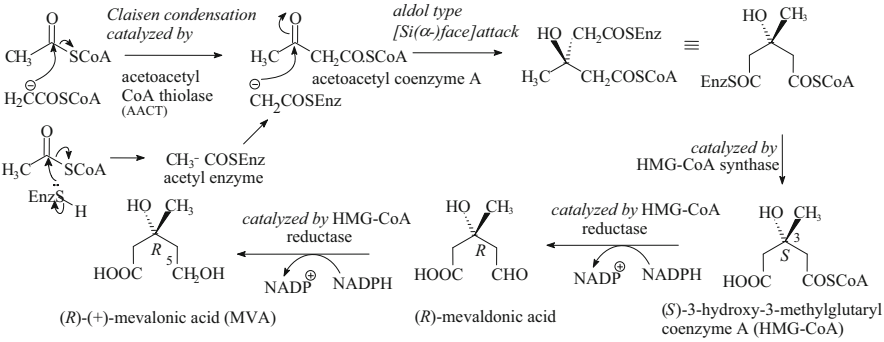


Fig. 5.2 Biosynthesis of (*R*)-(+)-mevalonic acid

important of all: . . .and it is not utilized for any metabolic process other than that of isoprenoid biosynthesis" [13].

5.2.3 Conversion of (R)-(+)-*MVA to IPP*

The conversion of mevalonic acid to IPP requires three molecules of ATP, though the direct evidence of the involvement of the third molecule of ATP is not available from the intermediates formed on the way. Two molecules are involved in pyrophosphorylation of C_5–OH of mevalonic acid to yield mevalonic acid 5-pyrophosphate (MVAPP) in two steps, releasing two molecules of ADP (Fig. 5.3). It is assumed that during decarboxylative dehydration of MVAPP, the latter undergoes phosphorylation at the 3-OH during its elimination. This conjecture has been proved by labeling 3-OH oxygen by ^{18}O (*i.e.*, using 3-^{18}OH), and the eliminated inorganic phosphate (Pi) is found to contain ^{18}O. Such phosphorylation may be realized by its interaction with γ-phosphate of the third ATP molecule (Fig. 5.3). The double bond of IPP is formed in a concerted elimination process and not by dehydration followed by decarboxylation. This type of enzymatic reactions is not observed elsewhere in enzyme chemistry. Here, we find the conversion of a C_6 compound to a C_5 one through the destruction of the chiral center. Further, it has been found that (*R*)-(+)-mevalonic acid is the obligatory precursor in this conversion. If the system is fed with (*S*)-(−)-mevalonic acid, it will not be accepted by the enzyme mevalonate kinase. Again, if (*RS*)-(±)-mevalonic acid is fed, the system will accept only the (*R*)-(+) variety while the (*S*)-(−) variety will remain unattended and will be eliminated as such from the system, being metabolically inert. Thus, this enzyme acts as "an efficient stereochemical filter." Cornforth (NL 1975) commented [14–16], "This is fortunate, since optical resolution of the synthetic acid is difficult: labeled mevalonic acids are usually racemic."

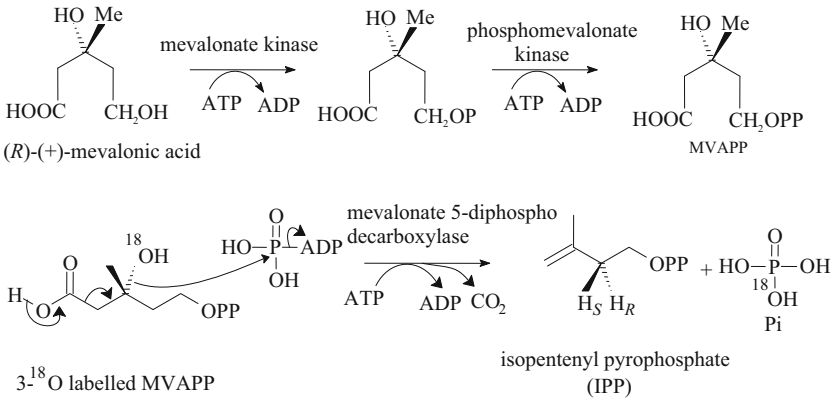
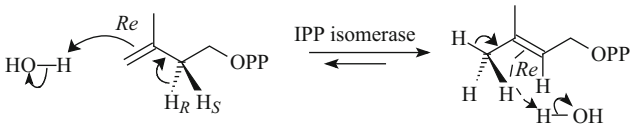


Fig. 5.3 Conversion of (*R*)-(+)-mevalonic acid to isopentenyl pyrophosphate (IPP)



for *Re,* H_R and H_S see **Chapter 2**

(Sections 2.9.5 and **2.9.6)**

Fig. 5.4 Isomerization of IPP to DMAPP

5.2.4 Isomerization of IPP to γ,γ-Dimethylallyl Pyrophosphate (DMAPP): Stereochemical Implications

The isomerase for converting IPP to DMAPP and the prenyl transferase that catalyze the head to tail condensation between IPP and DMAPP have been discovered by Lynen et al. [14] in 1959. The above isomerization is represented in Fig. 5.4. The equilibrium is inclined more towards γ,γ-dimethylallyl pyrophosphate (DMAPP), its double bond being more substituted. The stereochemistry of this isomerization will be defined by analyzing the following points: (a) which of the two prochiral allylic hydrogen atoms (H_R or H_S) (vide Sect. 2.7) will be eliminated to form a new double bond and (b) to which *enantiotopic face* the hydrogen will be accepted to saturate the existing double bond leading to the formation of a methyl group. In this study, isotope labeling of the key prochiral hydrogen by deuterium or tritium serves as the sure guide towards the stereochemical aspect of the process [13–21].

5.2.5 *Formation of Chiral Acetic Acid (7) [13, 17, 19] from 2-T-MVA Pyrophosphate (1)*

The aforesaid types of enzymatic experiments were elegantly designed and executed by Cornforth [13, 17, 19]. (2*R*,3*R*)-(2-T)-Mevalonic acid pyrophosphate (**1**) is subjected to similar decarboxylative dehydration in presence of the specific enzyme catalyst and ATP (cf. Fig. 5.3) to form the *Z*-isomer of T-labeled IPP (**2**) (Fig. 5.5). The stereochemistry of the decarboxylative dehydration step involving the elimination of the antiperiplanar OH [reacting with ATP to give the labeled IPP (**2**), ADP, and inorganic phosphate] and COOH groups is also shown. The labeled IPP (**2**) then undergoes isomerization in D_2O in the presence of IPP isomerase (cf. Fig. 5.4), which catalyzes the antarafacial 1,3-allylic rearrangement (consistent with the stereoelectronic control) to give the labeled DMAPP (**3**).

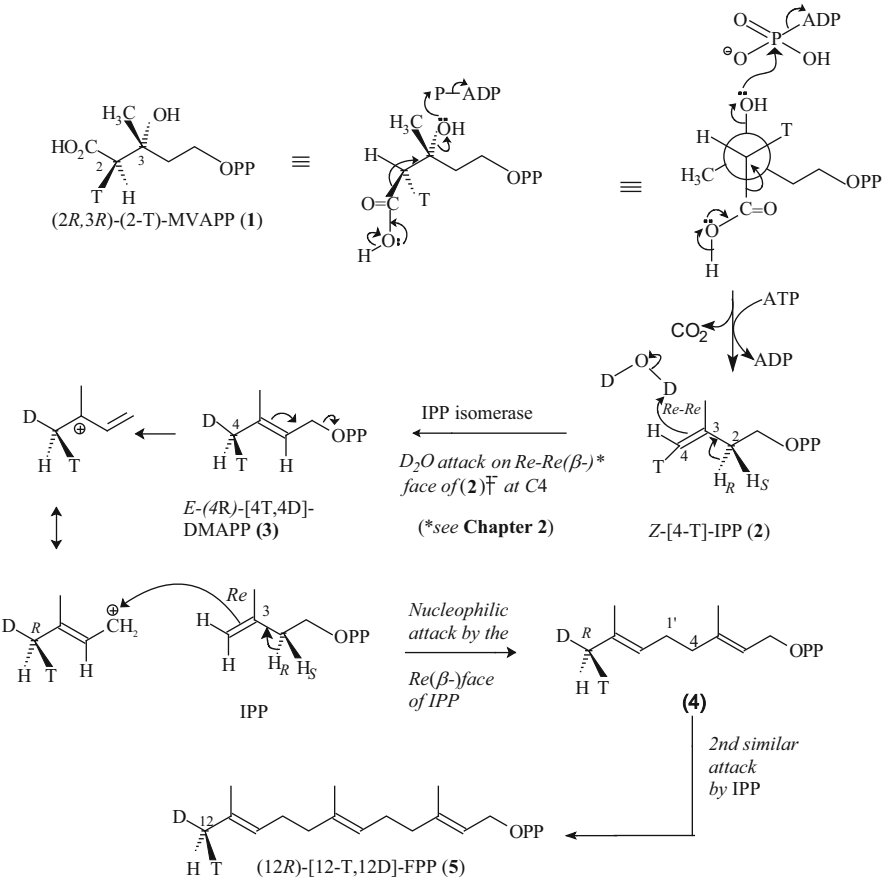


Fig. 5.5 Steric course of the reactions forming labeled IPP (**2**), DMAPP (**3**), and FPP (**5**)

Cornforth unambiguously established by elegant experiments (stated in the sequel) that the electrophilic addition of D^+ takes place at C_4 of (**3**) (Fig. 5.5) on the enantiotopic *Re–Re* (front) face (vide Sect. 2.7) [the priority sequence of ligands at C_3 being $C_2 > C_4 > CH_3$ (cf. Sect. 2.6.2.3) and that of ligands at C_2 being $C_3 > T > H$ of compound (**1**)] of the 3–4 double bond in a concerted manner with the loss of H_R from C_2. The geometry of the double bond in (**3**) becomes *E*, the generated doubly labeled (by T and D) methyl group (with 4*R* configuration) being *anti* to the CH_2OPP group. This provides an example of elucidation of prochirality in a biochemical reaction; here, loss of H_R from C_2 and concerted addition of D from the *Re–Re* face of (**2**) generates the doubly labeled (*R*)-methyl of DMAPP (**3**).

5.2.5.1 Formation of Isotopically Substituted Chiral Farnesyl Pyrophosphate (FPP) (5) and Chiral Acetic Acid (7)

The successful implementation of this plan required overcoming the reversibility of the isomerization responsible for the loss of stereochemical integrity of the chiral methyl group. This problem is solved by using a soluble enzyme fraction (containing a suitable prenyltransferase) from pig liver to remove DMAPP (**3**) by converting it, as soon as it is formed, into the chiral farnesyl pyrophosphate (FPP) (**5**) containing the labeled methyl group (at its head) by nucleophilic attacks by two molecules of IPP on C_1 of (**3**) and (**4**) successively. The corresponding alcohol, farnesol, obtained by enzymatic hydrolysis of (**5**) with alkaline phosphatase (Fig. 5.6), is subjected to ozonolysis to convert the labeled terminal isopropylidene group to labeled acetone (**6**). The latter undergoes iodoform reaction with $KI–I_2$ to afford chiral (*R*)-acetic acid (**7**) without giving an opportunity for proton exchange between the methyl group of (**6**) and the solvent. The *R*-configuration of (**7**) is established by a configurational correlation with a synthetic sample obtained by Cornforth [13, 17, 19] using a wholly chemical sequence and resolution procedure. Arigoni and Retey [18] used a part-enzymatic synthesis of the labeled (*R*)- and (*S*)-acetic acid, which appeared in *Nature* as the paper next to that of Cornforth. A few years later Arigoni [20] developed an interesting chemical synthesis of both (*R*) and (*S*) forms of chiral acetic acid. It is also observed that the configuration of the labeled acetic acid mainly generated via *E*-[4-T] IPP (the geometrical isomer of (**2**),

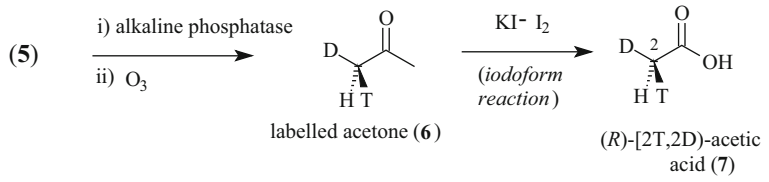


Fig. 5.6 Conversion of labeled farnesyl pyrophosphate (**5**) to (*R*)-[2T,2D]-acetic acid (**7**)

is *S*. It is thus established that the deuterium must have been added to the *Re–Re* (front) face of the double bond in *Z*-[4-T]-IPP (**2**).

It should be noted that chiral methyl compounds having no other dissymmetric part would exhibit very small optical rotation because of very little polarizability difference of H, D, and T. Moreover, even if all molecules of such a compound possess same chirality, they are usually mixed with majority of achiral methyl molecules. Hence, optical rotation measurement is clearly not practical.

5.2.5.2 The Absolute Configuration of [HDT]-Acetic Acid

The absolute configuration (*R* or *S*) of [HDT]-acetic acid produced is determined by bioassay methods using stereochemistry of known enzymatic reactions, *e.g.*, malate synthase reactions. [13, 17–21] In this method, the chiral acetic acid (as acetyl coenzyme A) and glyoxylate are condensed irreversibly on the enzyme malate synthase when they produce malates as shown in Fig. 5.7. This enzyme reaction is known to proceed with inversion of configuration. Tritium is displaced at the least rate, and deuterium is displaced three or four times less easily than protium when H, ^{2}H, and ^{3}H are present in the same methyl group (chiral methyl). The ratio of the abundance of the ^{2}H and ^{1}H in the products is equal to their kinetic isotope effects. Consequently, the products malates of this reaction with (*R*)-acetyl conenzyme A contains two tritiated species in unequal amounts, the major one retaining ^{2}H and the minor one retaining ^{1}H. Likewise, from (*S*)-acetyl coenzyme A two other tritiated malate species are formed (Fig. 5.7). The enzyme-catalyzed C–C bond formation takes place by attack on the *Si*-(α-) face of the glyoxylate to form 2*S*-malate in both cases (Fig. 5.7, last column).

Treatment of the malates with the enzyme fumarase affect the anti elimination of the elements of water. It is obvious from the figure that the major fumaric acid from the (*R*)-enantiomer will contain ^{3}H of the chiral methyl while the minor one is devoid of ^{3}H. In the case of the (*S*)-enantiomer, major fumaric acid will contain ^{2}H and the minor one carry the ^{3}H (Fig. 5.7).Thus, from the isotope analysis of the fumaric acids, the chirality of the unknown chiral acetic acid could be ascertained [13].

It is also found that catalyzed by the specific enzyme, (3*R*,4*R*)-[4-D]-MVA (**8**) is converted[2] through labeled IPP (**9**) and labeled DMAPP (**10**) into [2,6,10-D_3]-farnesyl pyrophosphate (**11**) which is enzymatically hydrolyzed to [2,6,10-D_3]-farnesol (**12**) (Fig. 5.8). The deuterium content of the isolated farnesol, as determined by mass spectroscopy, is consistent with the retention of the deuterium in (**8**)

[2] Actually (3*RS*, 4*R*)-[4-D]-MVA was used since only 3*R* (and not 3*S*) isomer got incorporated in the biosynthesis, as mentioned earlier.

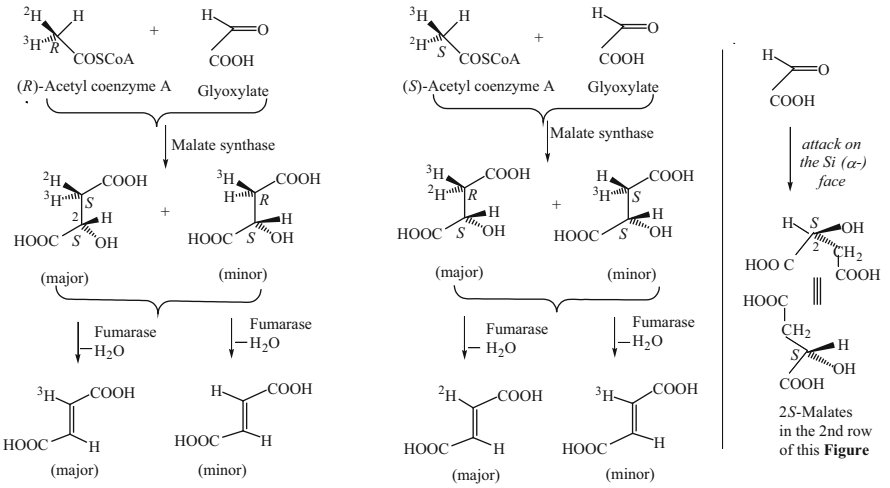


Fig. 5.7 A system for discriminating between the two enantiomeric chiral acetic acid [13]

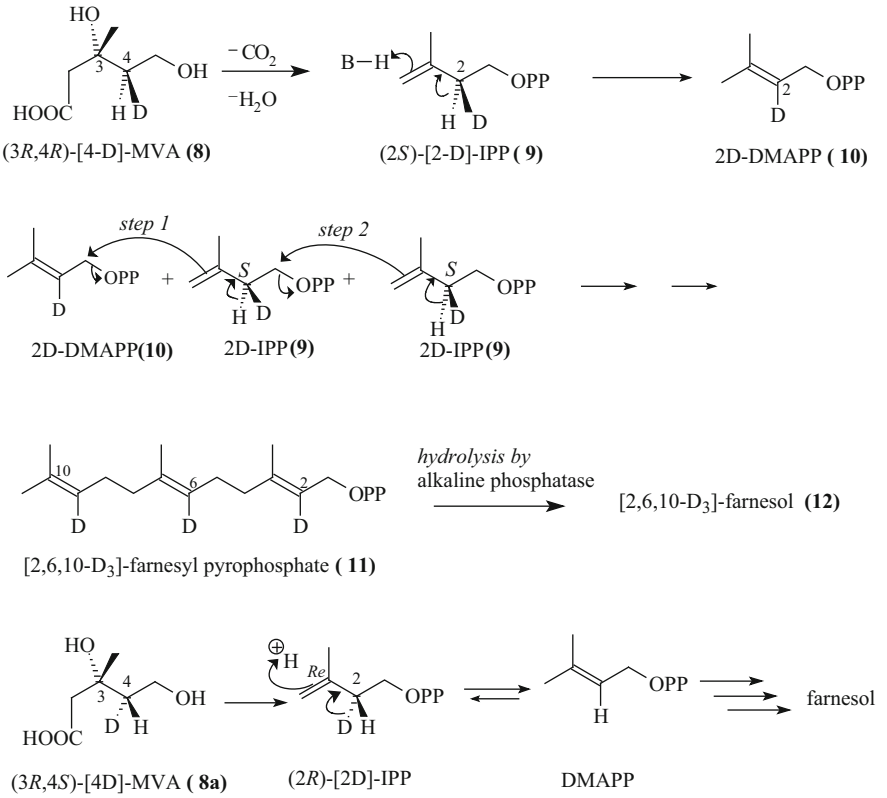


Fig. 5.8 Conversion of (3*R*,4*R*)-[4-D]-MVA (**8**) into [2,6,10-D_3]-farnesol (**12**) and of (3*R*,4*S*)-[4D]-MVA (**8a**) into farnesol (Cornforth's concept)

after decarboxylative dehydration and isomerization to give [2D]-DMAPP (**10**), proving that during isomerization, 2-H (α-) is lost. As expected (3*R*, 4*S*)-[4-D]-MVA (**8a**) loses virtually all of its deuterium during conversion into farnesol through the same sequence of reactions,[3] confirming that 4α-D is lost from the rear face during the isomerization (Fig. 5.8).

5.3 Non-Mevalonoid (Rohmer) Pathway {1-Deoxy-D-Xylulose-5-Phosphate (DXP or DOXP): Mevalonate Independent [22–25]

The (*R*)-(+)-mevalonic acid, thought to be the obligatory precursor in the entire biogenetic pathway of terpenoids, remained unchallenged since the discovery (1950s) of this route in the plants. However, comparatively recently (starting from late 1980s) *poor incorporation* of the labeled acetate/mevalonate in the expected manner was observed during the biosynthetic studies of various terpenoids, especially in a number of monoterpenes like *geraniol* and diterpenes like *taxol*, *ginkolides*, etc. The labeling patterns were inconsistent. This inconsistency questioned the compatibility and the universal acceptability of the mevalonic acid pathway as the cornerstone for the biosynthesis of terpenoids. The authors working on geraniol and other monoterpenes [26] and taxol and taxane skeleton [27] thought for an alternative pathway discovered by Rohmer [22, 23]. He discovered that the C_5 framework of the isoprenoid gets its carbon atoms out of the condensation of a C_2 unit, derived from pyruvic acid, on the carbonyl of a triose, D-glyceraldehyde-3-phosphate. Two major groups [11, 22–25] worked on the non-mevalonate pathway for the formation of isoprenoids.

5.3.1 Formation of DXP from Pyruvic Acid and D-Glyceraldehyde-3-Phosphate

The newly discovered route involves 1-deoxy-D-xylulose-5-phosphate (DXP or DOXP) in the early stage of IPP formation. This C_5-sugar is generated (Fig. 5.9) by a transketolase catalyzed addition of C_2 unit (CH_3CO–), derived from pyruvate decarboxylation (vide Fig. 3.22), to D-glyceraldehyde-3-phosphate. The DXP thus formed is transformed into 2-C-methyl-D-erythritol-4-phosphate (MEP) by skeletal rearrangement as stated in Sect. 5.3.2.

[3] Here the (3*RS*, 4*S*) compounds may be used giving farnesol, devoid of deuterium.

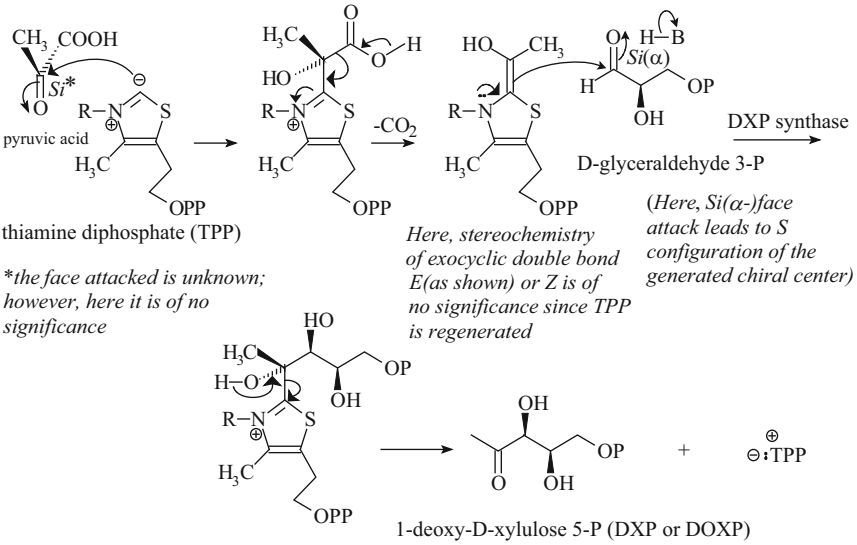


Fig. 5.9 Formation of 1-deoxy-D-xylulose 5-P (DXP or DOXP) (*non-mevalonoid pathway*)

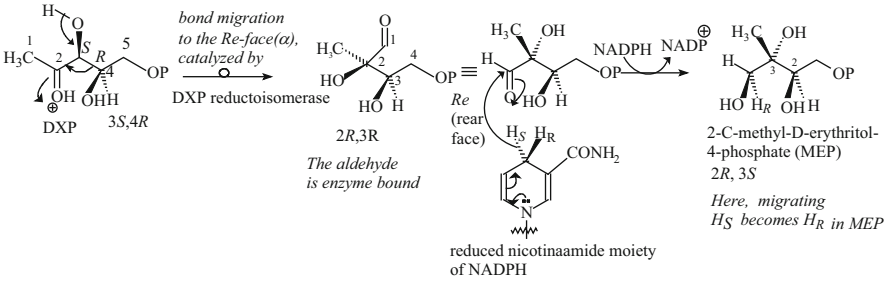


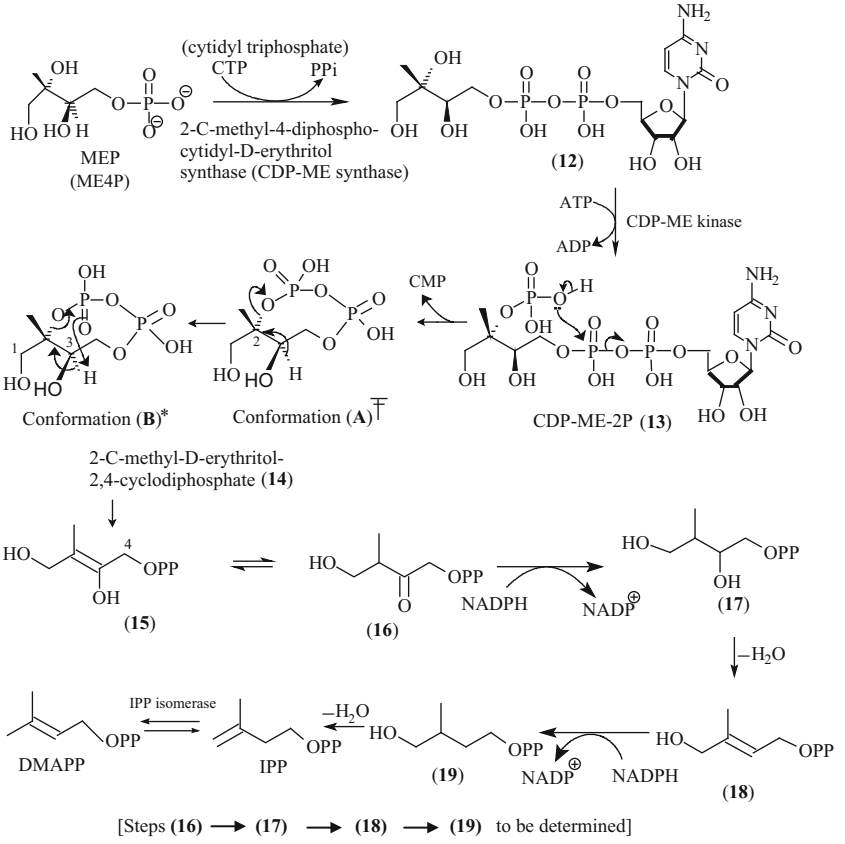
Fig. 5.10 Conversion of DXP to MEP

5.3.2 *Conversion of DXP to 2-C-Methyl-D-Erythritol-4-Phosphate (MEP or ME4P)*

The following transposition reaction is catalyzed by DXP-reductoisomerase. It resembles the pinacol–pinacolone type rearrangement. The aldehyde remains bound to the enzyme and uses NADPH for its reduction to 2-C-methyl-D-erythritol-4-phosphate (MEP) (Fig. 5.10). It is inconsequential to know which of the two diastereotopic hydrogens from the reduced nicotinamide moiety of NADPH effects the reduction, since the –CHO group is reduced to $–CH_2OH$ group and no chiral center is generated (Fig. 5.10). However, through selectively labeled NADPH it has been demonstrated that H_R is delivered to the *Re* (α-) face of the aldehyde and occupies ***pro-R*** position in the product MEP as shown.

5.3.3 *Conversion of MEP to IPP Via* a *Cyclic Diphosphate: Its Ring Opening, Followed by Repeated Reduction and Dehydration [11]*

MEP is converted into 2-C-methyl-D-erythritol-2,4-cyclodiphosphate (**14**) through the intermediacy of 4-diphosphocytidyl-2-methyl-D-erythritol (**12**) and its 2-phosphate (**13**) as shown in Fig. 5.11. The cyclic diphosphate then opens up at C_2 and undergoes loss of the hydroxymethine proton at C_3 to form the 3-keto-4-diphosphate (**16**) via its enol (**15**). At this point, it is pertinent to mention that C_3–H



∓*Since in this conformation 3-H and 2-O bonds are cis (syn-clinal), it is not expected to undergo E-2 elimination involving these bonds, as shown.*

**Cyclic cis-elimination can take place in this conformation having 3-H and 2-O bonds in cis orientation (syn-clinal) and O of P=O and 3-H in proximity, as shown.*

Fig. 5.11 The non-mevalonate/DXP pathway of isoprenoid biosynthesis starting from MEP

as well as C_2–O bonds are *syn*-clinal and hence E_2-elimination, as shown in conformation (**A**) [11], may not be stereoelectronically compatible.

However, *we think that it may undergo ring opening through the participation of an enzyme*, *or by cis-elimination through a 6-membered cyclic transition state as shown in conformation* (**B**). The next biological events are reduction and dehydration [11] to form the linear diphosphate (**18**). The latter upon repetition of the same biological events yields IPP, which is in equilibrium with DMAPP in presence of IPP isomerase.

An alternative hypothetical mechanism [11] for the conversion of (14) **to** (18)
It has been shown that all the hydrogen atoms of the C–H bonds present in the cyclic diphosphate (**14**) are preserved in the IPP and DMAPP molecules. This observation suggests [11] that (**14**) is converted to the linear diphosphate (**18**) through a series of reactions (Fig. 5.12) by direct involvement of the enzyme (this possibility has already been mentioned), and not through a proton loss at C_3 as shown in conformations (**A**) and (**B**) (Fig. 5.11). In the first step, the cofactor $NADP^+$ of the enzyme abstracts the hydride from the methylene of the $–CH_2OH$ group of (**14**) and is converted to NADPH. The resulting aldehyde makes its adjacent C_2 more electrophilic due to its inductive effect. Nucleophilic attack on the quaternary C_2^* by the thiol (−SH) group or thiolate ($-S^-$) anion of the enzyme resident cysteine displaces the diphosphate moiety and thus opens up the ring. The terminal aldehyde provides the necessary electron sink and enolizes through the cleavage of the newly formed S–C bond with the concomitant formation of a disulphide bond. The resulting enolate anion triggers the β-elimination of the water molecule. In the last step, the NADPH delivers back the same hydride to C_1 that it got in the first step and thus restores its original oxidation state, leading to the formation of (**18**) (Fig. 5.12). Reductive cleavage of the disulphide bond is necessary to regenerate the cysteine moiety of the enzyme for multiple turnovers.

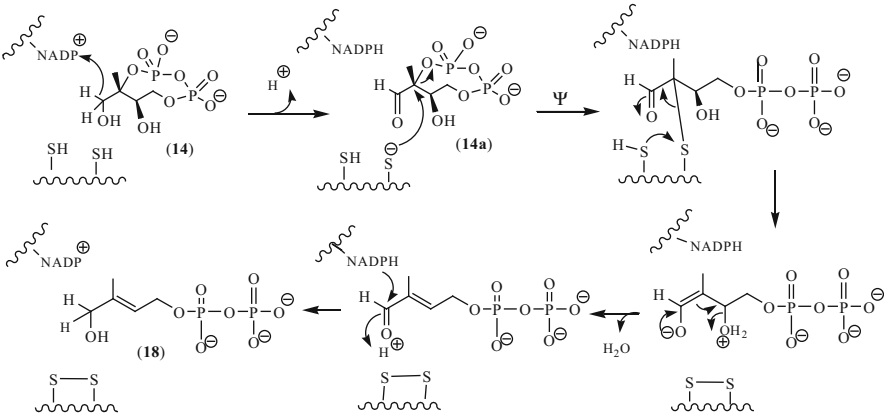


Fig. 5.12 Hypothetical mechanism for the conversion of (**14**) to (**18**) in the DXP pathway

However, it appears to us that such an S_N2 attack on a crowded quaternary carbon with a bulky nucleophile is less likely, and an S_N1 opening of the cyclic diphosphate (**14a**) with the release of the pyrophosphate anion at C_2 even after complexation with Mg^{2+} is also less likely because of the unstable nature of the resulting carbocation due to its attachment to the electron-deficient C_1 (see step ψ in Fig. 5.12).

An alternative hypothetical mechanism has also been suggested [11] that involves a radical process in each step and constitutes the same sequence of reactions as shown in Fig. 5.12. In this pathway, the hydrogen radical abstractor thiolyl radical (−S•) and the hydrogen radical donor thiol (−SH) group of the enzyme have been used instead of the hydride acceptor $NADP^+$ and the hydride donor NADPH. Thus, here bond formation and bond breaking in each step are initiated by a radical and are homolytic.

It has been shown [25, 26] that the IPP and DMAPP molecules are also independently produced in the DOXP pathway (Fig. 5.13) since the depletion of the gene specifying the IPP isomerase does not affect the non-mevalonate pathway biosynthesis. Thus, DMAPP and IPP may be formed independently from (**18**), also in absence of IPP isomerase via the resonance stabilized allylic carbocation (**18a**) (Fig. 5.13). Arigoni and others [24] have shown that in the conversion of DXP to IPP and DMAPP *isp*-proteins *viz.*, *isp* C, *isp* D, *isp* E, *isp* F, *isp* G, and *isp* H—which are specified by the corresponding genes—are involved. They have carried out these experiments in vitro also. We thus find a dual origin of IPP. The discovery of the DXP pathway is a major shift in our understanding of terpenoid biosynthesis. The mevalonate-independent pathway is operative in the plastids (chloroplasts).

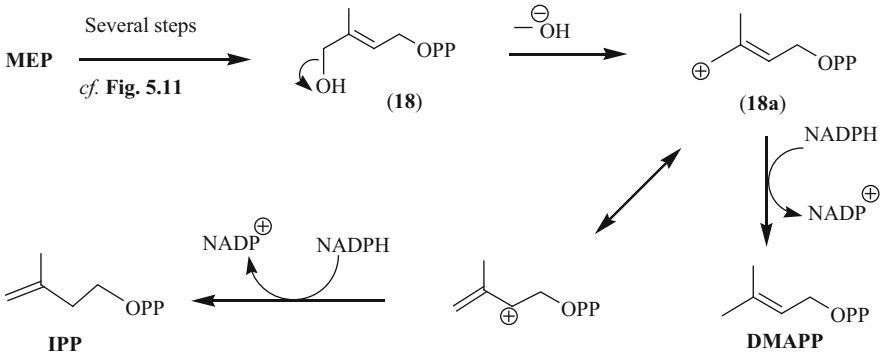


Fig. 5.13 Independent routes for the conversion of MEP to DMAPP and IPP in absence of IPP isomerase

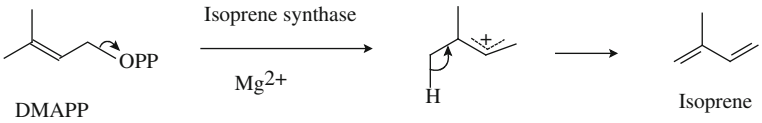


Fig. 5.14 Biosynthesis of isoprene from DMAPP

5.3.4 Emission of Isoprene from Some Plants [28]

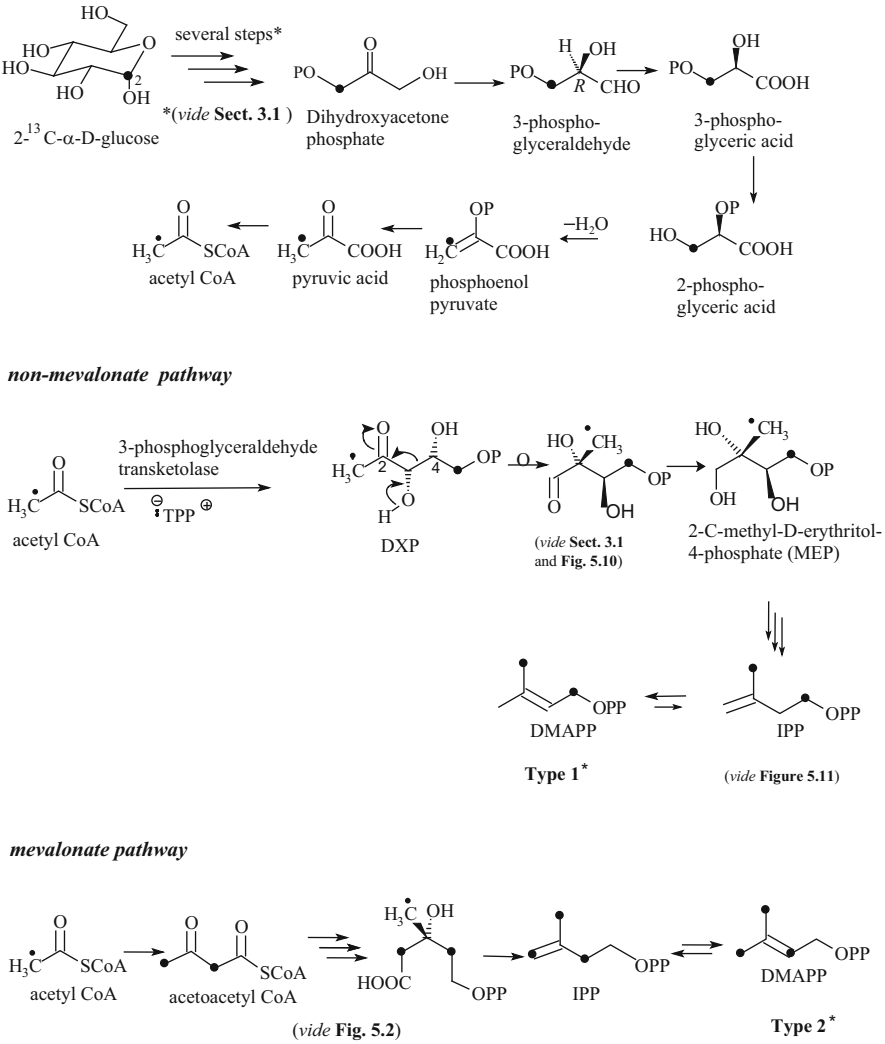
Incidentally, it may be mentioned that leaves of some plants, such as oak, poplar, spruce, etc., are capable of producing the *hemiterpene* isoprene (b.p. 33°), which escapes as gas into the air during the day at a temperature $\geq$ 30°. As a consequence, certain percentages of photosynthetically fixed carbon in those plants are emitted as isoprene. It is synthesized in plastids from the non-mevalonoid derived DMAPP via the action of *isoprene synthase* which catalyzes the elimination of the pyrophosphate [11, 28] in presence of Mg^{++} (Fig. 5.14). The global isoprene emission is estimated to be about as high as the global methane emission. There are indications that low amounts of isoprene provide a stabilizing effect against high temperature damage of photosynthetic membrane. In fact, the blue haze sometimes observed over forests at high temperatures is due to emission and accumulation of isoprene from trees.

Incidentally, it may be mentioned that C.G. Williams discovered isoprene in 1860 by dry distillation of rubber and named it without any explanation. Though isoprene does not occur in the free state in nature, several natural C_5 compounds, called *hemiterpenes*, e.g., isopentenyl pyrophosphate (IPP), γ,γ-dimethylallyl pyrophosphate (DMAPP), isoamyl alcohol (3-methylbutan-1-ol), isovaleric acid (3-methylbutanoic acid), tiglic acid (*E*-2-methylbut-2-enoic acid), angelic acid (*Z*-2-methylbut-2-enoic acid), and β-furoic acid (furan-3-carboxylic acid) are known to have the isoprene skeleton.

5.4 Dual Origin of IPP: Labeling Patterns of IPP Derived from Labeled Glucose by Two Different Routes

$^{13}C_1$-D-Glucose has been chosen to show these two distinct pathways (mevalonate and mevalonate-independent DXP pathways) for the terpenoid biosynthesis, since the former forms metabolites in two different routes [29] to yield IPP in which the distribution of the ^{13}C label indicates the path followed (Fig. 5.15).

The MVA pathway is operative in the cytoplasm and is responsible for the biosynthesis of sesquiterpenes, triterpenes, and sterols, whereas DXP pathway is reported to take place in the chloroplasts requiring photosynthetic machinery and is responsible for the formation of various groups of monoterpenes and diterpenes. Such compartmentation (*i.e.*, different subcellular locations) is based on incorporation of various labeled compounds in feeding experiments. However, exchanges



*The type of labelling will be relayed in the products and will indicate which pathway has been followed.

Fig. 5.15 Different labeling patterns of IPP and DMAPP obtained (feeding 2-^{13}C-α-D-glucose) in non-mevalonate (Type 1) and mevalonate (Type-2) pathways

[11, 25, 29] of common intermediates such as IPP, GPP, and FPP might occur between the two compartments as described by the "Cross-Talk Theory" [29]. Perhaps, prior to the discovery of the non-mevalonate pathway, this was the reason for the incorrect conclusion that the mevalonate pathway was the universal pathway for terpene biosynthesis [11].

5.5 Chain Elongation in Terpenes (Prenyl Transfer) [30]

Chain elongation from C_5 to C_{10}, C_{15} and beyond is an important biosynthetic phenomenon. The prenyl chain elongation is catalyzed by a set of enzymes called '*prenyltransferase*'. Sixteen such different prenyl transferases with different catalytic functions, *i.e.*, chain length specifications, have been identified [30] till 1998. Chain elongation process in terpenes is summarized in a general way in Fig. 5.16.

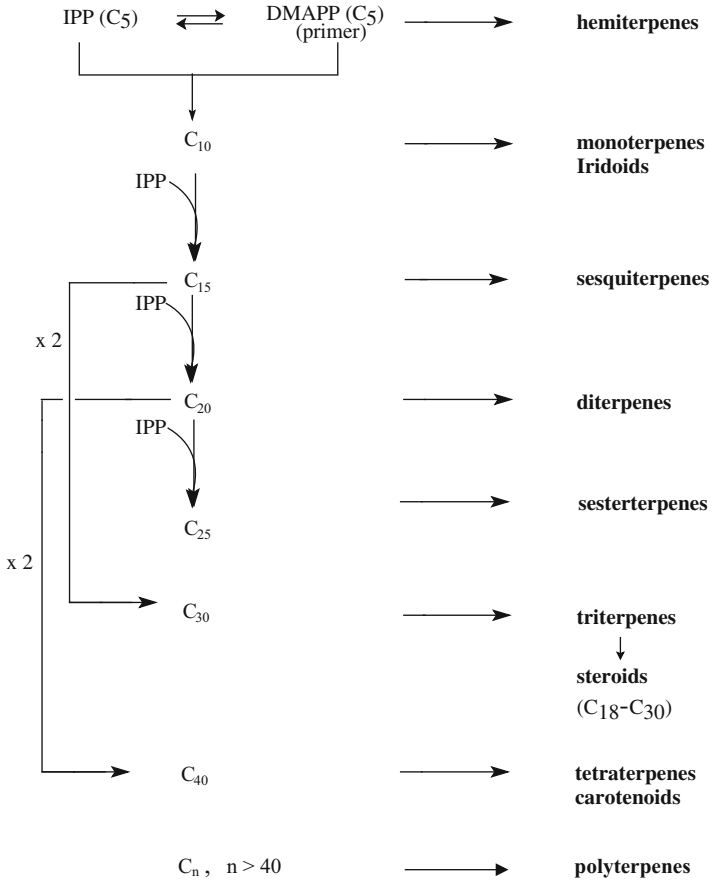


Fig. 5.16 Chain elongation process in the terpenes

5.5.1 *Cornforth's Concept and Its Modification by Poulter and Rilling*

The mechanism of chain elongation has been extensively studied by Cornforth [8, 14–16] and subsequently by Poulter and Rilling [8, 31]. According to Cornforth, the double bond of IPP is activated by the attack of a nucleophile from the appropriate enzyme XE to trigger its attack on the C_1 of DMAPP from which the pyrophosphate group is eliminated. This is followed by the loss of H_R from the original IPP part and release of XE. The whole process is suprafacial and kinetically S_N2' type (Fig. 5.17). The S_N2' type process demands inversion at C_1 of DMAPP moiety which is observed according to this proposition. However, a time lag between the bond breaking and bond formation was evident later on. This observation is not mechanistically compatible with S_N2'type kinetics. This unexpected experimental result prompted a reinvestigation of the existing mechanism for chain elongation.

Poulter and Rilling [8, 31] modified Cornforth's concept of chain elongation. According to them, the rate determining step in the enzymatic prenyl transfer involves the ionization of the allylic pyrophosphate substrate (S_N1 type) followed by a nucleophilic attack of IPP with the stereospecific removal of its H_R from the allylic methylene in tandem to form a C–C and a C=C bonds (Fig. 5.18). The S_N1 type metal catalyzed generation of dimethylallyl carbocation (DMA^+) and its electrophilic addition to IPP with concomitant stereospecific elimination of H_R can take care of the time lag between the bond breaking and bond formation. However, S_N1 type kinetics expects a randomization at C_1 of the added DMA $^{(+)}$, and loss of its stereochemical integrity, and thus apparently cannot explain the observed inversion at C_1 of the $DMA^{(+)}$ carbocation. The expected randomization is prevented by the rotational barrier (~28 kcal/mole) around C_1–C_2 bond of the allylic carbocation [8, 31]. In the absence of definitive idea about the topology of the enzyme–substrate conformation, it has been assumed that the inversion is imposed by the compatible prenyltransferase enzyme itself which holds the $DMA^{(+)}$ carbocation and the proximate cosubstrate IPP in such cooperative conformations that the electrophilic addition to C_4 at the *Re*-face (at C_3) of IPP takes place on the face of the $DMA^{(+)}$ opposite to that from which the OPP departs

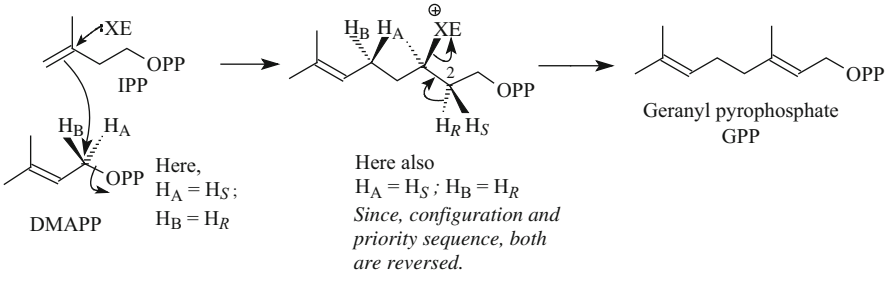


Fig. 5.17 Cornforth's concept of chain elongation (Formation of GPP)

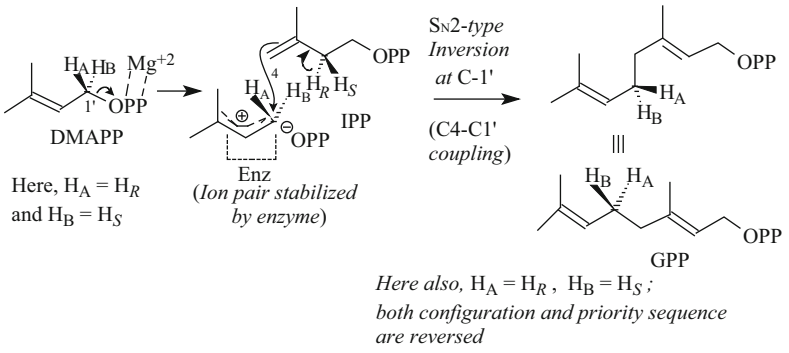


Fig. 5.18 Modification of chain elongation pathway. Formation of GPP (Rilling and Poulter)

(Fig. 5.18). So, basically the process remains suprafacial, as originally suggested by Cornforth, 2-H_R and 1′-OPP being eliminated from the same face.

5.5.2 *Formation of C_{10}, C_{15}, C_{20}, C_{25}, C_{30}, and C_{40} Linear Terpenoids and Natural Rubber*

The general paradigm associated with the elongation of isoprenoid chain (C_{10}, C_{15}, C_{20}, and C_{25}) involves repetition of the above-mentioned processes: (1) ionization of the allyl pyrophosphate, (2) addition of IPP to the carbocation thus formed at the face opposite to that from which 1'-OPP is eliminated, and (3) stereospecific elimination of 2-H_R from the IPP during its each addition. In summary, ionization, condensation, and elimination take place stereospecifically for each C_5-addition, *i.e.*, 1′–4 coupling reaction [31] leading to the linear terpenoids (see Fig. 5.19).

Here, it is assumed that the atoms $C_1{}'$ (of DMAPP serving as the electrophilic center) and C_4 (of IPP) are adjacent in the enzyme–substrate complex. Moreover, the formation of the *E*-C_2, C_3 double bond in IPP part requires that the dihedral angle (ω) between H_R−C_2 and C_3=C_4 bonds should ideally approach 180° (antiperiplanar), pushing the double bond to the β-face (Fig. 5.18). Since OPP binds with Mg^{+2}, it is assumed that the metal is assisting in the catalytic step. The enzyme can ionize the allylic substrate without anchimeric assistance by IPP. The electrophilic attack at the allylic carbocation takes place to the *Re* (or, β-) face of the C_3, C_4 double bond of IPP, and H_R from the α-face is lost. Hence, the allylic substrate must be located on the *Re* face of the trigonal center C_3. Molecular orbital calculations and ^{13}C NMR data give definitive evidence for the formation of C_{10}-allyl cation during FPP biosynthesis [32].

Rubber Biosynthesis [33] Natural rubber is produced in a simple extension of the ubiquitous isoprenoid pathway from IPP and allylic-PPs, namely DMAPP, GPP, and FPP. The allylic-PPs are synthesized by soluble enzymes in the same *cytosol*

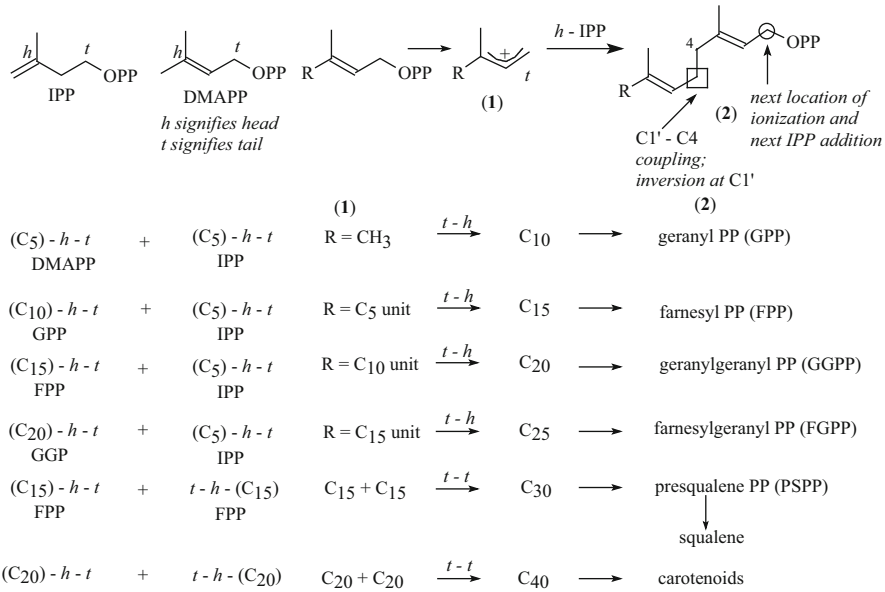


Fig. 5.19 Mode of attachments in chain elongation leading to C_{10}, C_{15}, C_{20}, C_{30}, and C_{40} compounds

compartment as the rubber transferase enzyme in the rubber producing evolutionarily divergent plant species (mainly *Hevea brasiliensis*) and could be used by the enzyme. The polymerization (chain elongation) of IPP, probably derived from the mevalonate pathway (Sect. 5.2), is brought about by rubber transferase present in *cytosol*. However, IPP derived from non-mevalonate DOXP pathway (Sect. 5.3) may diffuse from the *plastids* and join the *cytosolic* pool for the rubber biosynthesis [33].

5.5.3 *Ruzicka's Nomenclature: Terpene or Terpenoid, Head and Tail Parts of Acyclic Terpene Pyrophosphates (Diphosphates)*

At this stage, it is necessary to explain the terminologies '*terpene*' and '*terpenoid*'. The term terpene and for that matter, different classes of terpenes (mono-, di-, tri-, etc.) are considered to be synonymous with terpenoids—monoterpenoids, diterpenoids, triterpenoids, etc. in the literature.

Ruzicka held the opinion [5–7] of retaining the term terpene and the classes as monoterpenes, diterpenes, etc. According to him, '**oid**' has been introduced in terpene compounds by analogy with '**steroids**' "which denotes a group of

compounds having an irregularly varying number of carbon atoms. In the terpenic field, the expression terpenoid should be reserved by analogy for compounds in which the number of carbon atoms varies irregularly, in contrast to the terpenes proper, where the number of carbon atoms is always a multiple of five" [5–7]—a unifying feature. However, the term 'terpenoid' has deeply percolated through the veins of terpene chemistry literature, and it is difficult to avoid the '**oid**' nomenclature. Hence, both the terminologies '***terpenoid***' and '***terpene***' have been used in this book.

Further, in defining the '**head**' and '**tail**' parts of the acyclic terpene pyrophosphates (C_5, C_{10}, C_{15}, etc.) (Fig. 5.19), some confusion has been observed in the literature. Most of the earlier literatures label the terminal carbon atom carrying the pyrophosphate group as the **tail** part, while the isopropenyl/isopropylene group as the **head** part of the molecule. *In this book, this widely used convention of Ruzicka will be followed.* However, others [34–36] are also justified in the sense that *the functional group (OPP) containing carbon (C_1) should be recognized as the* **head** part of the molecule.

Here, two other points should be noted. (i) The importance of pyrophosphate group (OPP) lies in its ability to form a complex with Mg^{+2} or Mn^{+2}; this complexation confers better leaving group ability to OPP, a situation needed for generating a carbocation for chain elongation and cyclization. (ii) In the literature, some authors have designated OPP as 'diphosphate', which seems to be proper, instead of 'pyrophosphate'. The term 'pyrophosphate' has been used in the earlier literatures and is also in use in the current literature along with the 'diphosphate'; we have mostly used the old term pyrophosphate, and sometimes the other term 'diphosphate'.

5.5.4 *Formation of Squalene (C_{30}) via Presqualene Pyrophosphate (Involving Cyclopropane/Cyclobutane Ring Opening)*

The chain elongation to C_{30} takes place by a tail to tail union of two FPP molecules (Fig. 5.18), and not by an IPP addition to a C_{25} terpene residue. As discussed in the sequel, squalene ($C_{30}H_{50}$) is biosynthesized in a multistep pathway (Fig. 5.20). As expected, a stable intermediate, presqualene pyrophosphate [37–41] (PSPP) is formed in the key step. PSPP was first isolated [38] by Rilling in 1966. Presqualene alcohol was synthesized in 1971 by Rilling et al. [40] and two other groups [42, 43].

Squalene (**6**) is a highly branched (tentacled with methyls) symmetrical hydrocarbon having a C_2-axis. It results from an asymmetric process, for which a number of mechanisms has been proposed and debated. A simple mechanism for the biosynthesis of squalene from farnesyl PP has been proposed on the basis of a π-complex (between farnesyl carbocation, $F^{(+)}$ (**1**) and FPP) (*The numbering of the structures in this figure is independent of the earlier figures*) theory and supported

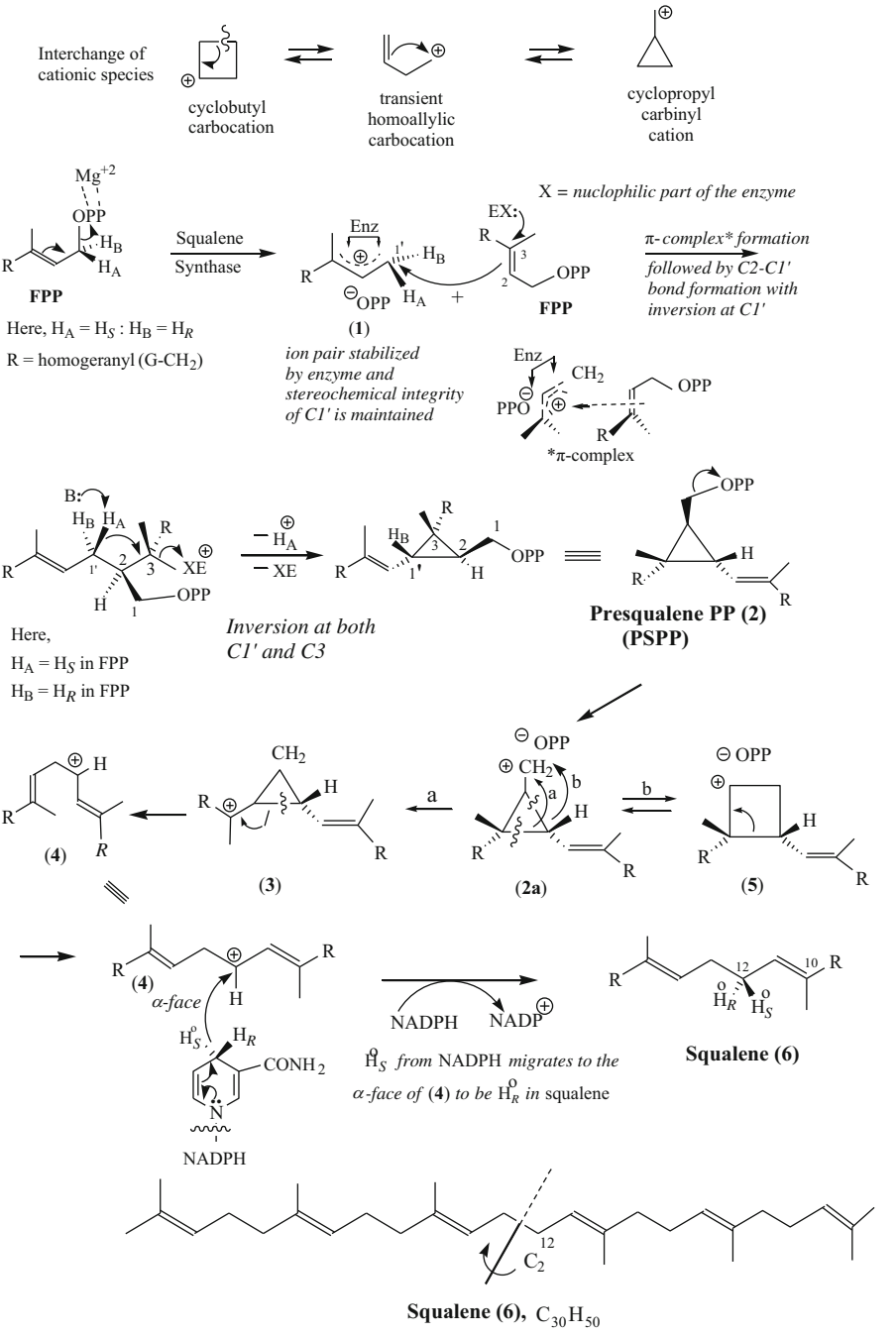


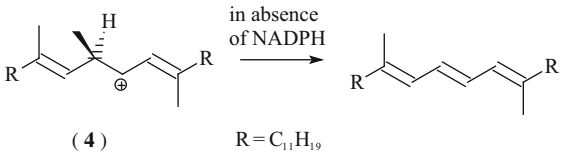
Fig. 5.20 Biosynthesis of squalene from FPP *via* presqualene PP (**2**)

by theoretical calculations [37]. The mechanism, which transpires from these calculations, is based on the ionization of one of the two participating FPP molecules *in tail to tail condensation*. The pyrophosphate group leaves with the help of Mg^{+2}; the generated resonance stabilized farnesyl cation (**1**) is then trapped by the enzyme squalene synthase, which can function only in presence of Mg^{+2}. It is unlikely that the free carbocation $F^{(+)}$, in the absolute sense, participates in this reaction. In all probability, it remains as an ion pair (**1**) with the counter ion $^{(-)}$OPP, the ion pair being stabilized by an appropriate enzyme. The trapping of the farnesyl carbocation by enzyme is followed by an S_N2 type interaction with the second molecule of FPP to form a π-complex, an outcome of theoretical calculations [37]. Real S_N2 type reactions are rare in biological systems. Rapid stereospecific deprotonation $\{-H_A(-H_R)\}$ gives PSPP (**2**), the structure and stereochemistry of which has been confirmed [38–43] by degradation and an unambiguous synthesis of its alcohol. Since one of the allylic methylene protons is stereospecifically eliminated, the deprotonation should be a fast process, being brought about by a base of appropriate strength. Literature provides such examples of cyclopropane ring formation [35]. Of the eight possible stereoisomers (four diastereomers and their optical antipodes) of PSPP (**2**) possessing three unlike chiral centers, only one with absolute stereochemistry defined as (**2**) is formed (as shown mechanistically in Fig. 5.20) and is found to be biologically active. Incorporation of various mixtures of isomers of (**2**) into yeast homogenates containing NADPH results in the efficient conversion of the active isomer (**2**) to squalene (**6**) through the steps delineated below.

It has been suggested [38–41, 44] that PSPP (**2**) suffers ionization (S_N1 type) to form the primary cyclopropylcarbinyl carbocation (**2a**) which rearranges to yield the cyclopropylmethyl tertiary carbocation (**3**) by the bond migration shown by arrow *a* in preference to the secondary cyclobutyl carbocation (**5**) [35] by the bond migration shown by arrow *b*. The rearrangement of (**2a**) to form the carbocation (**3**) is expected both from the theory of ring closing kinetics and formation of the more stable carbocation. The cyclopropane ring opening of (**3**), as shown, forms the more stable squalene allylic carbocation (**4**), which might also have been produced from the cyclobutyl carbocation (**5**), if at all formed, by migration of the bond shown. The conversion (**2**) to (**4**) via (**2a**) and (**3**) is catalyzed by squalene synthase. That the tertiary carbocation (**2a**) and not the cyclobutyl carbocation (**5**) is involved in this conversion has been proved by using a nitrogen analogue of cyclopropylcarbinyl cation as an inhibitor to squalene formation [37].

However, in view of the cation exchange as shown in the equilibriums (top of the Fig. 5.20), the opening of the cyclopropane ring prior to its conversion to squalene may also be considered to proceed via cyclobutyl carbocation (**5**) [35, 44]. Use of chemically and structurally equivalent inhibitors may show whether cyclobutyl carbocation is involved in the ring-opening path.

As has been delineated in Fig. 5.20, Cornforth has defined *the following steric course of the bioreactions leading to the formation of squalene from two molecules of FPP* by selective labeling experiments [14–16].



vide **Figure 5.17**

Fig. 5.21 Dimer of FPP with an additional double bond

(i) The enantiotopic hydrogen H_S from C_1 of one FPP molecule is lost and is eventually replaced by a hydrogen from NADPH (the last step).
(ii) The diastereotopic hydrogen H_S at C_4 of NADPH is delivered to the carbocation (**4**) at C_{12} and it occupies the ***pro-R*** position in squalene (**6**), *i.e.*, it becomes H_R in squalene.

It is further concluded that the delivery of the hydride from NADPH takes place at the final step, and in absence of NADPH, the squalene cation is quenched through the loss of adjacent proton to yield a dimer, 12,13-dehydrosqualene, $C_{30}H_{48}$ with one extra double bond (Fig. 5.21).

5.5.5 *Different Phases of Terpenoid Biosynthesis*

All terpenoids are oligomeric/polymeric products of IPP and DMAPP in which DMAPP serves as the primer.

Terpenoid biosynthesis may be sequentially divided into the following four phases, brought about by specific enzymes present in the plant cells:

(i) The origin of the isoprene units, IPP and DMAPP (mevalonoid and non-mevalonoid pathways).
(ii) Stepwise linear polymerization of the isoprene units to form the acyclic polyprenyl precursors like geranyl PP, farnesyl PP, geranylgeranyl PP, and farnesylgeranyl PP; however, squalene (via presqualene) and C_{40} terpenoids are formed by the dimerization of C_{15} (FPP) and C_{20} (GGPP) linear terpenoids, respectively.
(iii) Proper folding, cyclization, rearrangements of these polyprenyl precursors, and also sometimes shedding from and addition to the carbon skeletons to form an array of innumerable terpenoids of diverse skeleton patterns.
(iv) In most cases, functionalization (e.g., various regiospecific and/or stereospecific hydroxylations, oxidations, reductions, etc.) on the skeletal frames in presence of the specific enzymes leading to varied natural terpenoids.

In the present chapter, the first two phases have been discussed in depth. The other two phases will be discussed in the respective chapters for different classes of

terpenoids, as mentioned in the Contents. We have discussed the phases (iii) and (iv) in the biogenesis/biosynthesis of monoterpenoids (Chap. 6) sesquiterpenoids (Chap. 7), diterpenoids (Chap. 8), sesterterpenoids (Chap. 9), triterpenoids (Chap. 10), steroids (Chap. 11), and carotenoids (Chap. 12), starting from the corresponding acyclic precursors included in this chapter.

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Chapter 6
Monoterpenoids (C_{10})

6.1 Geranyl pyrophosphate, the Universal Precursor of Monoterpenoids

Geranyl pyrophosphate (GPP) is the first member of the elongated isoprenoids (Fig. 6.1). It is the universal precursor molecule for the biosynthetic formation of all acyclic and cyclic monoterpenes (C_{10}-terpenoids). It is formed by the combination of DMAPP and IPP, catalyzed by the appropriate prenyl transferase (see Sect. 5.5.1, Figs. 5.14 and 5.15).

6.1.1 Biosynthetic Formation of C_{10}-Acyclic Terpenes

It has been shown [1, 2] with labeled experiments that labeling always resides in the IPP portion of the labeled GPP molecule. On the basis of this observation, Battersby suggested that in the feeding experiments the externally added labeled **IPP** is trapped by the pool of endogenous DMAPP prior to IPP's isomerization to the labeled **DMAPP**.

A major problem in studying the biosynthesis of monoterpenes using labeled precursors is the difficulty in achieving the incorporation in the product. This may be explained by the fact that *the living organisms probably prevent the exogenous labeled precursors to reach the sites of synthesis*. However, with some selective cooperative plants, some biosynthetic studies on monoterpenoids have been possible. Thus, incorporation of labeled geraniol by way of secologanin in the terpene-indole alkaloids (see Sect. 6.1.6), suggested that the IPP and DMAPP part of geraniol are biosynthesized [3, 4] in the non-mevalonate (DOXP) pathway (see Sect. 5.3). Further, from the study of site-specific natural abundance of hydrogen isotopes ratio measurements [5] using deuterium NMR on several monoterpenes (e.g., geraniol) the hydrogen genealogy of the parent hydrocarbon could be elucidated. This finding will predict *the derivation of GPP from the condensation of IPP*

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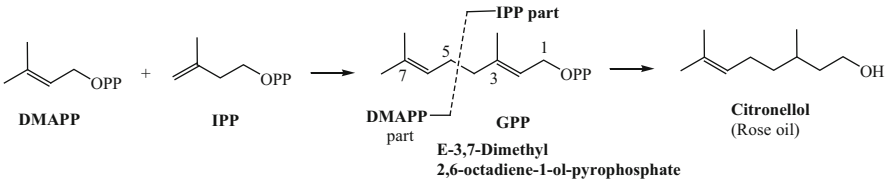
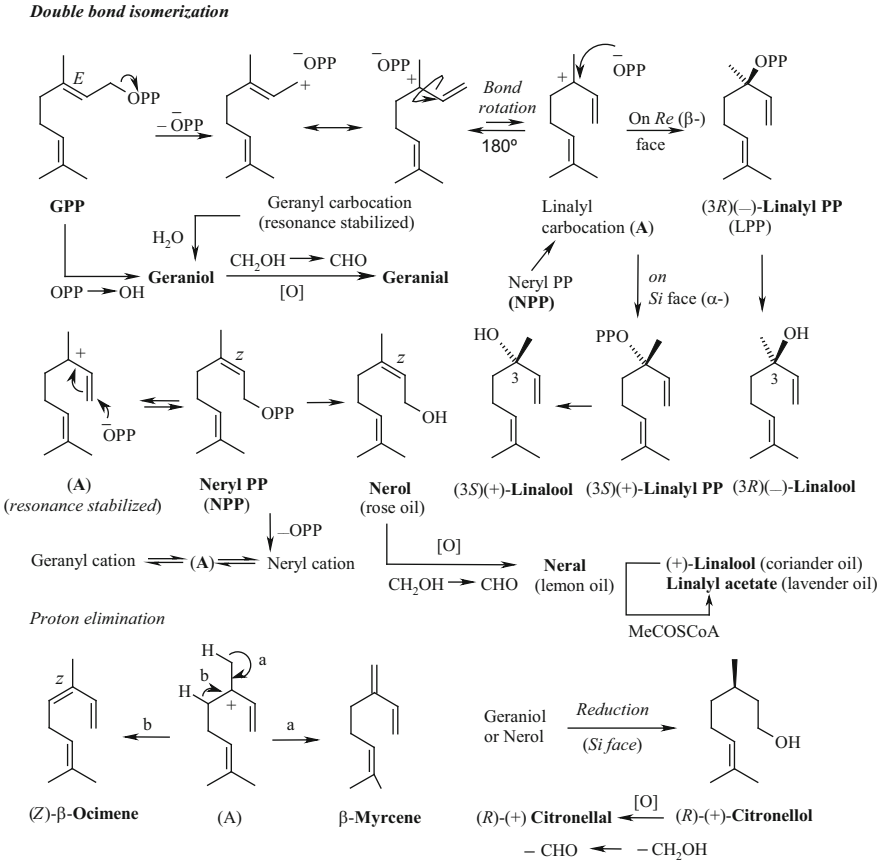


Fig. 6.1 Formation of geranyl pyrophosphate (GPP) and citronellol



(i) Every carbocation carries its counterpart $^{(-)}$OPP anion
(ii) The corresponding oxidation products of the alcohols like aldehydes (e.g., geranial, neral, citronellal) double bond reduced products (e.g., citronellol), and proton elimination products (e.g., β-ocimene and β-myrecene) are also known natural products.

Fig. 6.2 Acyclic monoterpenes arising from geranyl pyrophosphate (GPP)

and independent/isomerized DMAPP and their affiliation to DOXP pathway. That is to say, ". . ..isotope ratios at individual sites in geraniol can be traced back to the corresponding sites in GPP, then to sites of the IPP and DMAPP building blocks, then to pyruvate and glyceraldehydes-3-phosphate DOXP active molecules and finally to the carbohydrate photosynthetic precursor" [5].

The formation of acyclic monoterpenoids from GPP is delineated in Fig. 6.2.

6.1.2 Biosynthetic Formation of Cyclic Monoterpenes

The generated carbocations participate in the cation–olefin (cation-π) cyclization to yield mainly natural monocyclic and bicyclic and sometimes tricyclic monoterpenoids. The geranyl carbocation is capable of yielding several cyclic skeletal patterns under catalytic influences of suitable enzymes. Those of common occurrences are shown in Fig. 6.3. The plausible pathways for their formation are shown in the sequel (Figs. 6.4 and 6.5). Mechanistically the processes involve 1,2 anti-periplanar hydride or methyl or alkyl shift by way of a C–C bond shift to allow a new site for the carbocation and finally quenching the carbocation either through the loss of an appropriate proton or by the addition of a nucleophile like hydroxyl group. *The Wagner–Meerwein rearrangement initiated by the carbocation and stereochemically suited bond migration is almost a monopoly of terpenoid chemistry, especially of lower terpenoids. The enzymes provide compatible folding and environment for cyclization, which along with the cation chemistry is responsible for products formation*. Sometimes, a number of products in different amounts are formed by a single enzyme.

The topicity, i.e., the active site geometry, and the prochirality (*vide* Sect. 2.9) play a significant role in the biosynthesis of natural products, especially of terpenoids. The face at the location of attack and the prochirality of the hydrogen atom involved in the processes are important which in the presence of specific enzymes lead to the stereospecific products. The concerned C1 methylene hydrogen atoms in geraniol are enantiotopic which become diastereotopic in chiral environment in association with the specific enzymes or more specially in chiral cyclic monoterpenes.

6.1.2.1 Monocyclic Monoterpenes. Menthane Skeleton

The plausible pathways for the formation of some common monocyclic monoterpenes are delineated in Fig. 6.4. The significance of the double bond isomerization (E, Z) in cyclization is not obvious, as either isomer may serve as the precursor of the cyclized products, e.g., the **linalyl** carbocation (**A**) may be generated from **geranyl pyrophosphate** (E) or **neryl pyrophosphate** (Z), as shown (Fig. 6.2).

It is to be noted in Fig. 6.4 that (−)-menthol may be conceptually biosynthesized from GPP (i) via (4*R*)-limonyl carbocation (**B′**) or (ii) via (4*S*)-limomyl carbocation (**B**). The sequence of the biosynthesis of (4*S*)-(−)-limonene via (3*S*)(+)-LPP and

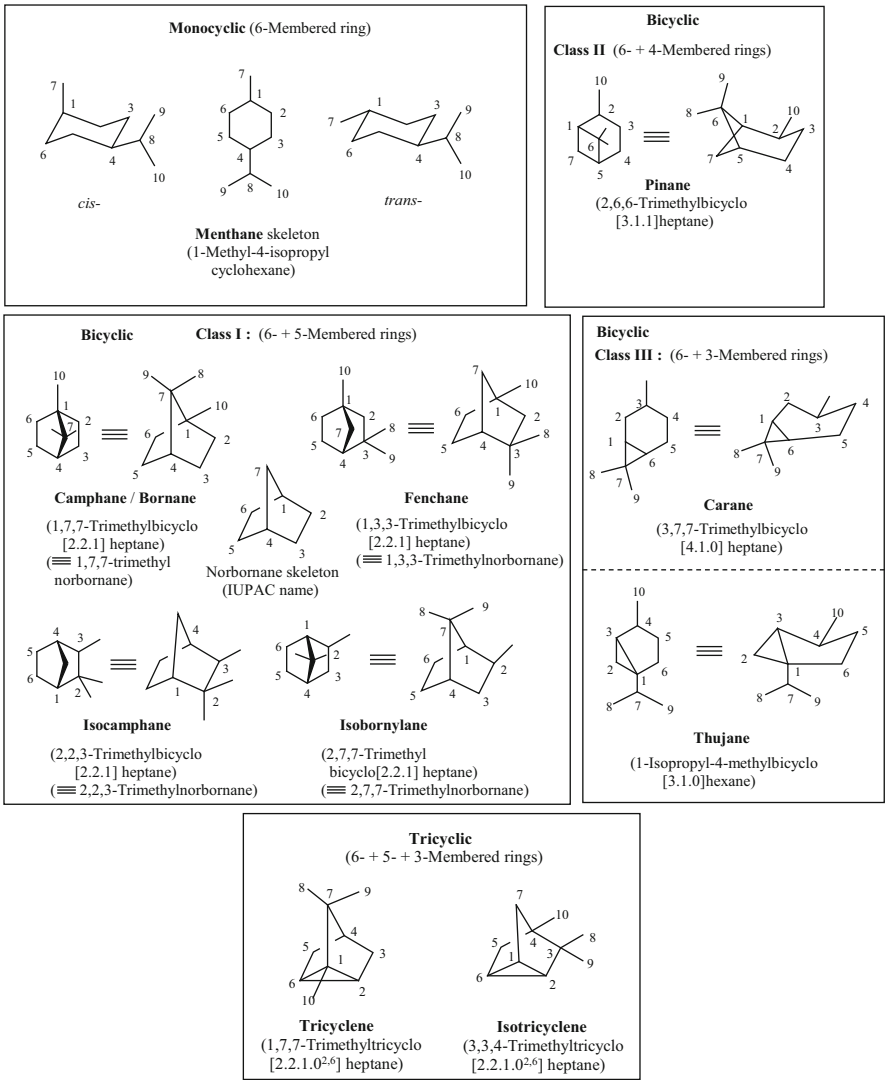


Fig. 6.3 Common skeletal patterns of cyclic monoterpenes and their nomenclatures

the sequence of the biosynthesis of (4*R*)-(+)-limonene via (3*R*)-(−)-LPP, caused by the mirror image (superposable) folding of the GPP in presence of specific enzymes, have been delineated in Fig. 6.6.

A small group of aromatic compounds, e.g., **p-cymene** and the phenol derivatives **thymol** and **carvacrol** (Fig. 6.4), occur in thyme (*Thymus vulgaris*, Labiatae). They are produced in nature from monoterpenoids, rather than via *acetate or*

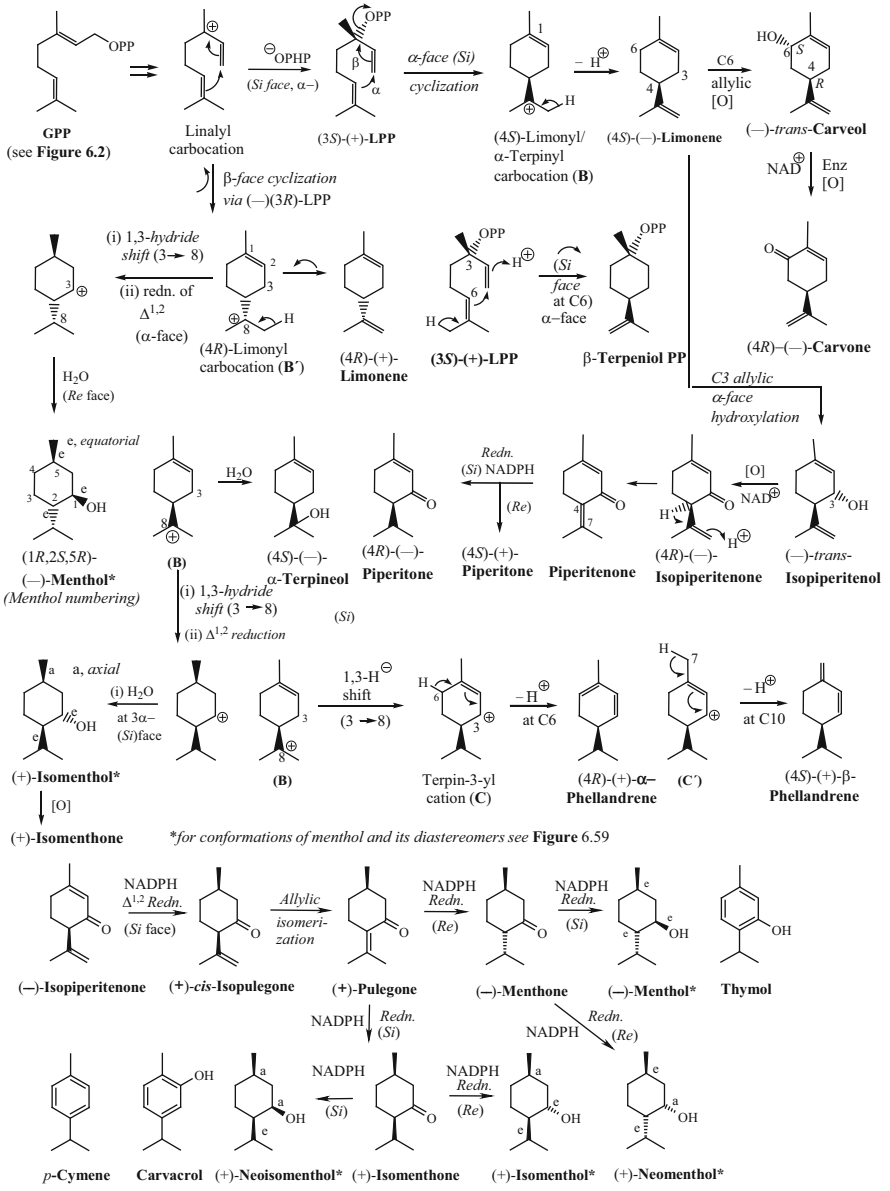


Fig. 6.4 Probable metabolic relations of monocyclic monoterpenes formed from GPP in plants

shikimate pathway, which is the more common route for aromatics. They have the same carbon skeleton as that of monocyclic monoterpenes and are structurally related to menthone, carvone—suggesting that additional dehydrogenation reactions have taken place.

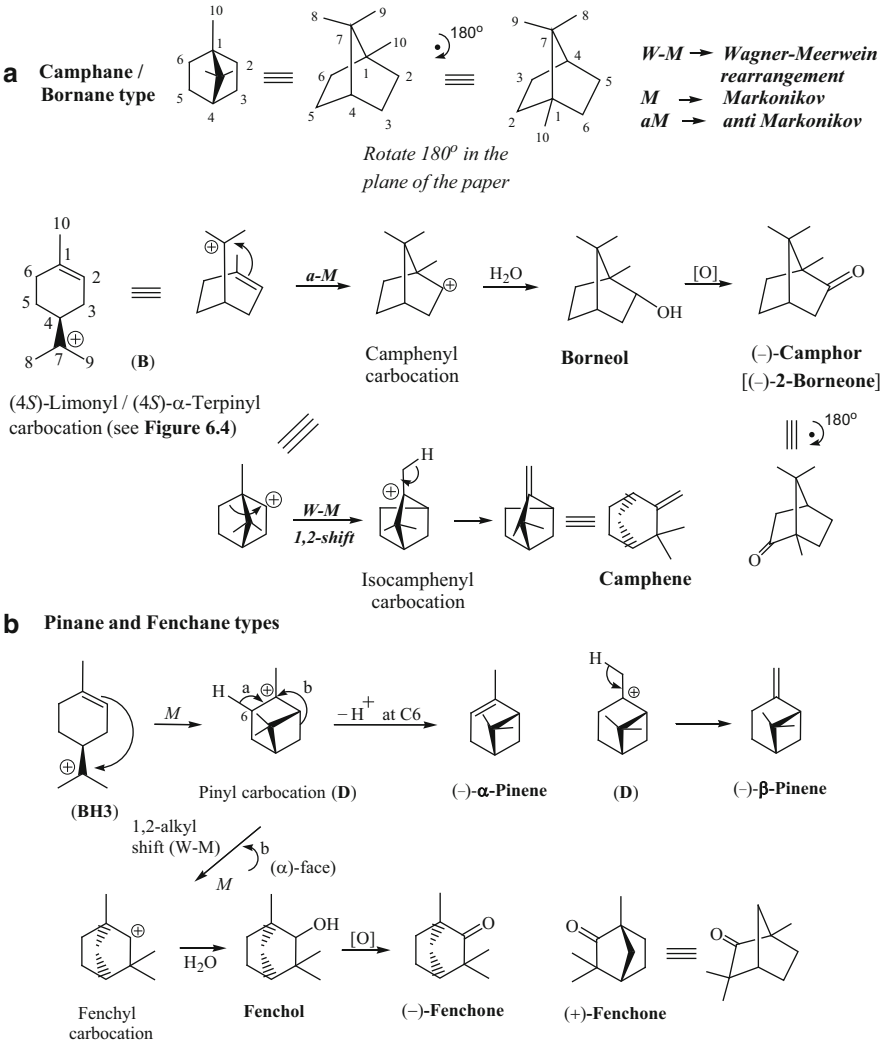


Fig. 6.5 (continued)

6.1.2.2 Bicyclic Monoterpenes. Formation of Some Familiar Skeletons (Camphane/Bornane, Pinane, Fenchane, Carane, and Thujane)

All these different types of bicyclic monoterpenes and their biosynthetic pathways in the presence of specific enzymes from (4*S*)-limonyl carbocation (**B**) are shown in Fig. 6.5. In the presence of the necessary specific enzymes the enantiomers of these bicyclic monoterpenes may be formed via the enantiomeric (4*R*)-limonyl carbocation (**B′**) (Fig. 6.4).

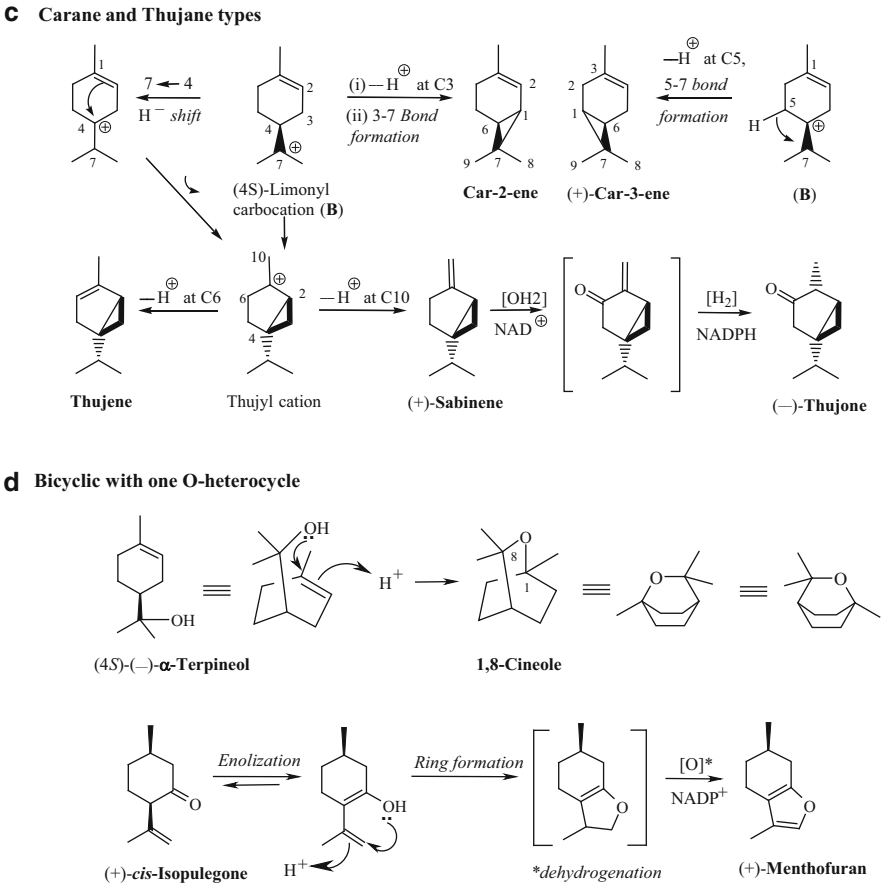
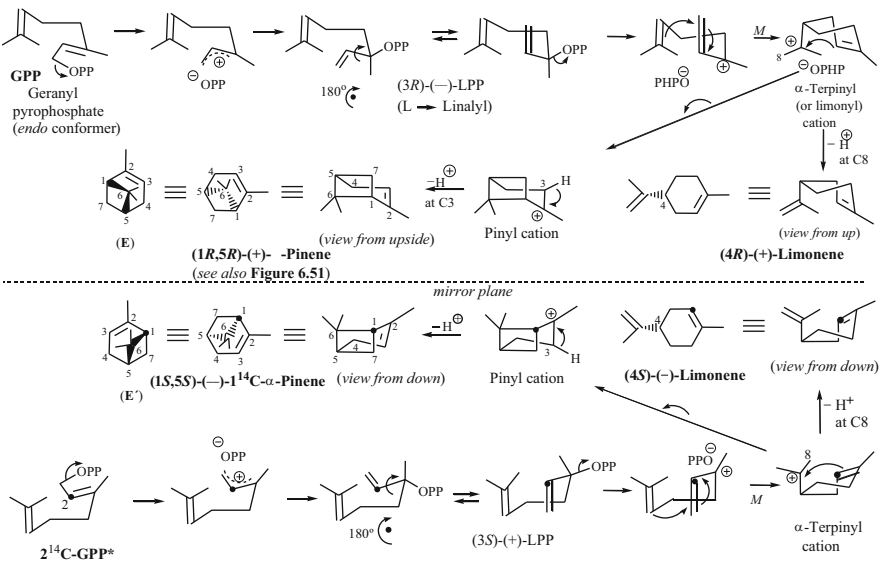


Fig. 6.5 Formation of bicyclic monoterpenes from limonyl carbocation

Generation of two *bicyclic monoterpenes with one O-heterocycle*, (i) from 4-*S*-α-terpineol by acid catalyzed ring formation (**1,8-cineole**) and (ii) from *cis*-isopulegone by acid-catalyzed ring formation followed by oxidation (**menthofuran**), are shown in Fig. 6.5.

6.1.3 Occurrence of Monoterpene Enantiomers, their Biological Responses and Biosynthesis

Individual enzyme systems present in a particular organism/plant cells control the folding of the substrate molecule, formation of a particular carbocation, and specific cyclization leading to the structure and stereochemistry of the final product. Most



GPP is folded and fixed by the specific enzymes in two superposable mirror image forms but nonsuperposable when complexed with the specific enzymes, thus leading to the formation of enantiomeric LPP molecules. Although the different specific enzymes generate enantiomeric monoterpenes, they themselves are not stereoisomers.
2^{14}C-Geraniol when fed in **Salvia officinalis forms 2^{14}C-GPP which is biosynthetically converted to (1S,4S)-(–)-2^{14}C-camphor via 1S,5S-(–)-1^{14}C-α-pinene (see also **Figure 6.51**)*

Fig. 6.6 Biosynthetic pathways for the stereospecific formation of enantiomers of α-pinene and limonene from GPP through enantiomers of LPP

monoterpenes are optically active. Enantiomers of the same compound have been isolated from different plants, e.g., (+)-**carvone** from caraway (*Caram carvi*, Fam. Umbelliferae) and (−)-**carvone** from spearmint (*Mentha spicata*, Labiatae) or (+)-**camphor** from sage (*Salvia officinalis*, Labiatae) and (−)-**camphor** from tansy (*Tanacetum vulgaris*, Compositae). Some enantiomeric monoterpenes are found to occur in the same plant, e.g., (+)-**limonene** and (−)-**limonene** in peppermint (*Mentha piperita*, Labiatae) and (+)-**α-pinene** and (−)-**α-pinene** in pine (*Pinus* species, Pinaceae). The enantiomers can give different biological responses, especially towards olfactory receptors in the nose. Thus characteristic odor of caraway is due to (+)-carvone whereas that of spearmint is due to (−)-carvone. (+)-Limonene smells of oranges, whereas (−)-limonene smells of lemons (see Fig. 32.2).

6.1.3.1 Biosynthesis of Pinene, Limonene, and Camphor Enantiomers

Biosynthesis of the enantiomers of limonene and α-pinene is illustrated in Fig. 6.6. It will be seen that the same achiral substrate *GPP is folded by two specific enzymes in two superposable mirror image* ways leading finally to two enantiomers. The optically pure substrates, (3*S*)- and (3*R*)-enantiomers of linalyl pyrophosphate

(LPP), catalyzed by (+)- and (−)-α-pinene synthases resulted in the predicted stereospecific transformations—(3*R*)-LPP to (+)-α-pinene and (3*S*)-LPP to (−)-α-pinene, respectively (Fig. 6.6). The reactions were demonstrated by labeling experiments and enzyme studies to proceed through *syn* isomerization of the primary allylic pyrophosphate to the tertiary allylic pyrophosphate, followed by rotation about C2–C3 single bond and *anti-endo*-cyclization [6–8]. The enantiomers (3*R*)-(−)-LPP and (3*S*)-(+)-LPP when catalyzed by different enzymes, (+)-limonene and (−)-limonene synthases present in plants, are converted into (+)- and (−)-limonenes, respectively, via loss of $H^{\oplus}$ from the corresponding enantiomeric α-terpenyl or limonyl cations (Fig. 6.6) [6–8]. The limonyl or α-terpenyl cation in the presence of (+)- and (−)-camphor synthases forms (+)-camphor and (−)-camphor, respectively, directly or through the corresponding pinyl cations. Biosynthesis of camphor has been discussed in Sect. 6.3 (Fig. 6.51).

Peppermint (*Mentha piperita*, Labiatae) produces (−)-**menthol**, with smaller amounts of the diastereoisomers, (+)-**neomenthol**, (+)-**isomenthol**, and (+)-**neoisomenthol** (Fig. 6.4). (For conformations and relative stability of these diastereomers see Fig. 6.59.) Oils from various *Mentha* species also contain significant amounts of (−)-**menthone**, (+)-**isomenthone**, (+)-**pulegone**, and (−)-**piperitone**. The metabolic relationships of these monoterpene alcohols and ketones and various other monoterpenes have been established as shown in Fig. 6.4. Enantiomers in many cases have different odors [9].

6.1.4 Occurrence of Monoterpenes in Plant Families

Monoterpenes are commonly isolated from various volatile or essential oils obtained mostly from different species of the families Rutaceae (several *Citrus* species), Labiatae (*Lavandula*, *Mentha*, *Rosmarinus*, *Salvia*, *Thymus*, etc. species), Pinaceae (*Pinus*), Myrtaceae (*Eucalyptus*), Umbelliferae (*Carum*, *Coriandrum*, *Anethum*), Lauraceae (*Cinnamomum*), Zingiberaceae (*Zingiber*), Compositae (*Chrysanthemum cinerariaefolium*). The oils are obtained by steam distillation or solvent extraction of fresh leaves, fresh or dried fruit peels/dried or fresh flowers/ripe fruit/fresh flowering tops/dried ripe berries/heartwood, etc. Useful data on volatile oils isolated from various plant materials are available in a tabular form [10].

6.1.5 Cyclopropyl Monoterpenes (C_{10}): Their Biosynthesis

The carbon skeletons of some monoterpenes, isolated from pyrethrum flower heads of a Compositae plant *Chrysanthemum cinerariaefolium*, are of irregular type, different from the regular head-to-tail coupling mechanism. They possess a cyclopropane ring (Fig. 6.7). Among them **chrysanthemic acid** and **pyrethric acid**, found as a number of esters, are especially important because of their insecticidal properties. These

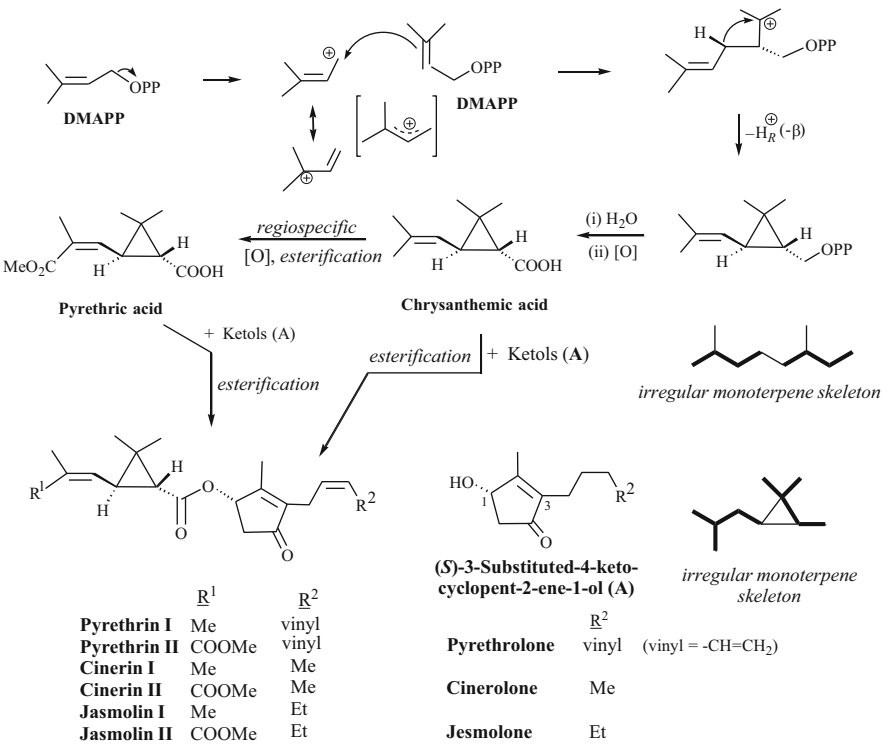


Fig. 6.7 Cyclopropyl monoterpenes: their biosynthesis from two molecules of DMAPP

cyclopropyl monoterpenes are not generated by regular head-to-tail coupling mechanism via GPP, like the regular monoterpenes discussed earlier. *They are biosynthesized from two molecules of* **DMAPP**, joining by a mechanism shown in Fig. 6.7, terminating into a cyclopropane ring formation. Chrysanthemic acid and pyrethric acid are esterified by (*S*)-4-keto-cyclopent-2-ene-1-ols (**A**), viz., +pyrethrelone, cinerolone, and jesmolone, to produce the corresponding cyclopropyl monoterpene esters. The origins of these 4-keto-1-ols are not known; it may be speculated that they are formed by cyclization of modified fatty acid derivatives.

6.1.6 *Biosynthesis of Secologanin (via Loganin), the Monoterpenoid Part of Some Indole and Quinoline Alkaloids. Iridoids*

The monoterpene **loganin** (Fig. 6.8), an iridoid glucoside, contains a cyclopentane ring fused to a six-membered oxygen heterocycle. It belongs to the iridoid system [11] occurring in nature, e.g., **nepetalactone** (Fig. 6.8) in *Nepeta cataria*

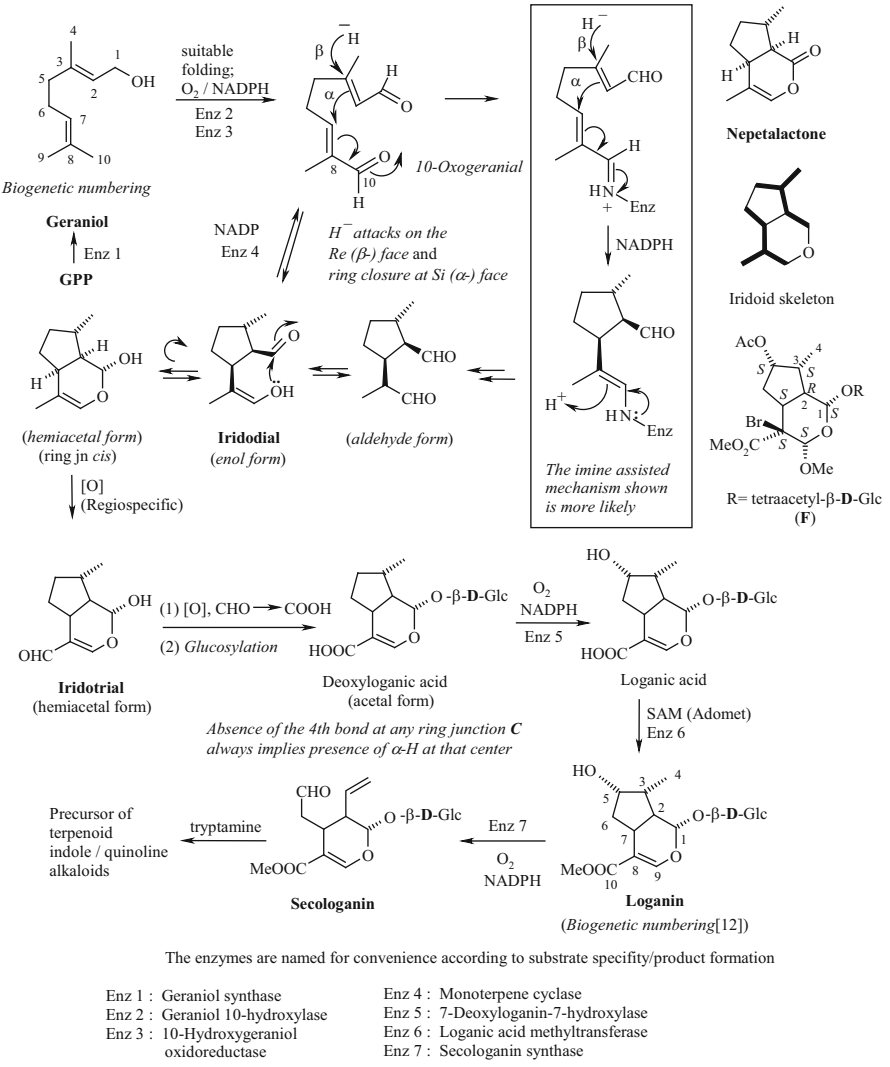


Fig. 6.8 Biosynthesis of secologanin, the monoterpene precursor of terpenoid indole and quinoline alkaloids

(Labiatae), a powerful stimulant and attractant for cats. Loganin is the key precursor for the monoterpenoid part of many complex quinoline and indole alkaloids (Chaps. 22, 25–27 and many alkaloids in Chap. 30). It is also the key intermediate in the biosynthesis of monoterpenes having iridoid structure.

It has been shown by feeding experiment that **secologanin** (Fig. 6.8) is derived from the non-mevalonate pathway [3, 6]. Hence IPP and DMAPP and, for that matter, geraniol are of triose phosphate or pyruvate origin (Sect. 5.3).

Biosynthesis of secologanin via loganin [12] involving a sequence of reactions has been delineated in Fig. 6.8. The iridoid system of loganin arises from geraniol after 10-hydroxylation and a type of folding different from the ones leading to the monoterpenoids already discussed (Figs. 6.2, 6.4, 6.5, and 6.7).

Secologanin unites with tryptamine derived from tryptophan to yield strictosidine, which rearranges to diverse indole alkaloidal skeletons in amazing fashions. The chemical structure of loganin as well as its absolute configuration has been settled by X-ray crystal structure of loganin penta-acetate monomethyl ether bromide (**F**) (Fig. 6.8) [4].

6.2 Geraniol

6.2.1 Occurrence, Structure Determination

Geraniol, $C_{10}H_{18}O$, b.p. 229–230°/757 mm, is a constituent of many essential oils, viz., citronella oil (20–40 %) from fresh leaves of *Cymbopogon winterianus* and *C. nardus* (Fam. Gramineae) (oil content 0.5–1.2 %)—also as acetate (~8 %) and rose oil (~20 %) from fresh flowers of *Rosa alba*, *R. centifolia*, *R. damascena*, and *R. gallica* (Rosaceae) (oil content 0.02–0.03 %). It is a colorless liquid possessing flowery rosy odor. Its structure has been determined in the classical way, i.e., derivatization, degradation, and synthesis. *The presence of two isolated double bonds, two methyls on sp^2 carbons, and an allylic methylene carrying a hydroxyl group at one of the termini made it an interesting small molecule of Nature*. Organic chemists could easily identify its chemical potential as a synthon and as a substrate for new reagents which needed the built-in structural characteristics possessed by it for studying their reagents' stereo- and regiospecificities.

Extensive work has been done on this small molecule. Both Sharpless (NL 2001) and Noyori (NL 2001) employed this molecule as an important substrate and achieved outstanding success in asymmetric synthesis with their reagents. Eventually, many chemists joined the endeavor of asymmetric synthesis with different reagents using geraniol as a substrate; thus lot of new chemistry evolved (*vide infra*).

Geraniol was subjected to the reactions shown in Fig. 6.9 for obtaining structural information.

These experimental results point to its being a primary alcohol with two isolated double bonds. One double bond carries a geminal dimethyl group, and the molecule can be represented as (**1**), which has been proved by its synthesis. Identical degradation products were also obtained from its isomer nerol, b.p. 225–226°, isolated from various essential oils, e.g., neroli oil, bergamot oil, etc. Thus they must be double bond isomers at the allylic double bond. The double bond configuration of geraniol has been settled by a simple cyclization reaction with dil. H_2SO_4

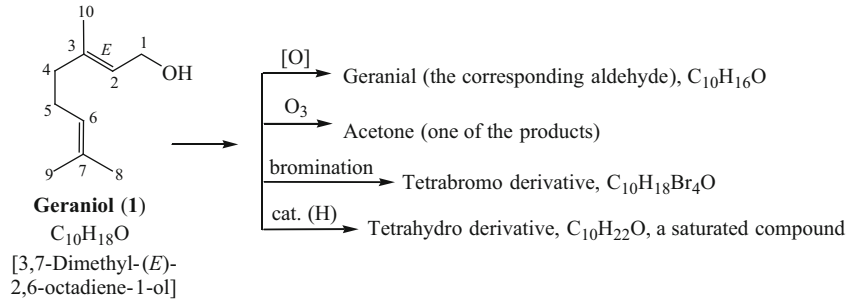


Fig. 6.9 Some reactions of geraniol

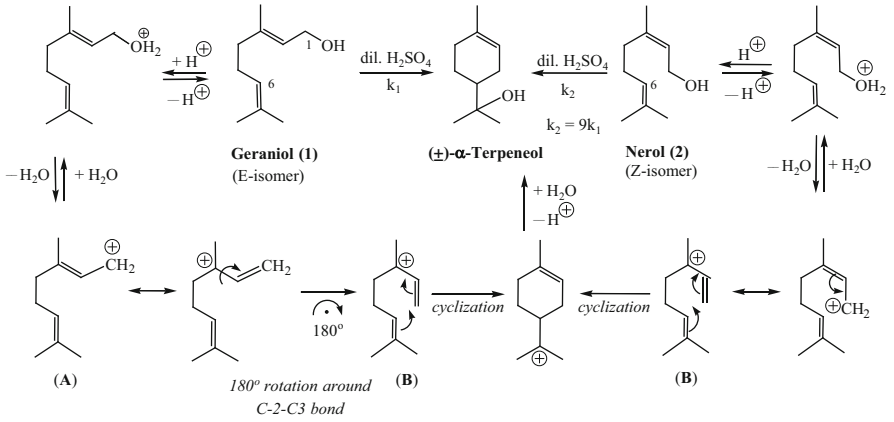


Fig. 6.10 Conversion of geraniol (**1**) and nerol (**2**) to (±)-α-terpeneol: the mechanistic sequences involved

to yield (±)-α-terpeneol, which is also obtained by cyclization of nerol with dil. H_2SO_4 (Fig. 6.10). The rate of cyclization of nerol is nine times faster than that of geraniol; this demands the proximity of the Δ^6-double bond and the hydroxyl group in nerol (**2**). On the basis of this observation *trans* (*E*) geometry is assigned to geraniol, while *cis* (*Z*) geometry to nerol (**2**).

The probable mechanistic sequence for the acid-catalyzed cyclization of geraniol and nerol involving the intermediate resonance stabilized allyl carbonium ions (**A**), and (**B**) is delineated in Fig. 6.10.

6.2.2 Spectral Properties

The IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data of geraniol are shown in Fig. 6.11.

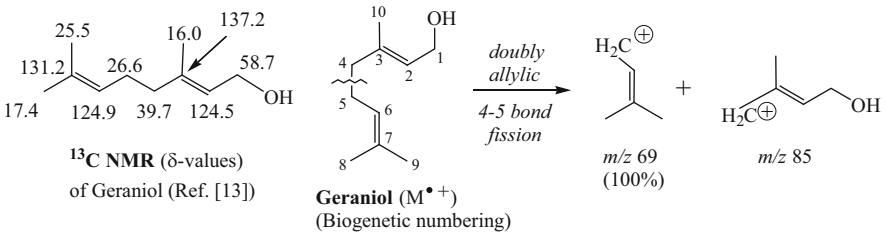


Fig. 6.11 IR and NMR spectral peaks and main mass fragments of geraniol

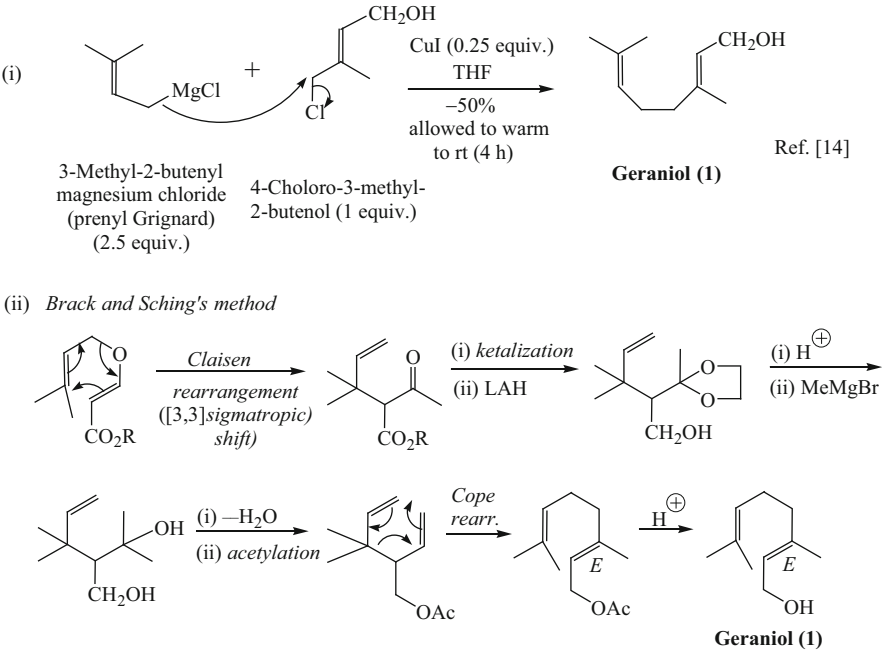


Fig. 6.12 Synthesis of geraniol (two methods)

6.2.3 Synthesis

Several methods of synthesis of geraniol are reported of which two are given in Fig. 6.12.

(i) One synthesis involves alkylation by an allyl Grignard reagent in the presence of CuI as a catalyst resulting in the *extensively regiospecific addition* of two C_5 units in a biosynthetic fashion [14]. The reaction is very slow in the absence of CuI [14].
(ii) Brack and Sching's method has also been delineated stepwise in this figure.

Selective reduction of geranial to geraniol has been achieved [15] by formic acid in the presence of a Ru catalyst (Fig. 6.13) in an excellent yield (99 %). Geranial has also been reduced by the transfer of a hydrogen from an alcohol diglyme, being catalyzed by a tin compound [16], in a moderate yield (67 %) (Fig. 6.13). Geranial occurs (along with neral) in lemon oil (2–3 %) obtained from the dried fruit peels of *Citrus limon* (Rutaceae) (oil content 0.1–3 %).

6.2.4 Synthesis of Chiral Geraniol-1-d

Synthesis of chiral (*S*)-(+)-geraniol-1-d (**5**) has been achieved by Noyori [17] by asymmetric reduction of geranial-1-d (**3**) with $LiAlH_4$-dihydroxybinaphthyl complex (**4**) in 91 % enantiomeric excess (*ee*) (Fig. 6.14). Isotopically labeled chiral geraniol and other related terpenic alcohols serve as the molecules in the studies of terpenoid biosynthesis.

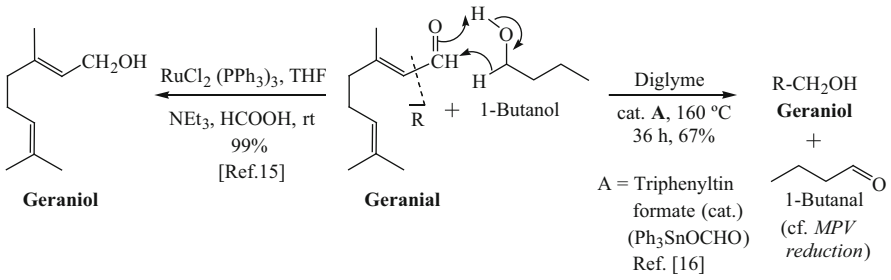


Fig. 6.13 Reduction of geranial to geraniol

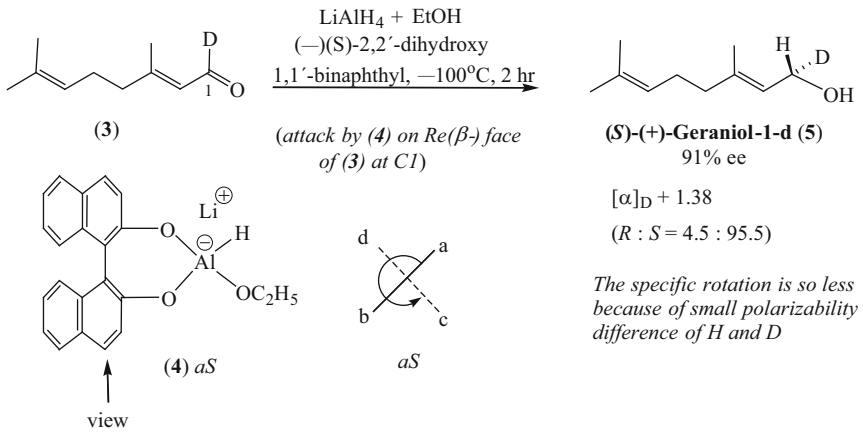


Fig. 6.14 Synthesis of (+)-(*S*)-geraniol-1-d (**5**)

6.2.5 *Reactions: (i)–(x)*

Several new methodologies have been developed as stated in the sequel.

(i)–(iii) ***Oxidation methods***. Several reagents have been employed for the oxidation of the alcoholic OH group [18, 19] [examples (i)–(ii)] and of the vinyl methyl group in a regiospecific manner [example (iii)] [20] (Fig. 6.15).

The success of the reaction (ii) [19] depends on the method of preparation of the reagent. Oxalyl chloride reacts violently with DMSO at room temp. The reagent is to be prepared at low temperature (−60 °C), when DMSO is successfully activated for the participation.

(iv) ***Geranic acid methyl ester***. Corey designed a novel method [21] for converting geranial to the methyl ester of geranic acid. This involves the reversible formation of the intermediate cyanohydrin and its oxidation by MnO_2 followed by reaction with methanol to from geranic acid methyl ester in one pot. A number of aldehydes were converted to the corresponding methyl ester by this method. Geraniol also can be converted to geranic acid methyl ester via geranial in one pot (Fig. 6.16).

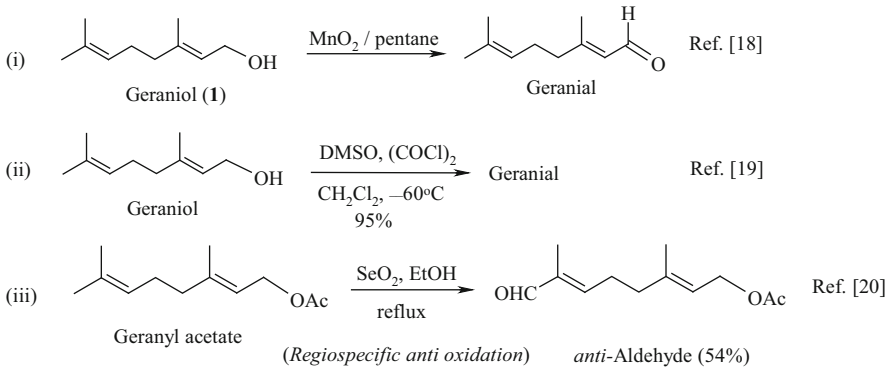


Fig. 6.15 Methods of oxidation of geraniol

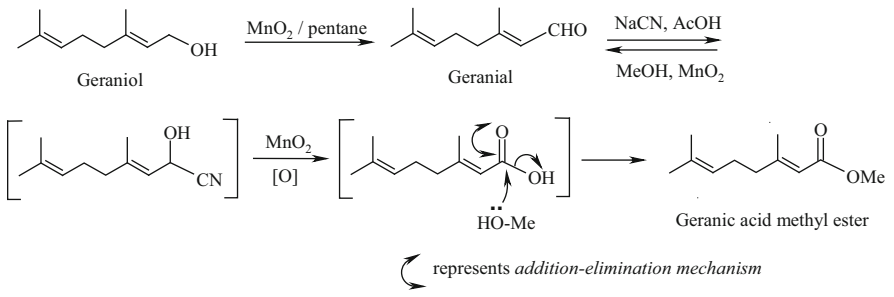


Fig. 6.16 Geranic acid methyl ester from geraniol

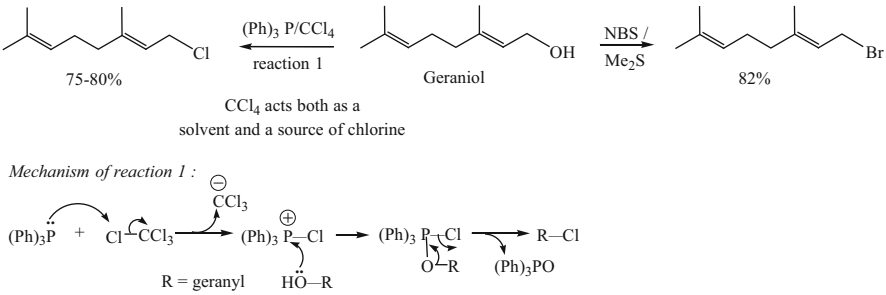


Fig. 6.17 Geranyl chloride from geraniol

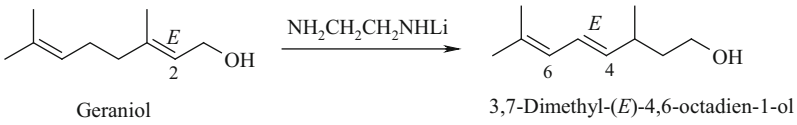


Fig. 6.18 Isomerization of Δ^2 of geraniol to Δ^4

(v) ***Conversion of geraniol to geranyl chloride (or geranyl bromide)*** (Fig. 6.17) Likewise, treatment with CBr_4 (instead of CCl_4) (Fig. 6.17) will convert geraniol to geranyl bromide.

(vi) ***Isomerization of the double bond*** of geraniol in the presence of *N*-lithioethylenediamine, a reagent discovered by Reggel et al. [22, 23], led to the formation of a conjugated double bonded C_{10}-alcohol in a good yield (Fig. 6.18).

(vii) ***Conversion of geranyl acetate into 1-alkene and 2-alkene.*** Palladium-catalyzed hydrogenolysis of the terminal allylic acetate with formic acid-triethylamine leads to the formation of 1-alkene. 1-Alkene and 2-alkene are formed under different condition [24] (Fig. 6.19). A greater selectivity is observed towards the formation of *E*-2-alkenes from allylic acetates (e.g., geranyl acetate) using $Pd^{\circ}SmI_3$ and 2-propanol as a hydrogen donor [24] (Fig. 6.19).

(viii) ***Conversion of geranial into carene*** [25] (Fig. 6.20)

(ix) ***Formation of the methyl ether by a mild method*** [26] (Fig. 6.21). The yield in this method is better than the usual drastic one (treatment with NaH and MeI).

(x) ***Olefin metathesis***. Tungsten hexachloride-tetramethyltin, WCl_6-$SnMe_4$, catalyzes olefin metathesis in the synthesis of terpenoids with 1-methylcyclobutene as the isoprene synthon. The following interesting metathesis involving geranyl acetate and the isoprene synthon yields farnesyl acetate. Both *E* and *Z* isomers are obtained but in very low yield (1–2 %) (Fig. 6.22) [27].

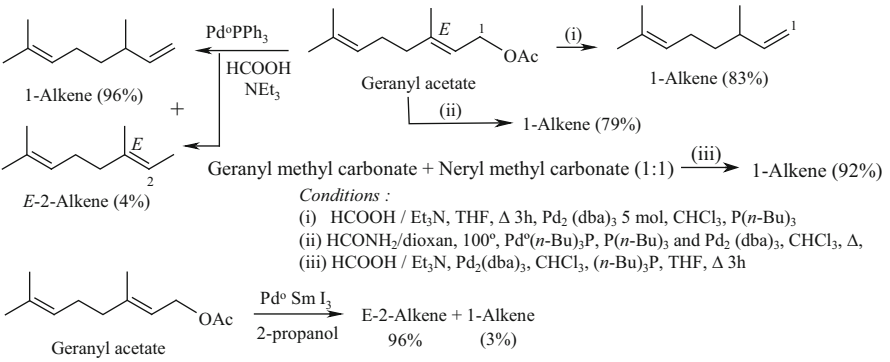


Fig. 6.19 Conversions of geranyl acetate into 1-alkene and 2-alkene

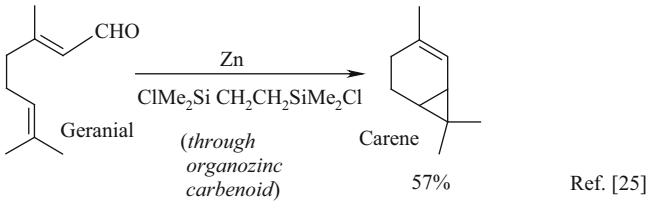


Fig. 6.20 Conversion of geranial to carene

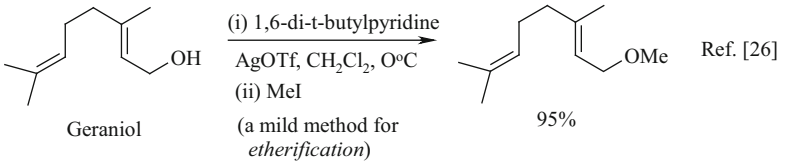


Fig. 6.21 Formation of the methyl ether of geraniol

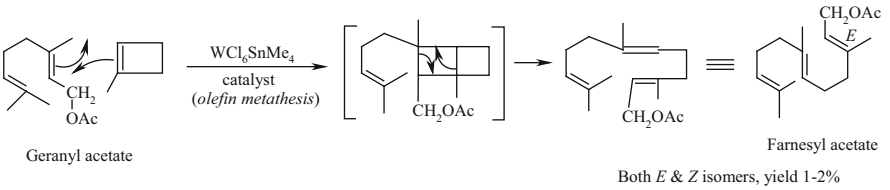
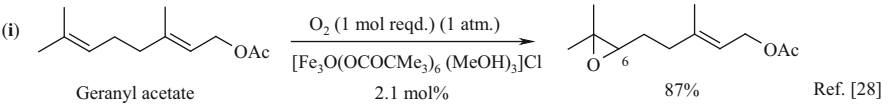


Fig. 6.22 Conversion of geranyl acetate to farnesyl acetate

6.2.6 *Epoxidation methods of Geraniol (Fig. 6.23)*

Five different epoxidation methods [(i)–(v)] are briefly stated.

(i) Geranyl acetate, when treated at 60 °C for 20 h with molecular oxygen in the presence of a catalyst having the structure $[Fe_3O(piv)_6(MeOH)_3]Cl$, is converted into 6,7-epoxygeranyl acetate (87 % yield) and the conversion is 82 %. Similarly neryl acetate also can be converted to the 6,7-epoxyneryl acetate (74 % yield, conversion 52 %). [28]



(ii) 2,3-Epoxyalcohols cannot be generally prepared from allylic alcohols with *m*-chloroperbenzoic acid (*m*-CPBA); however, geraniol-2,3-epoxide has been prepared regioselectively at the allylic alcoholic double bond with *m*-CPBA in an emulsion prepared by stirring a mixture of geraniol, *n*-hexane, *n*-octanol, water, NaOH, and dioctadecyldimethylammonium chloride [29]. Neither

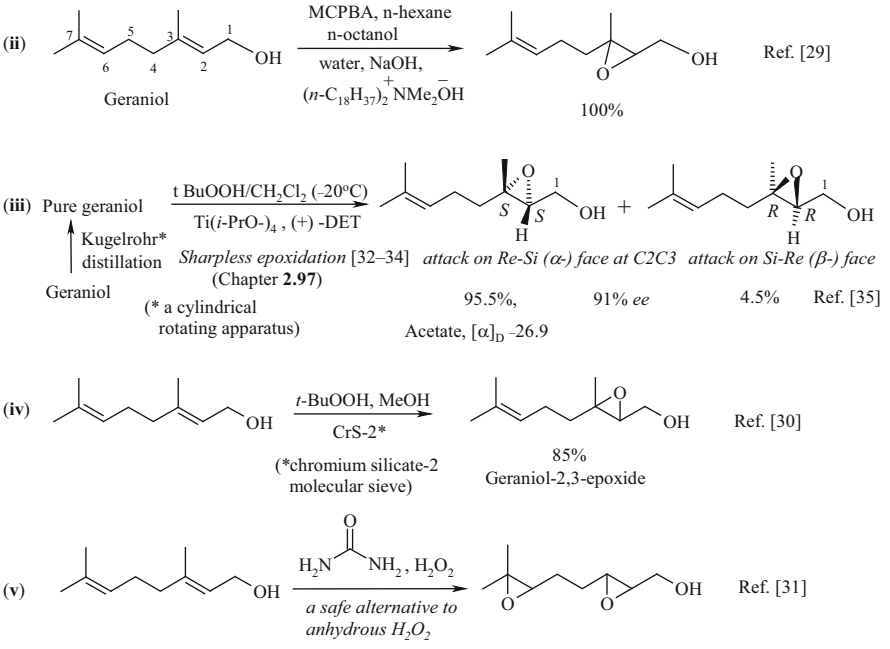


Fig. 6.23 Expoxidation of geraniol by different methods

6,7-epoxycompound nor 6,7-2,3-diepoxy-compound is formed. Such 100 % regioselectivity in epoxidation may be explained as follows: the orientation of the geraniol molecule in the emulsion system is such that due to well-known columbic attraction at micelle surface, the polar OH group will be facing the water, and *m*-CPBA will approach the substrate easily in this *cationic emulsion system*. 2,3-Double bond being close to the polar system is accessible to MCPBA for interaction, while 6,7-double bond, being away from polar head, is protected by the long alkyl chain of the surfactant or by hexane phase and hence escapes the attack by *m*-CPBA. Similarly nerol has been selectively epoxidized at 2,3-double bond in 93 % yield.

(iii) ***Entirely regioselective and highly enantioselective asymmetric catalytic epoxidation of geraniol*** [32–35] has been achieved by Sharpless (NL 2001, see Appendix A). Reaction of an allylic alcohol with t-butylhydroperoxide (TBHP) in the presence of Ti(*i*-PrO-)$_4$ and diethyl tartrate (DET) (*Sharpless epoxidation*) gives epoxy alcohol with high enantiomeric purity. Geraniol is epoxidized at the C2C3 double bond at the *Re–Si* face [α-,when written in the way shown in Fig. 6.23, (v)] producing the (2*S*,3*S*)-epoxide in 77 % overall yield (95 % *ee*) [35], when stoichiometric amount of titanium-(+)-DET complex is used. Use of (−)-DET leads to the preferential attack of oxygen at C2C3 double bond from the *Si–Re* face (β-) to give the enantiomeric (2*R*,3*R*)-epoxide with 90 % *ee*. By manipulating the experimental condition improvement of the overall yield to 95 % (with 95 % *ee*) has been possible. For the epoxidation of a *Z*-allylic alcohol (like nerol) in the presence of (+)-DET attack takes place on C2C3 double bond, again at the α-face (which is now *Re–Re* at C2C3) to produce the (2*S*,*3R*)-epoxide with ~95 % *ee*. *The Sharpless epoxidation has been used as the key step in the chiral syntheses of antibiotics* [33], *terpenes, carbohydrates, pheromones, and pharmaceuticals* [34]. For enantiotopic faces, see Sects. 2.9.5, 2.9.6, and for Sharpless epoxidation, see Sect. 2.11.7

6.2.7 Geraniol As a Synthon

A few examples of the use of geraniol and its functional derivatives as starting materials (synthons) for synthetic studies have already been described. Some more examples are now cited.

(i) ***Synthesis of farnesol:***

(a) Ruzicka [36] synthesized farnesol from geraniol. It constitutes the first total synthesis of farnesol (Fig. 6.24).

(b) Corey converted geranyl acetone into farnesol through the six steps shown in Fig. 6.25 [37, 38]

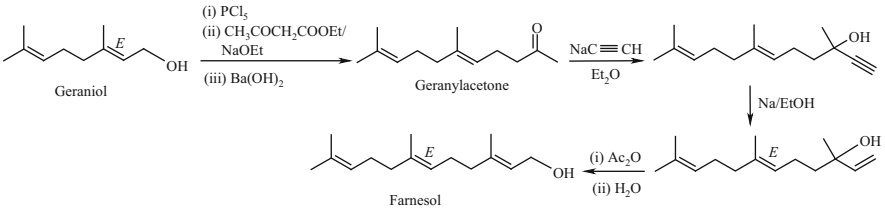
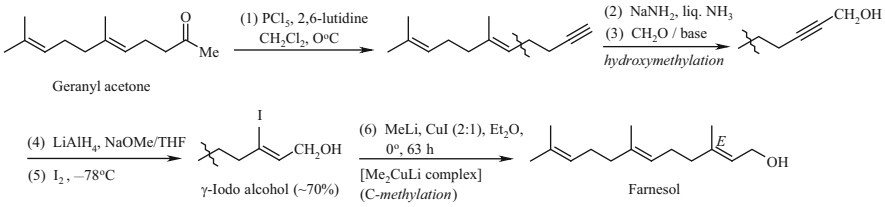


Fig. 6.24 Synthesis of farnesol from geraniol [36]



Step (1) leads to a mixture of chlorides which in step (2) undergoes dehydrochlorination to give the ethynyl derivative

Fig. 6.25 Stereospecific synthesis of farnesol [37, 38]

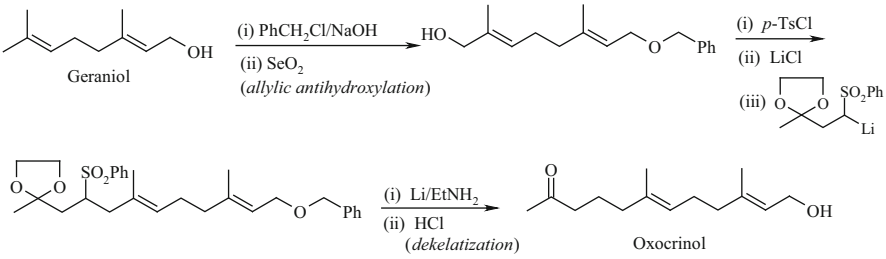


Fig. 6.26 Synthesis of oxocrinol [39]

(ii) ***Synthesis of oxocrinol.*** Kato et al. [39] synthesized oxocrinol, a norsesquiterpene, isolated from marine algae, using gernaiol as the starting material. The steps are quite straightforward (Fig. 6.26).

(iii) ***Synthesis of Gyrinidal.*** Gyrinidal [20], a defensive secretion of the whirligig water beetles, has been synthesized starting from geranyl acetate (Fig. 6.27).

(iv) ***Synthesis of E,E-α-farnesene.*** Chou et al. [40] have synthesized *E*, *E*-α-farnesene using geranyl bromide as an alkylating agent for an isoprene anion used in the form of 3-methyl-3-sulfolene. Thermolysis gave the product and the *E*-geometry of the double bond is enforced by [4 + 2] cheletropic elimination (Fig. 6.28).

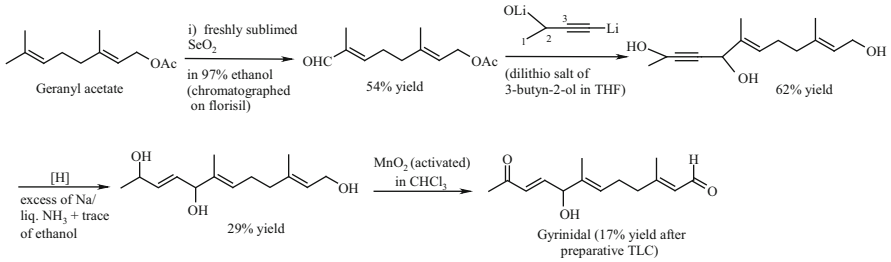


Fig. 6.27 Synthesis of gyrinidal [20]

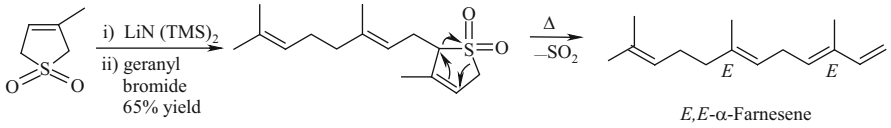


Fig. 6.28 Synthesis of *E,E*-α-farnesene [40]

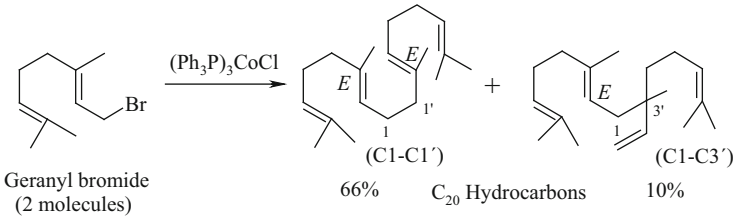


Fig. 6.29 Synthesis of C_{20} hydrocarbons

(v) ***Some more C–C coupling reactions:***

(a) Geranyl bromide undergoes allylic coupling and is converted into two C_{20} hydrocarbons (66 % and 10 %), formed by reductive couplings [41] of C1–C1′ and C1–C3′ of two molecules respectively; a Wurtz type of coupling takes place in the presence of chlorotris(triphenylphosphine) cobalt (Fig. 6.29).

(b) Transition metals like Ni or Fe catalyze the S_N2 type coupling between a selective Grignard reagent and a primary allylic diphenylphosphate. [42] However, in the presence of a catalytic amount of CuCN. 2LiCl S_N2'-type coupling takes place (Fig. 6.30). Highly activated Rieke-Mg [43] is used to prepare the allyl magnesium chlorides from geranyl chloride and neryl chloride at <-100 °C in situ.

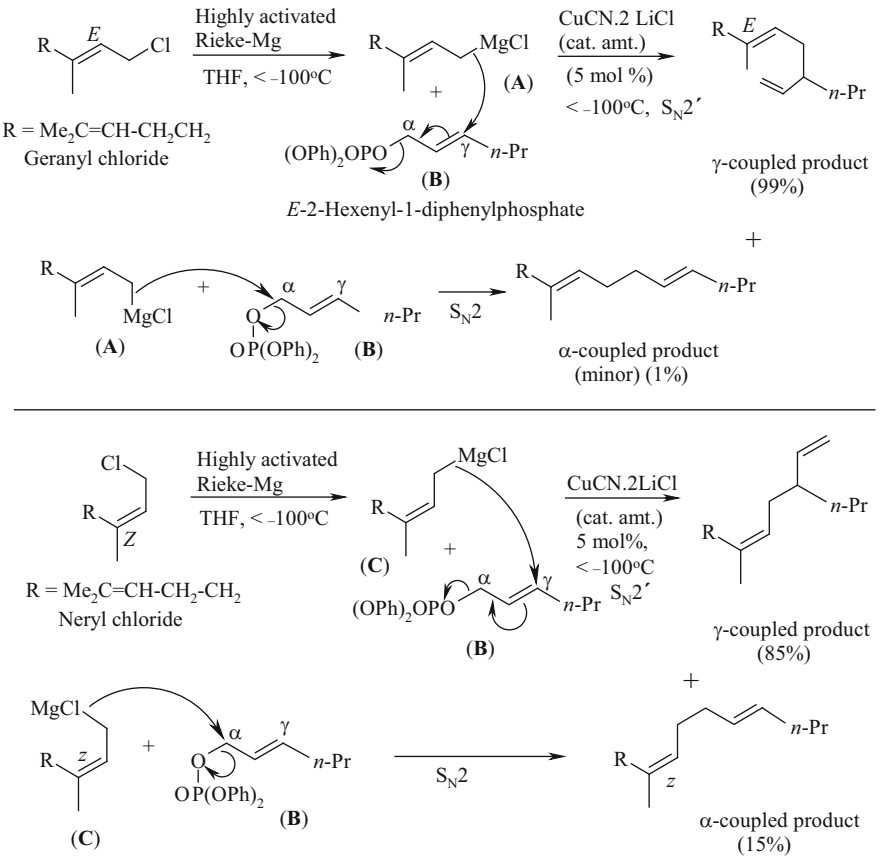


Fig. 6.30 Synthesis of α- and γ-coupled products from geranyl chloride and neryl chloride

6.2.8 Cyclic Products from Geraniol

In this subsection *five more examples of the use of geraniol as a synthon* are given.

(i) Oxidation of geranyl acetate with $KMnO_4$ proceeds stereospecifically and gives a tetrahydrofuran derivative via an intermediate 6,7-diol [44]. The stereochemistry of the product suggests the ring closure process to be suprafacial (Fig. 6.31).

(ii) OsO_4-catalyzed diastereoselective formation of *cis*-tetrahydrofuran derivative has been achieved using geranyl benzoate [45]. In this process the enantio-enriched 1,2-diol is formed from geranyl benzoate by Sharpless AD (asymmetric dihydroxylation). The diol-complexed Os(VI) species then undergoes an intramolecular cyclization to yield enantiopure (+)-*cis*-solamin (Fig. 6.32).

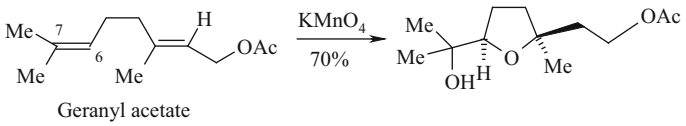


Fig. 6.31 Cyclization to a tetrahydrofuran derivative

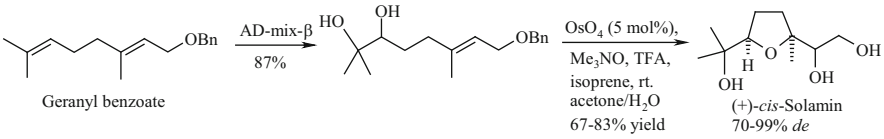


Fig. 6.32 Formal synthesis of (+)-*cis*-solamin

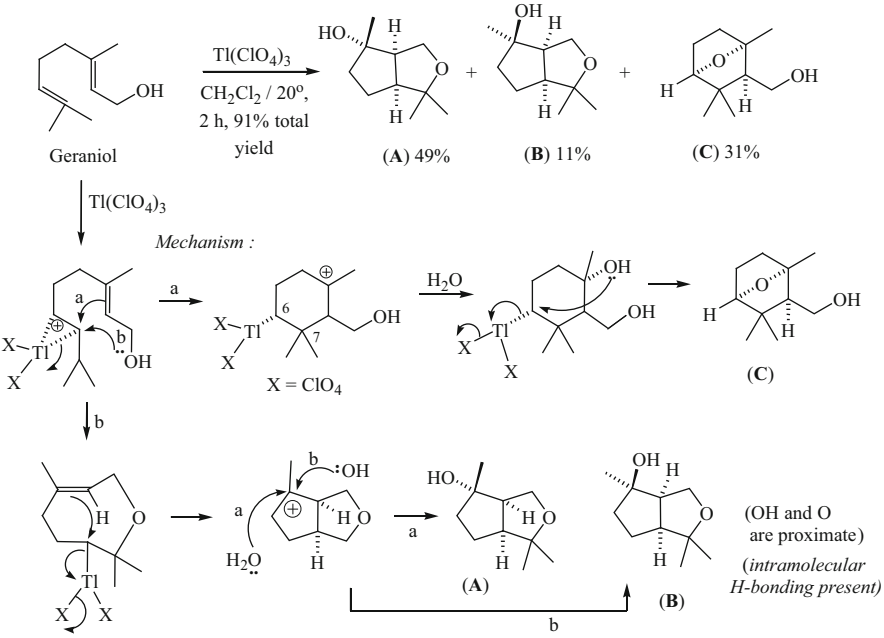


Fig. 6.33 A new type of olefinic cyclization of geraniol

(iii) A new type of olefinic cyclization of geraniol with thallium perchlorate Tl $(ClO_4)_3$ has been reported [46] (Fig. 6.33). The product formation (the connectivity and not the stereochemistry) can be rationalized as shown in the figure. These products possessing iridoid carbon skeleton may be useful in the synthesis and biogenesis of iridoid monoterpenes.

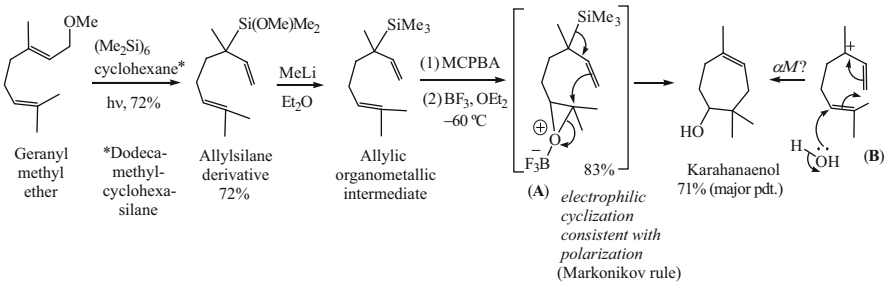


Fig. 6.34 Synthesis of karahanaenol, a seven-member ring monoterpene

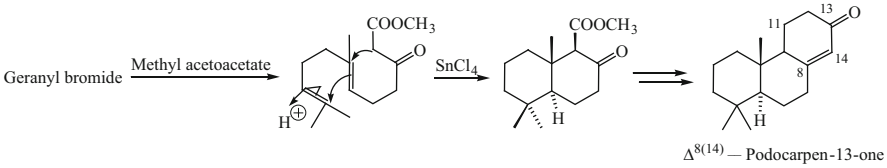


Fig. 6.35 Synthesis of $\Delta^{8(14)}$-podocarpen-13-one

(iv) From the methyl ether of geraniol, a seven-membered cyclic monoterpene *karahanaenol* [47] has been synthesized following the steps shown in Fig. 6.34.

This synthesis suggests the possibility that electrophilic cyclization of type (**A**) of an allylic organometallic type intermediate during enzymatic formation may account for the biogenesis of seven-membered ring monoterpnenes, karahanaenol, humulene, etc., without involving anti-Markovnikov (*aM*) cyclization like (**B**), as is usually thought.

(v) A novel synthesis of $\Delta^{8(14)}$-podocarpen-13-one [48] has been accomplished starting from geranyl bromide, as outlined in Fig. 6.35.

6.2.9 Molecular Recognition (Regio- and Stereoselective)

6.2.9.1 Molecular Recognition of Carbonyl Compounds [Examples (i)–(iii)]

(i) The α,β-unsaturated aldehydes like geranial (*E*-citral) and neral (*Z*-citral) showed regioselectivity at the γ-position when condensed with benzaldehyde in the presence of LDA and aluminum-tris(2,6-diphenylphenoxide (ATPH) (Fig. 6.36). Of the two available γ-positions with respect to carbonyl, a process of molecular recognition of carbonyl at the *anti* position seems to be operative, and the reaction takes place at two different γ-locations for two isomers [49]. Two more examples of molecular recognition of CO follow.

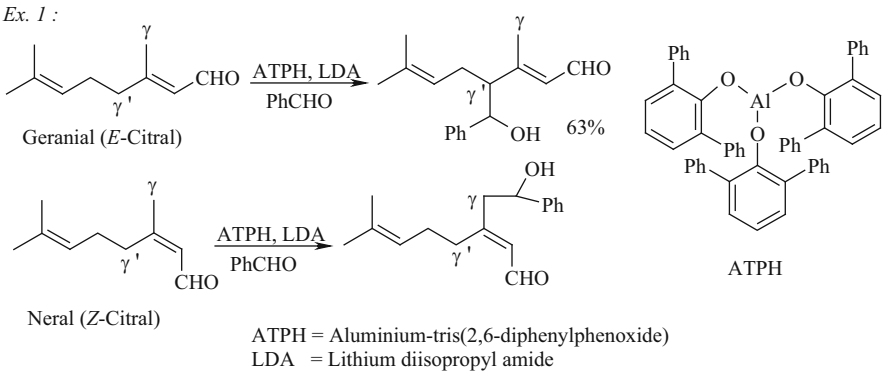


Fig. 6.36 Molecular recognition of CO group—an example [49]

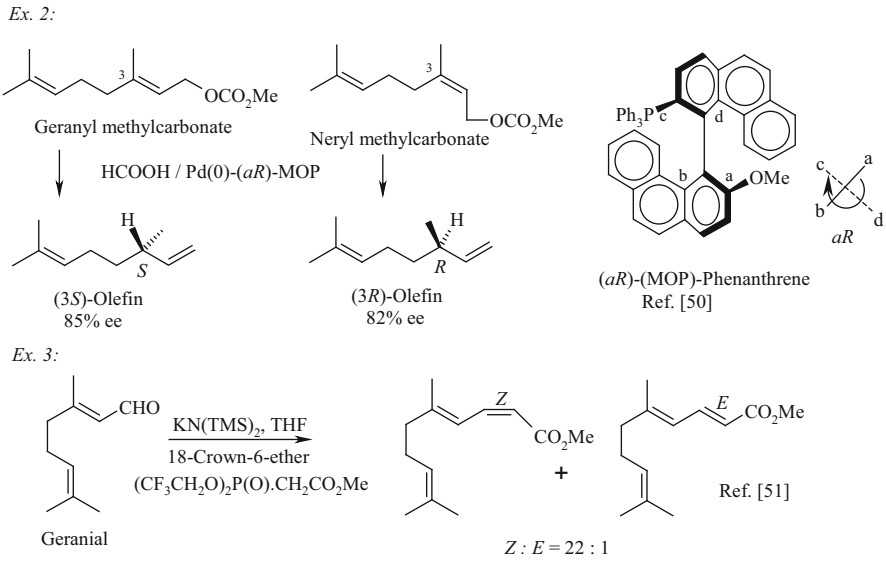


Fig. 6.37 Molecular recognition of CO group—two more examples

(ii) The reaction of geranyl methyl carbonate under the condition cited (Fig. 6.37) gives the reduced (3*S*)-olefin in 85 % *ee*, while the corresponding neryl methyl carbonate under the same reaction condition gives the reduced (3*R*)-olefin in 82 % *ee* [50].

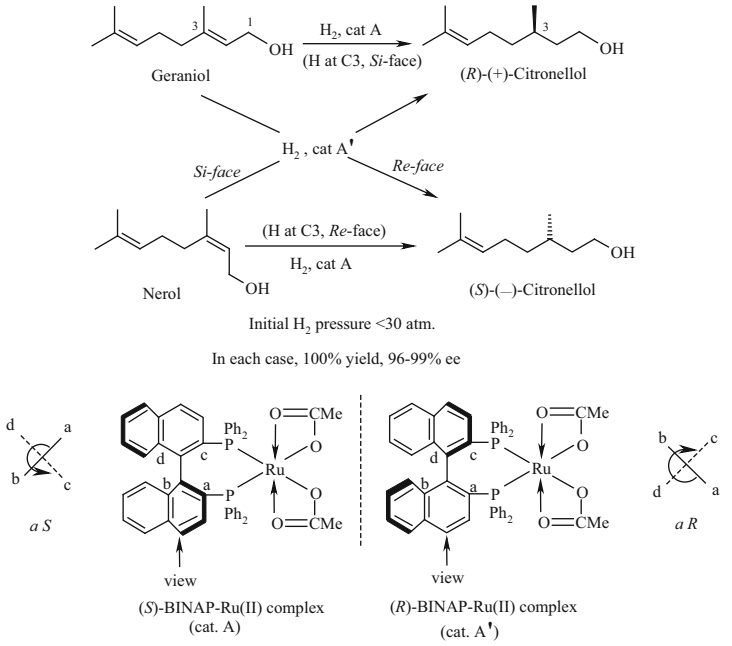
(iii) Geranial is converted [51] to the *Z*:*E* olefins in the ratio 22:1 under the condition specified in Fig. 6.37.

6.2.9.2 Molecular Recognition of Prochiral Allylic Alcohols

Catalysts **A** and **A′** (Fig. 6.38) have accomplished highly enantioselective and regioselective hydrogenation of prochiral allylic alcohols [52] like geraniol and nerol. Here, the chemical multiplication of chirality is based on catalyst/substrate intermolecular *asymmetric induction*. The advantages of this unique asymmetric catalysis [53] are (i) high chemical and optical yields, (ii) regioselective reaction avoiding hydrogenation of the C6=C7 bond, (iii) high substrate–catalyst mole ratio, (iv) lack of double bond migration or E/Z isomerization, and (v) easy recovery or reusability of the catalyst. Here, both (*R*)- and (*S*)-citronellols are accessible by either variation of allylic olefin geometry or choice of handedness of the catalysts which can differentiate the C3 enantiofaces.

6.2.10 Microbial Hydroxylation

Geranyl acetate undergoes deacetylation and regiospecific hydroxylation at the terminal allylic *anti*-carbon (C8) by a strain of *Aspergillus niger* to yield 8-hydroxygeraniol (40 %) along with geraniol (50 %) (Fig. 6.39) [54]. By this method acetates of



Note: *Only the BINAP part of either enantiomeric catalyst causes enantiomerism; thus, the catalysts A and A′ have aS and aR configurations respectively, as shown. The rest Ru complex part of the catalyst A or A′ possesses a horizontal plane of symmetry passing through Ru, and hence achiral.*

Fig. 6.38 Enantioselective catalytic hydrogenation of geraniol and nerol—molecular recognition

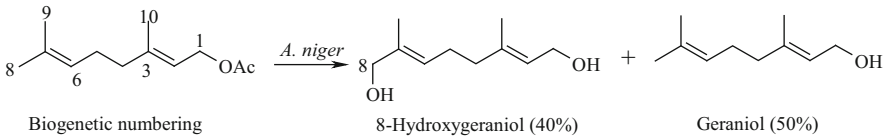


Fig. 6.39 Regiospecific hydroxylation of geraniol

citronellol (2,3-dihydrogeraniol) and linalool (3-hydroxy-2,3-dihydrogeraniol) have been regiospecifically hydroxylated at the terminal allylic *anti*-carbon (C8).

6.2.11 Metabolism of Geraniol in Grape Berry Mesocarp [55]

By using labeled geraniol (d_6-geraniol) it has been shown that in grape berry mesocarp of *Vitis vinifera*, geraniol acts as the precursor of the potent odorant *cis*-(2*S*, 4*R*)-rose oxide; its enantiomer [*cis*-(2*R*, 4*S*)] or two diastereomers [*trans*-(2*S*, 4*S*) and *trans*-(2*R*, 4*R*)] are not found. A scheme for metabolism of d_6-geraniol in grape berry mesocarp is presented in Fig. 6.40.

This metabolism takes place during the ripening of the fruits. The concentration of the flavor in berries increases by allowing the fruits to stay on the vine for an extended period. The labeled geraniol also suffers from stereoselective reduction, E/Z isomerization, oxidation, and glycosylation to give the products delineated in Fig. 6.40 [55].

6.2.12 Bioactivity and Uses

(1) Geraniol is bioactive against mosquitoes and is used as a mosquito repellent.
(2) It has been extensively used as a synthon for the synthesis of various interesting linear as well as cyclic compounds, as already stated in the previous subsections.
(3) Because of its sweet smell and since it has no harmful effect on human system—it is used in perfumery and in the preparation of sweets (confectionery).
(4) It is also used as an odorant component in some medicines and foods.

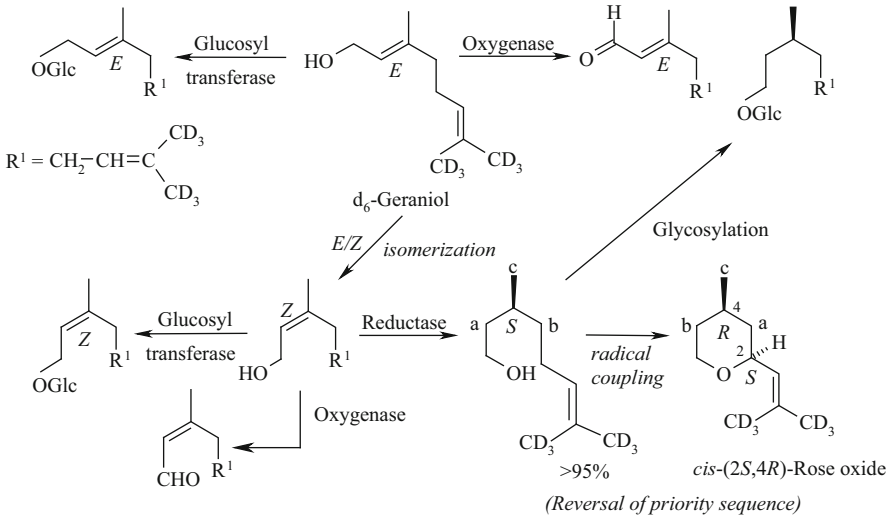


Fig. 6.40 A scheme for metabolism of d_6-geraniol in grape berry mesocarp

6.3 Camphor

6.3.1 Introduction

Camphor (Arabic, Kāfūr; Sanskrit, Korpur), $C_{10}H_{16}O$, m.p. 180°, +54.9 (EtOH), is the most well-known and classical example of biogenetically geraniol pyrophosphate-derived bicyclo[2.2.1]heptane compound. It is a volatile white crystalline solid, widely distributed in Nature, and found specially in all parts (particularly wood) of the camphor tree (*Cinnamomum camphora*, syn. *Laurus camphora*, Fam. Lauraceae) [56], a tree growing in Brazil, Japan, Borneo (hence its alternate name bornan-2-one), and some other places. It also occurs in some other related trees in the family Lauraceae, notably *Ocotea usambarensis*. The less abundant (−)-camphor, m.p. 179°, $[\alpha]_D$ −44.2 (EtOH), is present as a constituent in *Matricaria parthenium* (Fam. Compositae/Asteraceae). Camphor is present in camphor oil (25–47 %) from camphor tree and in rosemary oil (10–25 %) from fresh flowering tops of *Rosmarinus officinalis* (Fam. Labiatae/Lamiaceae), coriander oil (~5 %) from ripe fruits of *Coriandrum sativum* (Fam. Umbelliferae/Apiaceae), and sage oil (5–22 %) from fresh flowering tops of *Salvia officinalis* (Fam. Labiatae/Lamiaceae) and many other oils. Camphor is steam volatile and also volatile at room temperature [56]. Monoterpenes are abundant in biosphere but only rarely encountered in geosphere. Camphor and borneol were found in shale (sedimentary rock) from the Precambrian Ketilidian fold of South West Greenland [57]. Camphor possesses a characteristic aromatic odor. Several compounds are reported to have camphoraceous odor, but structurally they are widely different [58] (Fig. 6.41).

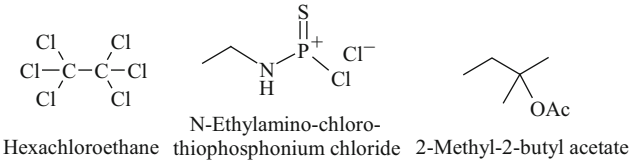


Fig. 6.41 Some compounds with camphoraceous odor

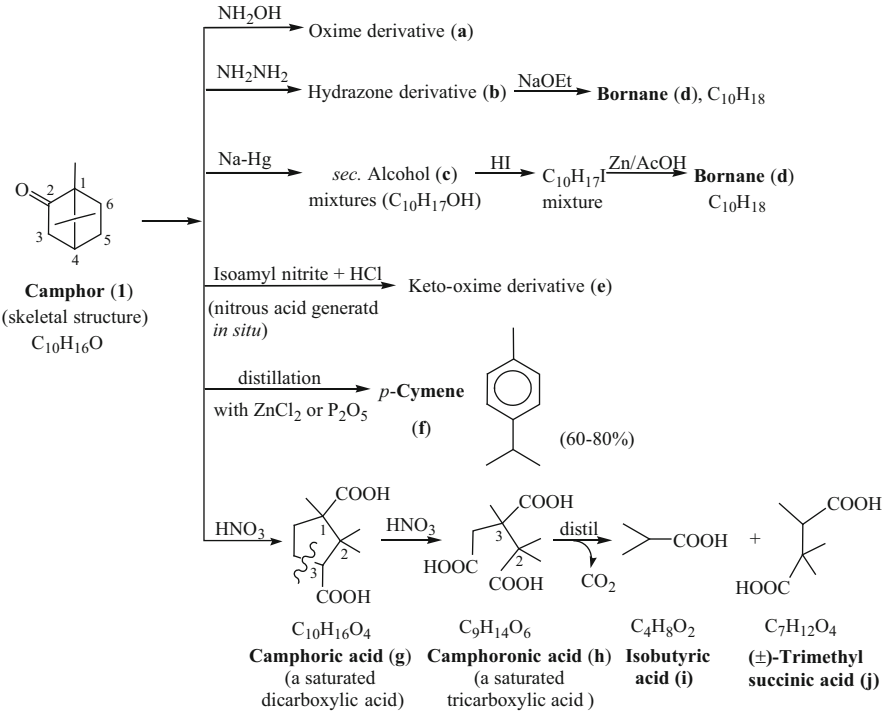


Fig. 6.42 Some degradative reactions of camphor

6.3.2 Structure Determination

The structure of camphor has been deduced from its degradative studies (done during 1894–1914) and confirmed by synthesis.

Some degradative studies are shown in Fig. 6.42. Formation of (**a**), (**b**), (**c**), and (**d**) (Fig. 6.42) suggested the presence of a ketocarbonyl group in the molecule; formation of (**e**) indicated the presence of a ketomethylene group. Further, the ketomethylene group is present as a part of a ring since the product (**g**) contains

the same number of the carbon atoms as its parent compound (**1**). Formation of p-cymene (**f**) indicates the presence of a C–Me and *gem*-dimethyl moieties in (**1**). The products (**i**), (**j**) and CO_2 vindicated 1,2,2-trimethylcyclopentane-1,3-dicarboxylic acid and 2,2,3-trimethyl-3-carboxymethyl succinic acid structures for camphoric acid (**g**) and camphorinic acid (**h**), respectively. The ratio of carbon to hydrogen atoms of (**d**) ($C_{10}H_{18}$), a saturated hydrocarbon, suggested it to be a bicyclic system. All these observations pointed to the structure 1,7,7-trimethylbicyclo[2.2.1]-heptane-2-one (**1**) for camphor. Nearly 30 different structures were proposed for camphor prior to Bredt's correct structure (without stereochemistry) in 1893.

6.3.3 Absolute Configuration and Conformation

(+)-Camphor with two chiral centers (bridgehead carbons) has been chemically correlated to (*R*)-(−)-2-isopropyl-2-methylsuccinic acid of known stereochemistry (Fig. 6.43). Thus, in (+)-camphor, the (*R*)-configuration of C1 fixes the (*R*)-configuration of the other bridgehead carbon C4 also. This correlation is consistent with the results obtained by quasi-racemate method. Additionally, X-ray studies [59] of some derivatives of (+)-camphor having heavy atoms like Br or Cl (Fig. 6.44) also confirmed this absolute configuration. Thus (+)-camphor is

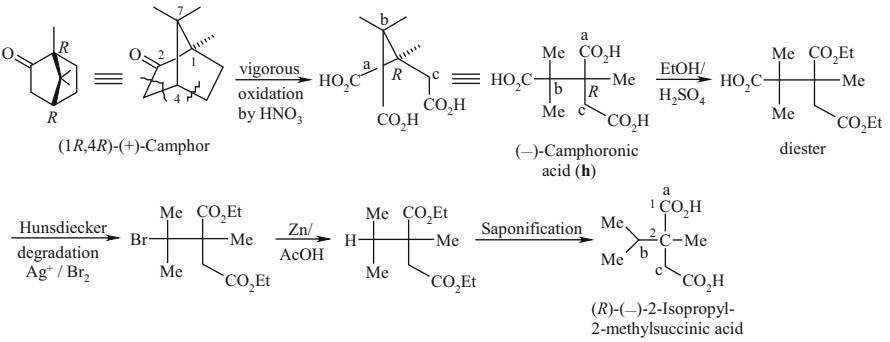


Fig. 6.43 Absolute configuration of (+)-camphor at C1 by chemical correlation

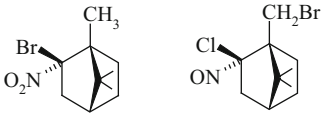


Fig. 6.44 Some (+)-camphor derivatives with Br atom (for X-ray)

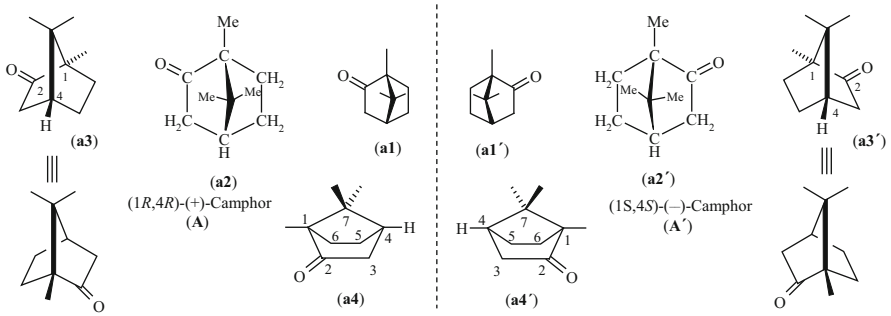


Fig. 6.45 Structural representations of (+)-camphor (**A**) and (−)-camphor (**A′**)

represented as (**A**) having (I*R*, 4*R*) configuration, and (−)-camphor is represented as its mirror image (**A′**) having (1*S*, 4*S*) configuration (Fig. 6.45).

Conformation. The bridgehead of the bicyclo[2.2.1]heptane skeleton imparts strain to the skeletal frame of camphor and locks its molecular conformation into a rigid one in which the cyclohexanone ringattains a true boat form while the bonds C1–C7 and C4–C7 are at its flagpole positions [see (**a4**) and (**a4′**) in Fig. 6.45]. The two cyclopentane rings attain *envelope* conformations [see (**a3**) and (**a3′**) in Fig. 6.45]. The two faces of the molecule—*endo* (concave) and *exo* (convex)—are diastereotopic. In camphor the *exo* (here top or β-) face of the C=O group is sterically more hindered than the *endo* face, since one of the overhanging geminal methyls is projected towards the CO group in the *exo* face. Consequently, $LiAlH_4$/$NaBH_4$ or any other nucleophile attacks the carbonyl carbon predominantly from the *endo* face forming the *exo* or β-alcohol, isoborneol as the major product (see reaction (i), Fig. 6.51). In case of norcamphor (=norborneone) predominant attack takes place from the less hindered *exo* face producing the *endo* (or α-) alcohol, norborneol as the major product (see Fig. 6.51).

6.3.4 Meaning of Structural Representation

Interestingly, it may be mentioned that Roald Hoffmann (NL 1981) selected camphor (globally household name with a long chemical and social history) molecule to explain how "We abstract a piece of reality to show it to another person" [60]. Commonly (+)-camphor (**A**) is expressed as (**a1**), which is a graphic shorthand for the structure (**a2**), which again can be represented in three-dimensional structures (**a3**) and (**a4**) (Fig. 6.45). Roald Hoffmann then moves

further by ascending the ladder of complexity in representation [60], and shows its ORTEP [61] diagram (X-ray), space-filling diagram, and finally considers the vibrations of the molecule and distribution of electrons in space which are not nailed down at static positions of the structures already represented. They are all representative models [60] of the (+)-camphor molecule. He thus elegantly explains the meaning of a structural representation of a molecule. The mirror image representations (**a1′**), (**a2′**), (**a3′**), and (**a4′**) of (−)-camphor (**A′**) molecule are also shown in Fig. 6.45.

6.3.5 Synthesis

(1) The first partial synthesis of (+)-camphor was achieved [62–64] by Albin Haller in 1896, starting from (+)-camphoric acid, a degradative product of (+)-camphor (Fig. 6.46). Komppa in 1903 completed the total synthesis of (±)-camphoric acid [65, 66] (Fig. 6.47) from which (+)-camphoric acid was obtained by resolution. So Komppa's synthesis constitutes the first formal total synthesis of (+)-camphor. It is interesting to note the following: The Na–Hg reduction step produces α-campholide (**B**) and not β-campholide (**C**) (the other possibility), as is evident from the ultimate synthesis of (+)-camphor (Fig. 6.46). In 1960 Otvös et al. have shown by using labeled $-CH_2{}^{14}COOH$ in homocamphoric acid that the labeled carboxyl group is lost in the pyrolysis of its calcium salt, as shown in Fig. 6.46 .

(2) (±)-Camphor has been synthesized [68] in a biomimetic fashion (Fig. 6.48) from (+)-dihydrocarvone via enol acetylation, followed by a brief treatment

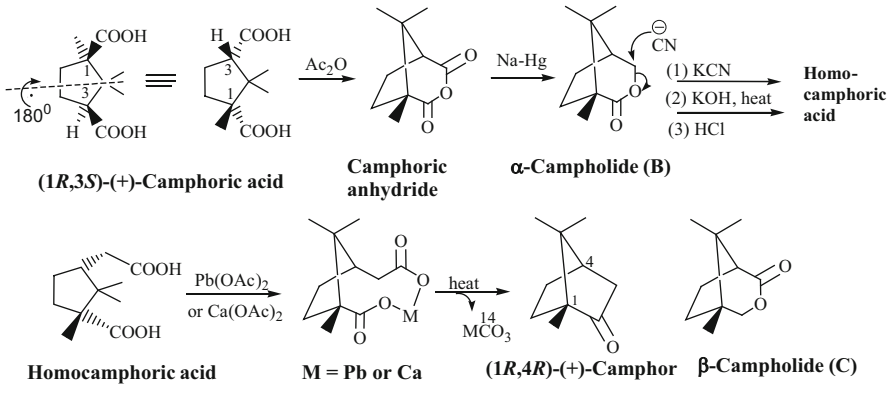
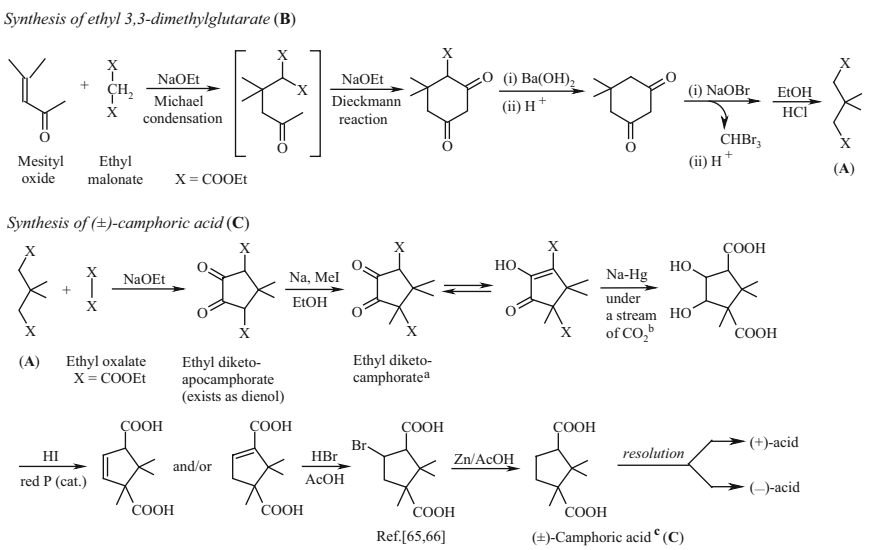


Fig. 6.46 Conversion of (+)-camphoric acid to (+)-camphor [63, 64]



[a] *Exclusive C -methylation (by Na, MeI) claimed by Komppa, but disputed by chemists for several decades who believed it O-methylation, was finally settled by NMR studies in 1968 [67].*

[b] *Double reduction of the diketone is mediated by Na -Hg, and concomitant hydrolysis of the ester groups takes place under a stream of CO_2.*

[c] *Ready formation of its anhydride indicates cis-orientation of the 1,3 COOH groups.*

Fig. 6.47 Synthesis of (±)-camphoric acid [65, 66]

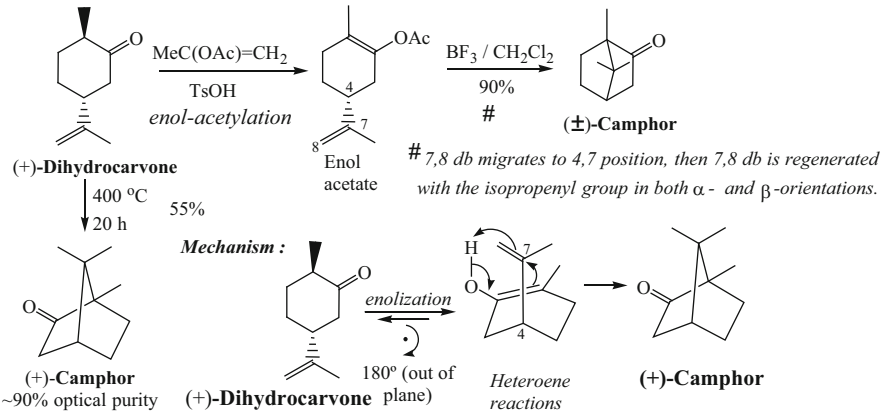


Fig. 6.48 Biomimetic synthesis of (±)-camphor [68] and (+)-camphor [69] from (+)-dihydrocarvone

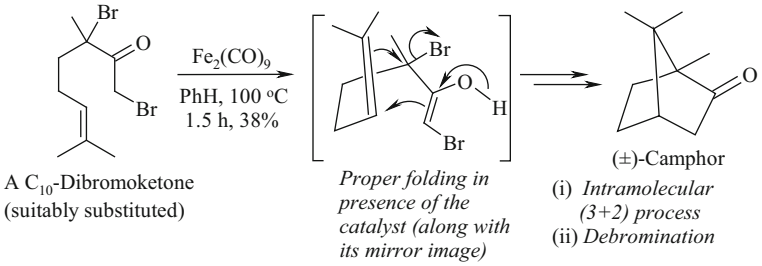


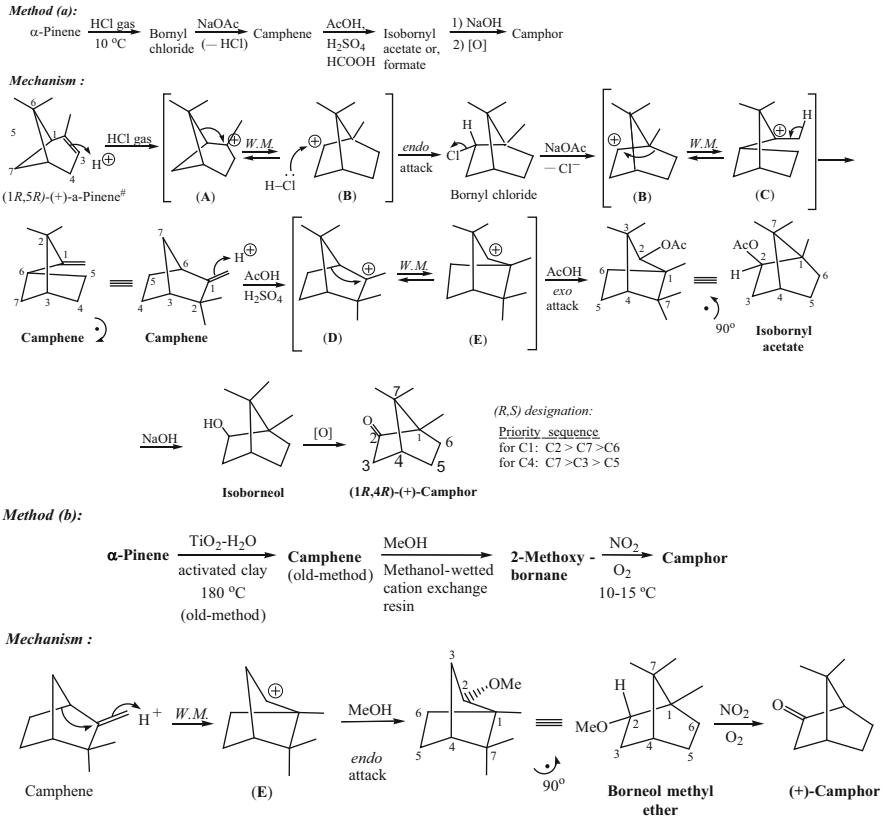
Fig. 6.49 Synthesis of (±)-camphor from a C_{10}-dibromoketone-iron carbonyl reaction [70]

with BF_3 in dilute CH_2Cl_2 solution. This reaction serves as a chemical analogy for the biosynthetic conversion of a monocyclic monoterpene into a bicyclic one. In this case racemization may be caused by the migration of the 7–8 double bond to the 4–7 position, followed by regeneration of the 7–8 double bond with the isopropenyl group in both α- and β-orientations, and formation of the bridge. However, (+)-dihydrocarvone when heated at 400 °C for 20 h gave (+)-camphor in 55 % yield (Fig. 6.48) [69]. This is an example of an *oxygen containing heteroene reaction*.

(3) (±)-Camphor has been obtained in a low yield from a C_{10}-dibromoketone (Fig. 6.49) [70].

6.3.6 *Industrial Preparation of Camphor*

Camphor, a very valuable material for commercial use, is now industrially prepared in thousands of tons from the readily available monoterpene α-pinene, which is abundant in the turpentine collected from pine tar (obtained from coniferous trees). The route followed involves generally Wagner–Meerwein (*W–M*) rearrangement via camphene. There are several modifications of this route [71] (Fig. 6.50) of which catalytic conversion with activated clay is the oldest one. Solid catalyst like TiO_2 has also been used. Camphene when treated with methanol in the presence of methanol-wetted cation exchange resin forms 2-methoxybornane which is subjected to catalytic oxidation by NO_2/O_2 at 10–15 °C to afford camphor. However, whatever may be the source of the catalyst, the structural framework must have been changed via W–M rearrangement (Fig. 6.50). In fact, Wagner (George Wagner, 1849–1903) first noticed the conversion of bornyl chloride and borneol to

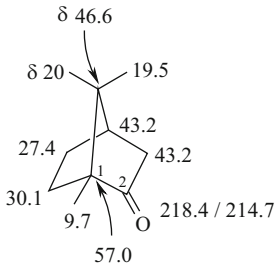


*The carbocations (**A**), (**B**), (**C**) are in equilibrium. In presence of HCl gas (**B**) undergoes attack by Cl^- from endo-face and forms Bornyl chloride. In presence of TiO_2 α-pinene forms camphene by loss of $H^{\oplus}$ from the carbocation (**C**). Cation (**E**), formed from (**D**), undergoes exo attack by AcOH (due to steric crowding of the endo face), whereas in presence of methanol-wetted cation exchange resin which perhaps blocks the exo face, it undergoes endo-attack by MeOH to form borneol methyl ether.*

Fig. 6.50 Methods (a) and (b) for industrial preparation of camphor and their stepwise mechanisms

camphene, and Meerwein (Hans Lebrecht Meerwein, 1879–1965) realized the need of the rearrangement of the skeleton in such a process. *This is the genesis of Wagner–Meerwein rearrangement (W–M)* (Chap. 31). Industrially (±)-α-pinene is usually converted to (±)-camphor. In Fig. 6.50 we propose probable mechanistic pathways for the conversion of (+)-α-pinene to (+)-camphor via three Wagner–Meerwein rearrangements by two methods, for clear understanding.

6.3.7 Spectral Data of Camphor



^{13}C NMR (CCl_4) spectral data [72]

UV (MeOH)	λ_{max} 288 nm (ε 36) (isolated C=O)
IR (CCl_4)	ν_{max} 1,740 (1,760) (C=O), 1,422, 1,050 cm^{-1}
^{1}H NMR (CCl_4)	δ 0.83, 0.85, 0.96 (3H s each, 3t-CH_3's)
MS	*m*/*z* 69, 83, 95, 108, 109 (M^+–CO–Me), 152 (M^+)
ORD and CD	1*R*, 4*R*)-(+)-Camphor exhibits the positive Cotton effect of a single isolated electronic transition.

6.3.8 Biosynthesis of Camphor [1, 2, 6]

The biosynthesis of the enantiomers (1*R*,5*R*)-(+)-α-pinene and (1*S*,5*S*)-(−)-α-pinene from GPP (endo conformer) by its specific enzyme-induced helical mirror image folding and enzyme-catalyzed six steps has already been discussed and illustrated in Fig. 6.6. Again (+)-α-pinene or (−)-α-pinene, being catalyzed by specific enzymes (+)- and (−)-camphor synthases, present in camphor producing plant cells, undergoes Wagner–Meerwein (W–M) rearrangement followed by successive nucleophilic attack of water on the incipient carbonium ion and enzymatic oxidation of the formed alcohol with the help of NAD^+ to generate (1*R*, 4*R*)-(+)-camphor or (1*S*, 4*S*)-(−)-camphor, respectively (Fig. 6.51).

2^{14}C-Geraniol was fed in the plant *Salvia officinalis*. 2^{14}C-Geranyl pyrophosphate (2^{14}C-GPP) formed in the cells underwent specific folding followed by the steps, shown earlier in Fig. 6.6 (lower half), and now shown with label in the lower half of Fig. 6.51, produced, as expected, (−)-2^{14}C-camphor [1, 2]. Thus incorporation of 2^{14}C-geraniol to produce 2^{14}C-camphor demonstrated the biosynthetic pathway of camphor, as shown.

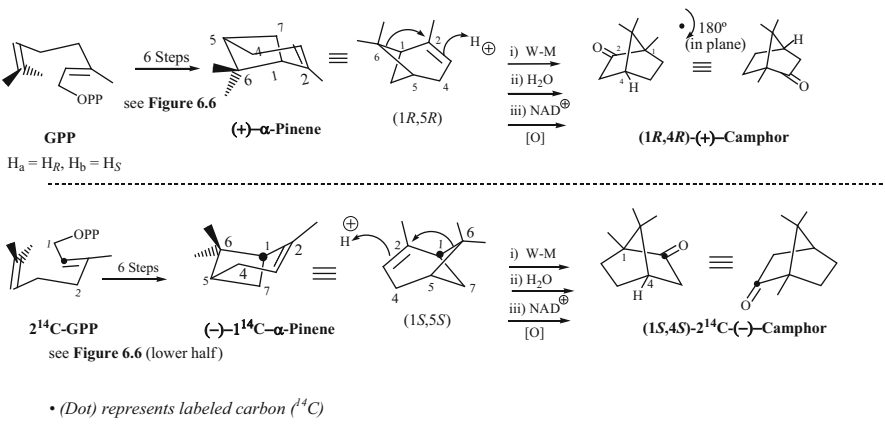


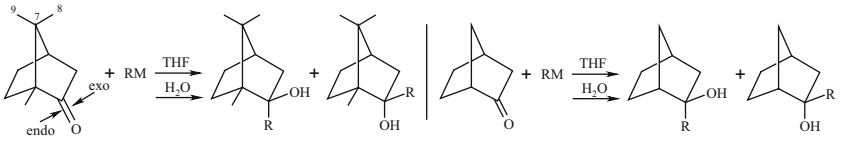
Fig. 6.51 Biosynthetic pathways for the stereospecific formation of (1*R*,4*R*)-(+)-camphor and (1*S*, 4*S*)-2^{14}C-(−)-camphor via (+)-α-pinene and (−)-α-pinene, respectively

6.3.9 Reactions

(i) *Stereochemistry of nucleophilic reactions* (metal hydride/Grignard reactions). A reversal of the stereochemical course of the reduction with $LiAlH_4$ or other metal hydrides, as apparent from the ratio of the products formed, is observed between norcamphor and camphor (Fig. 6.52) [73–77]. Norcamphor, lacking all three methyl groups, offers a less crowded *exo* face to the reagent and the reagent encounters one methylene bridge hydrogen and one pseudoequatorial C-3-methylene hydrogen for interactions in the transition state (TS). The *endo* face offers interactions with three pseudoaxial hydrogens in the TS. Hence the attack from the less sterically hindered *exo* face is preferred, forming the *endo* alcohol as the major product. While in camphor one of the C7-geminal methyls, the *syn m*ethyl, is protruded to the *exo*-face of C=O, making it crowded enough to cause preferential *endo* attack by the reagent. Hence *exo* alcohol appears as the major product in the product mixture. Reduction resulting from attack of the metal hydride from the less sterically hindered side is enhanced when the reagent is changed from lithium aluminum hydride to the more bulky lithium trimethoxyaluminium hydride, more so in a concentrated metal hydride solution indicating that such a reagent aggregates into dimeric or trimeric species [73, 74, 76, 77]. Brown (NL 1979) introduced lithium triisoamylborohydride, a new sterically hindered reagent, for the reduction of cyclic ketones with exceptional stereoselectivity [75]. Two reviews [76] and [77] are available on this topic.

Reactions [(ii)–(ix)] of camphor are delineated in Fig. 6.52 showing reaction conditions and products as well as their stereochemistry, whenever applicable, mechanism, and references.

(i) *Stereochemistry of nucleophilic reactions (metal hydride/Grignard reactions)*



(–)-Camphor | (exo alcohol)[a] Isoborneol | (endo alcohol) Borneol | Norcamphor (Trisnorcamphor) | Norisoborneol (endo attack) | Norborneol (exo attack)[b]

(Nor stands for trisnor)

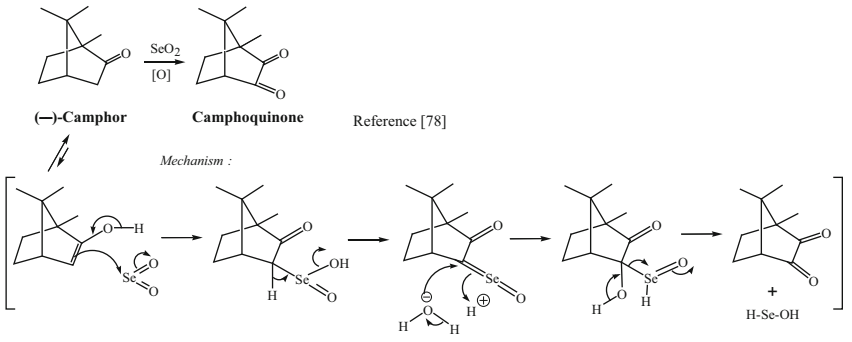
RM = $LiAlH_4$	92%	8%	9%	91%
RM = $LiAl(OMe)_3H$[c]	99%	1%	2%	98%
RM = $LiAl(o\text{-}t\text{-}Bu)_3H$[c]	93%	7%	7%	93%
RM=NaBH4, iPrOH (in i-PrOH)	86%	14%	14%	86%
RM = RMgX	100%	0%	3-0%	97-100%

[a]*In case of camphor approach to the top face is strongly hindered by the overhanging syn-Me gr at C7; so attack takes place almost exclusively from the much less hindered bottom (endo) face, the more so, the more bulky the nucleophilie is.*

[b]*In case of norcamphor there is no hindrance from the exo (top) face whereas hindrance to approach from the endo face (concave) remains, so nucleophiles approach almost exclusively or mainly from the exo face.*

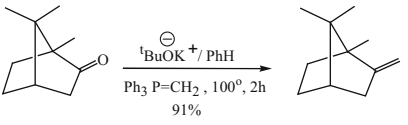
[c]*The stereoselectivity, is more in the case of the less bulky lithium trimethoxyaluminium hydride because of its tendency to aggregate into dimeric or trimeric species, whereas lithium tri-t-butoxyaluminium hydride remains monomeric and thus actually behaves like the less bulky nucleophile than the polymeric trimethoxyaluminium hydride.*

(ii) *Oxidation with SeO_2*



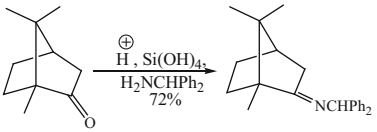
(—)-Camphor **Camphoquinone** Reference [78]

(iii) *Wittig reaction* (Georgi Wittig, 1897-1987)



(A') (—)-Camphor Reference [79] Wittig product

(iv) *Formation of a sterically hindered imine*



(A) (+)-Camphor A sterically hindered imine

Reference [80]

Fig. 6.52 (continued)

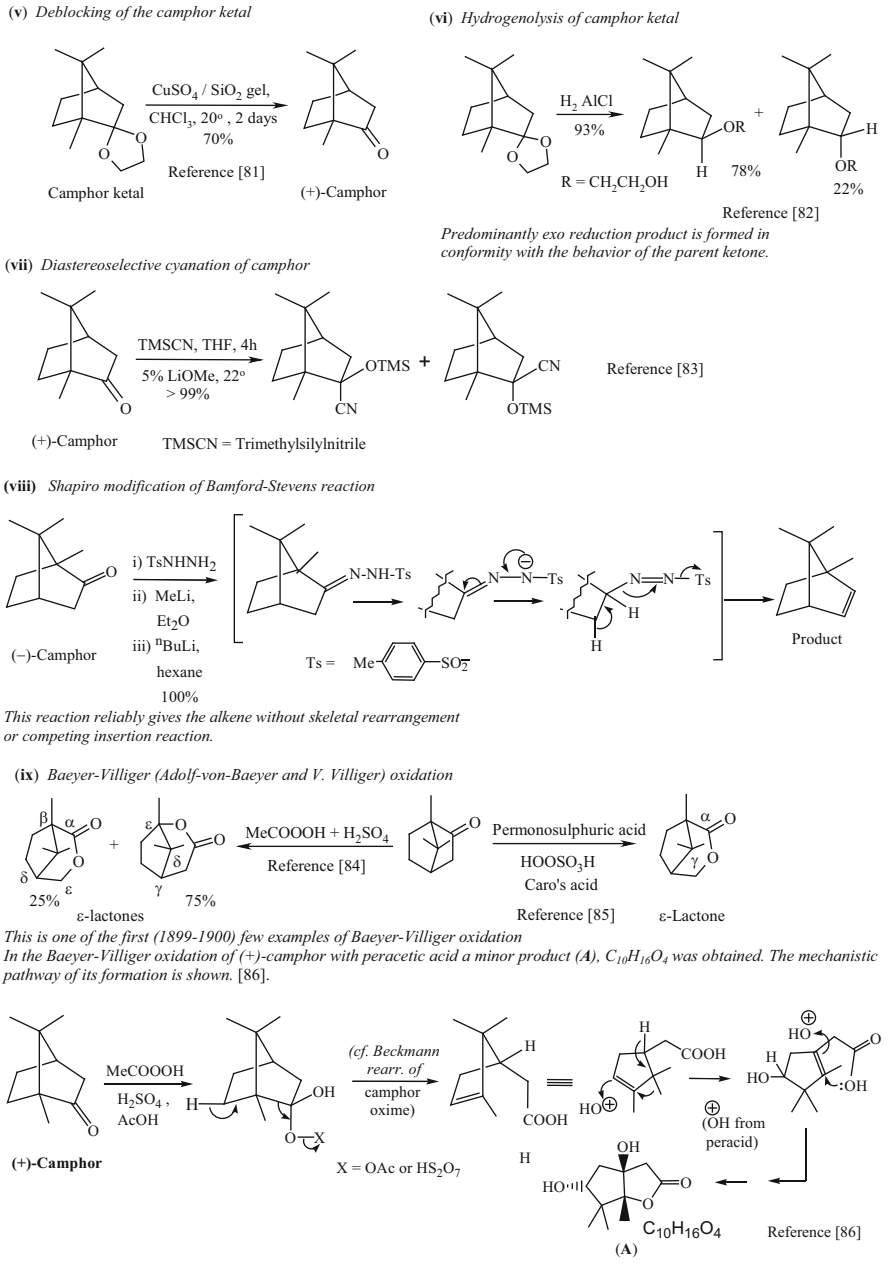


Fig. 6.52 Reactions (i)–(ix) of camphor

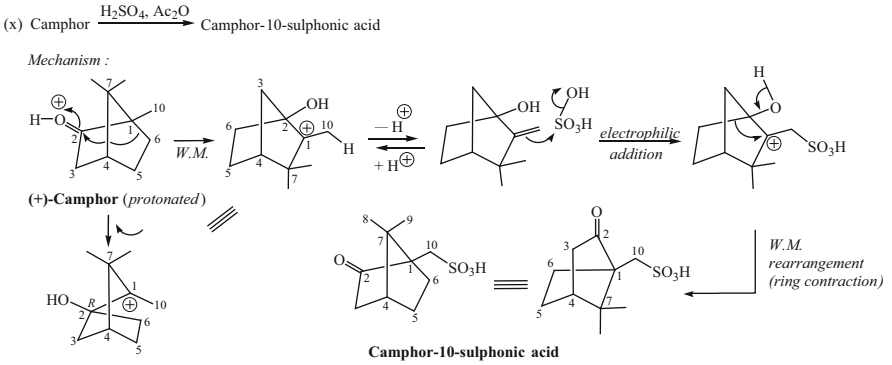


Fig. 6.53 Mechanism of formation of camphor-10-sulfonic acid from camphor

6.3.9.1 Functionalization of Camphor and Its Derivatives

Regiospecific and stereospecific (Reaction x) functionalizations of different carbon atoms of camphor and its derivatives, various bond cleavages, and rearrangements have been elegantly reviewed [87]. An example of such a stereo- and regiospecific functionalization leading to the preparation of camphor-10-sulfonic acid and the probable mechanism of the steps involved are shown in Fig. 6.53.

6.3.9.2 Ring Contraction Reaction of Camphor

Camphor on treatment with PCl_5 (or PCl_3) (Reaction xi) formed a rearranged chloro compound which upon ozonolysis and $NaBH_4$ reduction followed by treatment with NaH produced a ring contracted bicyclo[2.1.1]hexan-1-carboxaldehyde [88] (Fig. 6.54). The latter underwent Cannizaro reaction to form the corresponding primary alcohol and the carboxylic acid.

6.3.9.3 Ring Contraction by Photolysis

Photochemistry of various cyclic ketones and the elimination of CO promoted by radical formation have been reported [89]. The elimination of CO and formation of other products [89] from camphor may be explained as shown in Fig. 6.55. Photolysis of camphor is also reported to give campholenic aldehyde, a natural rearranged monoterpene [90] occurring in *Junisperus communis* [91]. The corresponding nitrile is also formed by heating the γ,γ-dimethylallyl ether of camphor oxime in 62 % yield (Fig. 6.55).

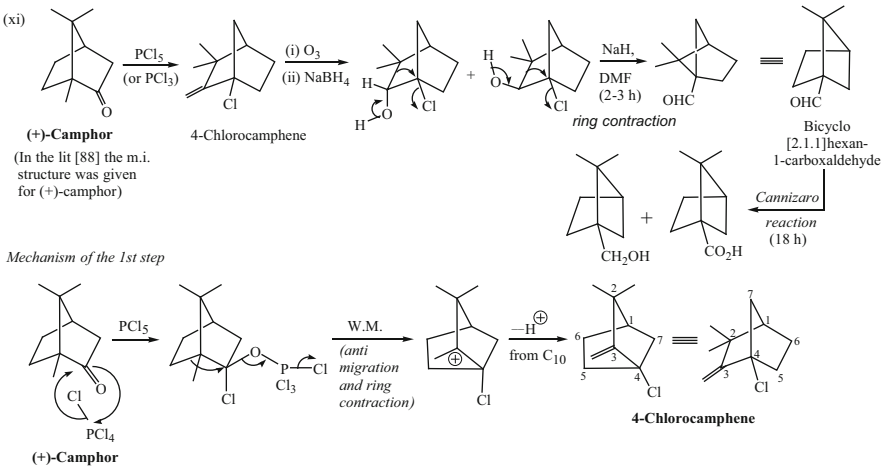


Fig. 6.54 Ring contraction reactions of camphor

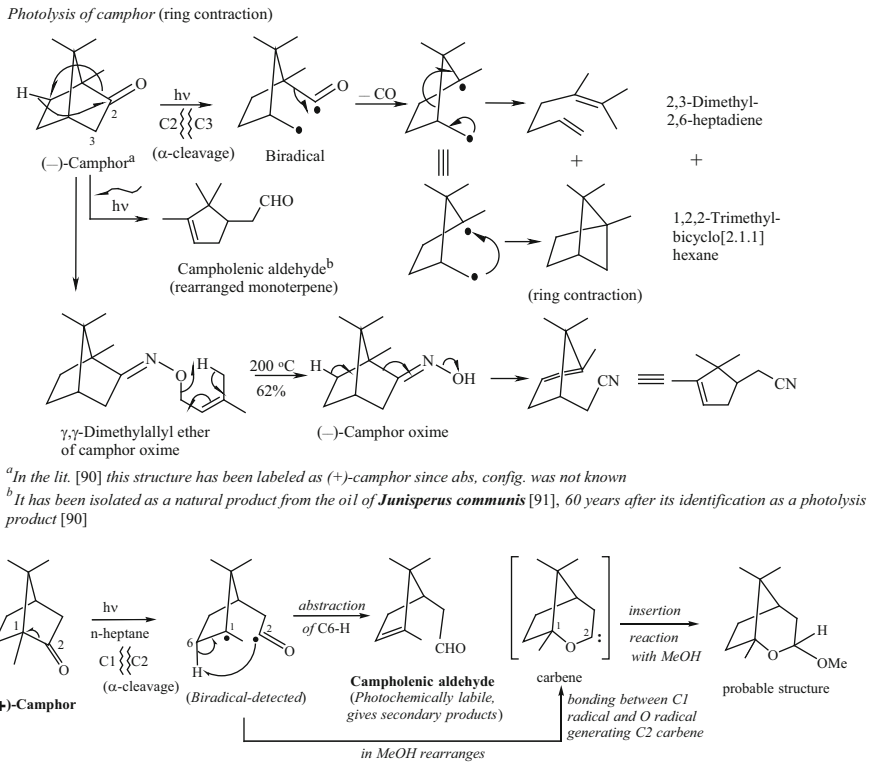


Fig. 6.55 Photolysis products of camphor [90]

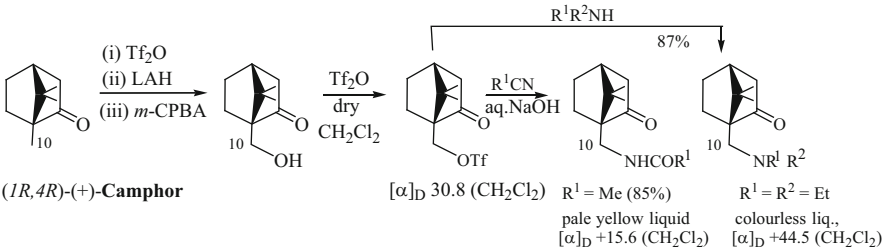


Fig. 6.56 (+)-Camphor as a chiral auxiliary

6.3.10 Camphor as a Synthon and a Chiral Auxiliary

Robert Horton in the start of his elegant route to the first synthesis [92] of taxol [93] (approved by US FDA for its use for the treatment of ovarian cancer and breast cancer, see Sect. 8.4 and Chap. 33), simultaneously with its first synthesis by Nicolaou's group, used (1*S*, 4*S*)-(−)-camphor as the cheap and readily available source of chirality to construct the eight-membered B-ring of taxol with some of its desired functionalities.

A number of enantiopure 10-aminocamphors (including amides) have been prepared in simple straightforward steps in overall good yields from (1*R*,4*R*)-(+)-camphor [94] (Fig. 6.56). They allow enantiospecific access to them and thus lead to the preparation of 10-*N*-substituted-camphor-based chirality transfer agents (chiral auxiliaries) (see Chap. 32) and drugs.

6.3.11 Bioactivity and Uses

Camphor has a long history of use in various religious rituals in different cultures and parts of the globe. Camphor is readily absorbed through skin and it imparts a cooling and a little anesthetic feeling like menthol. However, a large dose (>500 mg) when ingested is poisonous and can cause seizures, neuromuscular hyperactivity, irritability, and mental confusion. Generally 2 g causes toxicity and 4 g is potentially lethal.

It is extensively used as a *preservative* or *an embalming fluid* and also for *medicinal purposes*, in several cough preparations, such as "Vicks vaporub" and "Buckleys," as a cough suppressant and a tropical analgesic. Having embalming properties it is used as an active ingredient in some anti-itch gel.

Its rigid bridged structure with two distinctive faces and availability of both (+) and (−) forms make it and its various derivatives ideal molecules to function as

chiral auxiliaries in asymmetric synthesis (Chap. 32). Modern uses include as a *plasticizer* for cellulose nitrate and as a *moth repellent* in clothes.

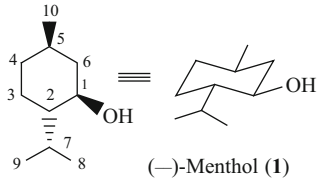
Recently carbon nanotubes were successfully synthesized using camphor in chemical vapor deposition process [95].

Camphor is mostly used as a flavoring agent for sweets in Asia. In ancient and medieval Europe it was widely used as an ingredient for sweets and in the dessert dish preparations in India, where it is known as "*Karpooram*." It is available in Indian grocery stores and is labeled as "edible camphor." It is used as a scent in religious rituals. In Hindu pujas (worships) and ceremonies crude camphor is burned in a ceremonial spoon for performing "*Aarti*" or prayer.

6.4 Menthol

6.4.1 Introduction

(−)-Menthol, also known as levomenthol, $C_{10}H_{20}O$, m.p. 42–45 °C, $[\alpha]_D$ −50 (racemic variety, m.p. 36–38 °C), b.p. 212 °C, white or colorless crystalline solid, occurs in various mint oils, especially peppermint oil (*Mentha piperita*) (Labiatae/Lamiaceae) along with small amounts of (−) menthone and (−)-menthyl acetate. It is one of the most used terpenoids. *Mentha arvensis* (Indian name podina/pudina), cultivated in China, is the primary species of mint used to make natural (−)-menthol crystals and flakes. This species is mainly grown in Uttar Pradesh in India.



(—)-Menthol (**1**)

(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexanol

Menthol has local anesthetic and counterirritant properties and is widely used to relieve minor throat irritation. Menthol with its characteristic odor when inhaled gives a soothing and relieving effect in nasal congestion, and hot menthol vapor as well as menthol-lozenges relieves the sore throat. In fact, plants of mint families have been used as a pain reliever for centuries. Various types of applications and uses of menthol have been stated in Sect. 6.4.8.

6.4.2 Reactions, Structure, Absolute Configuration

(−)-Menthol was subjected to the reactions shown in Fig. 6.57 for obtaining structural information. It is evident from the reactions that the gross structure of menthol is 2-isopropyl-5-methylcyclohexanol. It has been shown by correlation with D-glyceraldehyde and from a study of chemical and optical relationship and the Auwers–Skita rule that (−)-menthol has (1*R*,2*S*,5*R*) configuration (**1**). The structure of menthol has been confirmed by the synthesis of (±)-menthol via (±)-menthone. The absolute configuration of (−)-menthol at C1 was determined as (*R*)- by Prelog by applying his method (Sect. 2.11.5) [96, 97]. The phenylglyoxalate esters of (−)-menthol (**1**) and (−)-neomenthol (**3′**) [the 1-epimer of (+)-menthol (**1′**) (see Fig. 6.59)] upon reaction with methyl Grignard gave in each case after saponification predominant amount of (*R*)-(−)-atrolactic acid PhC(Me)OH. COOH indicating (*R*)-configuration of C1 in both diastereomers (2.11.5). The relative configuration of the diastereomers of menthol being known from chemical studies, the absolute configuration of (**1**) and (3′) at C1 determines the absolute configuration of all the diastereomers and their enantiomers at the chiral centers (at C1, C2, and C5) (see Fig. 6.59). The absolute configuration of (−)-menthol has also been established by X-ray crystallographic studies [98].

6.4.3 Spectral Data

IR: ν_{max} 3,335 (OH), 1,048, 1,020, 994, 977, 920, 876, 845 cm^{-1}

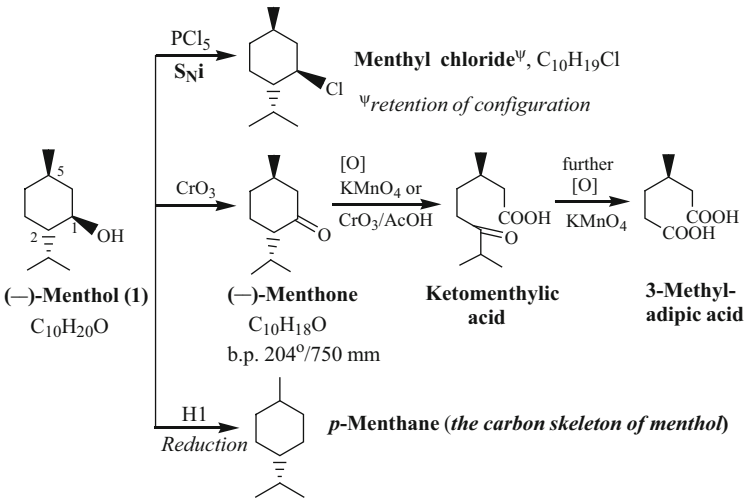
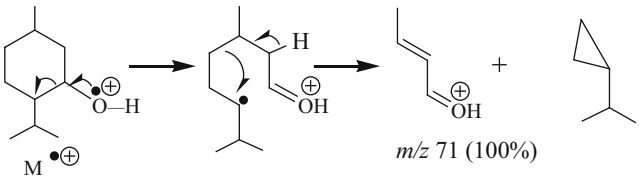


Fig. 6.57 Some reactions of menthol

^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, 5-CH_3), 0.90 and 0.93 (3H each, d, CH_3's at C7), 3.42 [1H, m, H1 (>CHOH)]
^{13}C NMR ($CDCl_3$): δ 71.5 (C1), 50.2 (C2), 23.3 (C3), 34.6 (C4), 31.7 (C5), 45.2 (C6), 25.8 (C7), 16.1 (C8), 21.9 (C9) and 22.2 (C10)
MS: *m/z* 156 ($M^{\bullet+}$), 123, 95, 81, 71 (100), 55, 43, 41



6.4.4 *Synthesis of (±)-Menthone and (±)-Menthol*

The structure of menthone has been confirmed by its synthesis by Kötz and Schwarz (1907) by the distillation of the calcium salt of 2-isopropyl-5-methylpimelic acid (**A**), the synthesis of which is delineated stepwise in Fig. 6.58. In each step racemic product was obtained, although the stereochemistry of each product shown corresponds to that of natural (−)-menthol.

(±)-Menthol may be obtained in good yield by reduction of (±)-menthone. This may be explained in terms of its conformation. Again (+)-pulegone of known structure and stereochemistry gives (−)-menthol on reduction.

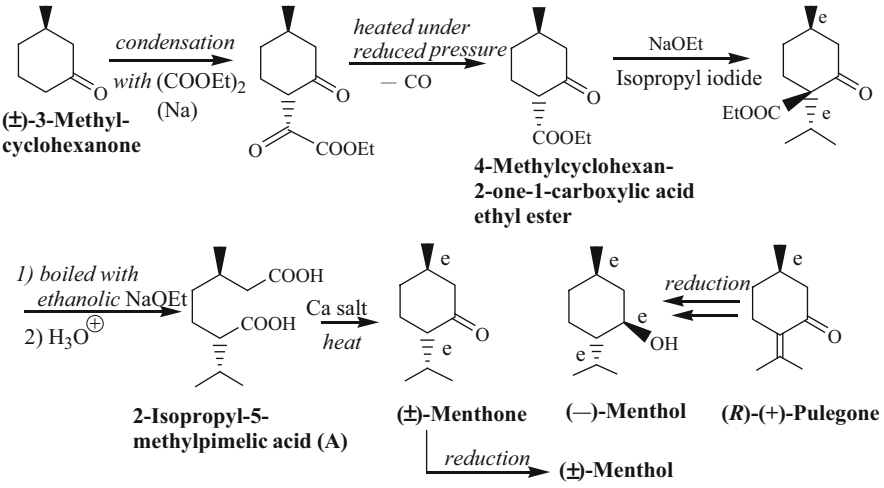
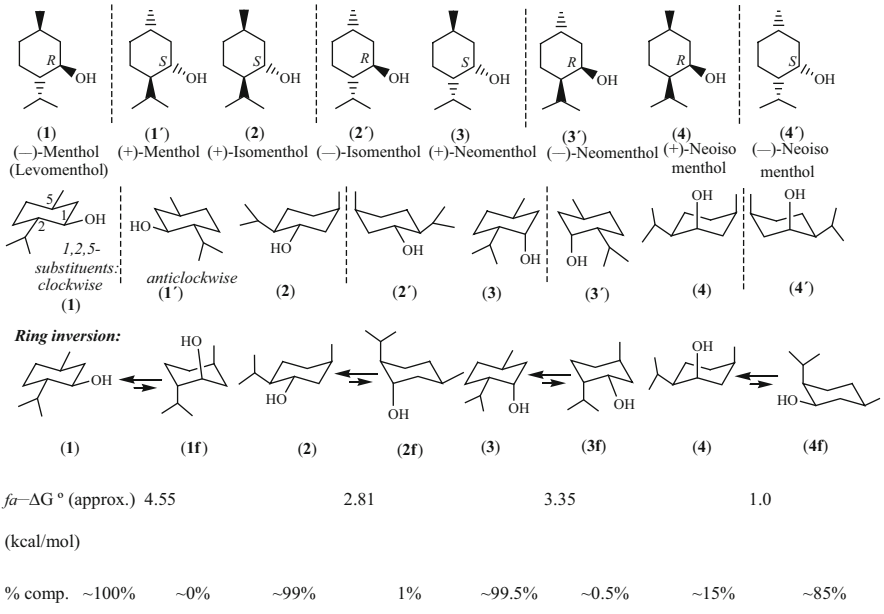


Fig. 6.58 Synthesis of (±)-menthone

6.4.5 *Stereoisomers of Menthol, Their Conformations, and Relative Stability*

The projection formulae of the four diastereomers of menthol having three dissimilar chiral centers and their enantiomers are shown in Fig. 6.59. Ideally we should draw their preferred conformational structures rather than their projection formulae, and by analogy with cyclohexane we expect the six-membered cyclohexane ring to exist in the chair form. The conformation of menthol is particularly interesting because all the substituents occupy equatorial (*e*) positions, and hence it is the most stable diastereomer. All eight stereoisomers are known and their configurations are as shown in Fig. 6.59.

The most stable diastereomer (−)-menthol occurs in various *Mentha* species in more amounts than other diastereomers. Menthol possesses *anancomeric* (meaning *fixed in one conformation*—the word is derived from Greek *anankein*, which means to fix by some fate or law, common example, 4-*t*-butylcyclohexanol) or biased conformation, as shown, its flipped conformer (**1f**) possessing all three substituents



ΔG° represents the conformational energies of the diastereomers, obtained by addition of conformational energies (–ΔG° values) of OH (.6 kcal/mol), Me (1.74 kcal/mol) and iPr (2.21 kcal/mole) [4] *for each conformer of any particular diastereomer. Taking the syn-axial interaction between OH and Me as 2.0 kcal/mole (approx), the –ΔG° in case of neoisomenthol (**4**) becomes ~1 kcal/mole in favor of the conformer (**4f**).*

Fig. 6.59 The configurations and conformations of menthol stereoisomers

in axial (*a*) orientation, being almost nonexistent. The isopropyl group in isomenthol and neomenthol is also equatorial (*e*) in the highly predominant preferred conformations (**2**) and (**3**), respectively, and these two diastereomers may also be regarded as anancomeric (see approximate $-\Delta G^{\circ}$ values [99] and equilibrium concentrations in Fig. 6.59). The conformer (**4f**) of (+)-neoisomenthol having axial isopropyl group, and equatorial methyl and OH groups [and having no *syn*-axial interaction between OH and Me groups as in conformer (**4**)], is more stable and hence predominant (~85 %).

Thus, in view of the relative stability of the preferred conformers of the four diastereomers (in terms of their substituents) their *stability order* is expected to be as follows:

$$menthol > isomenthol > neomenthol > neoisomenthol$$

To alleviate the *syn*-axial interaction between Me and OH groups in neoisomenthol the cyclohexane ring might exist in a distorted chair conformation, decreasing the conformational energy to some extent.

6.4.5.1 Relative Rates of Esterification

It is known that the equatorial (*e*) hydroxyl undergoes esterification by acid chlorides at a faster rate than the axial (*a*) hydroxyl at the same location (epimeric molecules) (*case of steric hindrance*). The rate of esterification of a flexible molecule depends upon the specific rates and mole fractions of its (*e*) and (*a*) conformers and is expressed by the Winstein–Holness equation:

$$k = keNe + kaNa,$$

where $k =$ specific reaction rate, *ke* and *ka* are specific rate constants of (*e*) and (*a*) conformers, and *Ne* and *Na* are the approximate mole fractions of these conformers, respectively.

Careful consideration of the approximate mole fractions (derived from % populations, Fig. 6.59) of the flipped conformers and their specific rate constants reveal that (−)-menthol (**1**), (+)-isomenthol (**2**), (+)-neoisomenthol (**4**), and (+)-neomenthol (**3**) (and their enantiomers) should follow (*e*), (*e*), (*ea*), and (*a*) pathways, respectively, in their esterification reactions. Thus, one can rationalize the following *relative rates of esterification* of these diastereomers, reported by Read et al. in 1934.

$$(-) - menthol(\mathbf{1}) : (+) - isomenthol(\mathbf{2}) : (+) - neoisomenthol(\mathbf{4}) : (+) - neomenthol(\mathbf{3}) = 16.5 : 12.3 : 3 : 1$$

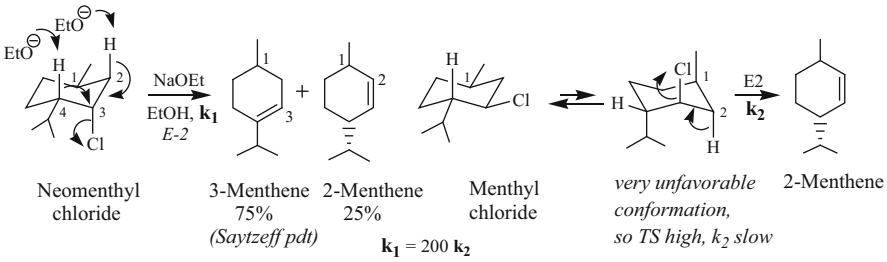


Fig. 6.60 Dehydrochlorination of neomenthyl chloride and menthyl chloride

6.4.5.2 Ionic Elimination Reactions of Menthyl Chloride and Neomenthyl Chloride

Neomenthyl chloride undergoes E2 elimination upon heating with ethanolic sodium ethoxide to produce the trisubstituted olefin, 3-menthene (75 %) along with the disubstituted olefin, 2-menthene (25 %) at a rate 200 times faster than does menthyl chloride, which under the same condition produces only 2-menthene (Fig. 6.60). These facts can be explained on the basis of the 1,2-diaxial eliminations (stereoelectronic requirement) in E2 reactions.

Neomenthyl chloride readily loses elements of hydrogen chloride at a fast rate involving axial Cl at C3 and axial H from C4 to produce predominantly 3-menthene, following the usual Saytzeff rule (double bond formed in the most substituted position); alternatively axial H from C2 may be lost to form 2-menthene, contrary to Saytzeff rule. On the contrary, menthyl chloride having equatorial chlorine can undergo dehydrochlorination only after ring inversion or flipping to an unfavorable conformation in which all three substituents including the chloride become axial. The only axial H next to the axial Cl is at C2, which is eliminated in an E2 fashion to produce the only product 2-menthene (anti Saytzeff product). Evidently, the rate in this case is much slower [99].

6.4.6 Commercial Synthesis of (−)-Menthol (Takasago Process)

Commercial synthesis of (−)-menthol is of great interest since this molecule is consumed on a massive scale in flavoring, pharmaceutical, and other applications and uses (Sect. 6.4.8). The Takasago process constitutes the major route to (−)-menthol.

6.4.6.1 Retrosynthetic Analysis and Strategy [100]

It was known that (−)-menthol (**1**) could be produced in one step through hydrogenation of isopulegol (**7**), which could arise from a Lewis acid-induced carbonyl ene cyclization of (R)-citronellal (**6**) (Fig. 6.61). The latter reaction is particularly productive because it simultaneously creates the six-membered ring and the two contiguous stereogenic centers through an ordered transition state (see TS, Fig. 6.62). Large-scale preparation of enantiomerically pure (*R*)-citronellal (**6**) with a single stereogenic center is possible through hydrolysis of (*R*)-citronellal-(*E*)-*N*,*N*-diethyl-enamine (**5**), the projected product of an enantioselective isomerization of prochiral *N*,*N*-diethylgeranylamine (**4**). Compound (**4**) could be constructed stereoselectively from myrcene (**3**) and diethylamine through telomerization. Myrcene could be obtained by the thermal cracking of (−)–βpinene,

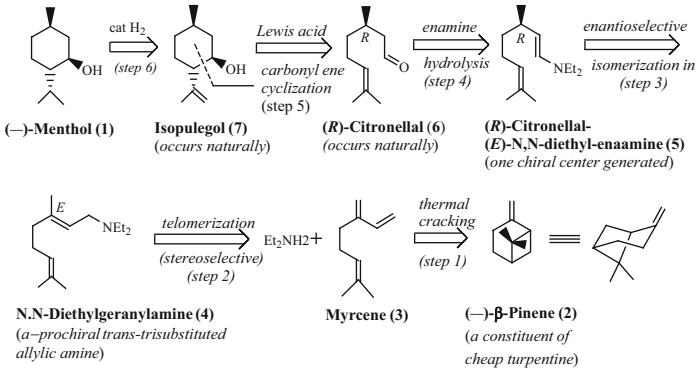


Fig. 6.61 Retrosynthetic analysis of (−)-menthol (for the Takasago process)

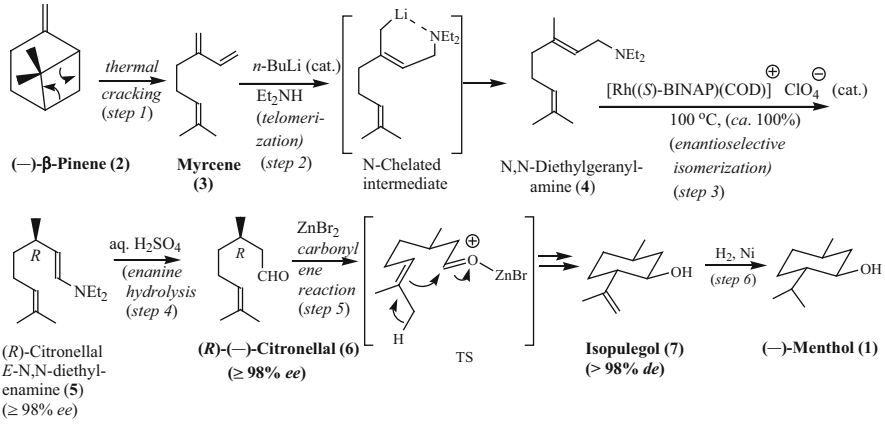


Fig. 6.62 Takasago process for the asymmetric synthesis of (−)-menthol

a major constituent of cheap turpentine. Takasago Corporation used this elegant plan to develop a highly practical and financially viable industrial process of manufacture of (−)-menthol, as delineated in Fig. 6.62.

6.4.6.2 Commercial Asymmetric Synthesis of (−)-Menthol Starting from (−)-β-Pinene (Fig. 6.62)

In this commercial synthesis [100] the *first step* constitutes thermal cracking of (−)-β-pinene (**2**), a constituent of cheap turpentine, to give myrcene (**3**). As was already known, *n*-butyllithium could catalyze the reaction of a 1,3-diene, here myrcene, with a secondary amine like diethylamine, to give regio- and stereoselectively diethylgeranylamine (**4**), a *trans*-trisubstituted allylic amine (*step 2*). This type of addition process is referred to as *telomerization* (Fig. 6.62).

The most important central step in Takasago process is the asymmetric double bond isomerization (*step 3*) of *N*,*N*-diethylgeranylamine (**4**) to (*R*)-citronellal *E*-diethylenamine (**5**) in quantitative yield (*ee* >98 %), upon treatment of the former at 100 °C with a small quantity of the catalyst [Rh((*S*)BINAP)(COD)]$^{+}ClO_4^{-}$. The catalysts [Rh((*S*)-BINAP)$(MeOH)_2$]$^{+}ClO_4^{-}$ and Rh((*S*)-BINAP)$(THF)_2$]$^{+}ClO_4^{-}$ are also equally effective. Process refinements permitted this reaction to be conducted on a ton scale at substrate:catalyst ratios of 8,000:1 to 10,000:1. The enantiomerically nearly pure enamine product (**5**) can be distilled directly from the reaction mixture at low pressure, and the (*S*)-BINAP-Rh (I) catalyst can be recycled. Using this effective catalytic asymmetric reaction as the central step the Takasago Corporation produces approximately 1,500 tons of (−)-menthol and other terpenes annually. It may be mentioned here that diethylnerylamine, the corresponding *Z*-isomer of (**4**), would also produce (**5**) by switching to the enantiomeric (*R*)-BINAP-Rh(I) catalyst (cf. Fig. 6.38 for enantioselective hydrogenation). This catalytic asymmetric process (*step 3*) is thus economical, efficient, and also very flexible. The remote $\Delta^{6,7}$ double bond is impervious to such asymmetric reactions.

The enamine (**5**) is then converted to (*R*)-citronellal (**6**) by the mild action of aqueous sulfuric acid (*step 4*). (*R*)-Citronellal (**6**) produced in this manner is of much higher enantiomeric purity (98–99 % *ee*) than the natural citronellal which is at best 80 % enantiomerically pure. In the next step (*step 5*) the neighboring C3 stereocenter having (*R*)-configuration in (−)-citronellal (**6**) guides the stereochemical course of the carbonyl ene cyclization to produce (−)-isopulegol (**7**). Thus, treatment of (*R*)-(−)-citronellal (**6**) with the Lewis acid $ZnBr_2$ (or $ZnCl_2$) forms (−)-isopulegol in >98 % *de*. Isopulegol (**7**) having all three ring substituents in equatorial orientations arises naturally from a chair-like transition state structure (TS), in which the C3 methyl group, the coordinated C1 aldehyde carbonyl, and the $\Delta^{6,7}$ double bond are all equatorial-like. This TS is expected to have lower energy than any other postulated transition state. The chemical and enantiomeric purity of

(**7**) is raised close to 100 % by low-temperature crystallization. Finally in the last step (*step 6*), catalytic hydrogenation of isopulegol completes the synthesis of (−)-menthol (**1**).

6.4.7 One-Pot Conversion of (R)-Citronellal to (−)-Menthol

Recently it has been reported [101] that cyclization of (*R*)-citronellal (**6**) over Lewis acid catalyst (step 5) may produce besides the desired (−)-isopulegol (**7**) three stereoisomers and other side products. One-pot full conversion of (*R*)-citronellal (**6**) to (−)-menthol (**1**) has been achieved [101] by treatment of (**6**) with beta zeotites impregnated with Ir (bifunctional catalyst) which effects consecutive acid-catalyzed cyclization and Ir-catalyzed hydrogenation at 80 °C, with 95 % selectivity for the menthol isomers, of which 75 % is the desired (−)-menthol. In this method catalyst/substrate ratio is also much less than earlier studies; 17 g (−)-menthol can be produced per g of catalyst in a single run. Presumably, this method has great industrial potential.

6.4.8 Applications and Uses

- (−)-Menthol is a substituted cyclohexanol derivative with three chiral centers and is used for the *resolution* of racemic acids via their diastereomeric esters.
- It is used as a *chiral auxiliary* in asymmetric synthesis.

  (−)-Menthol has low toxicity: Oral (rat) LD_{50}: 330 mg/kg; skin (rabbit) LD_{50}: 15,800 mg/kg. Menthol is included in many products throughout the world for various reasons as stated below:
- As a *topical analgesic* to relieve minor aches and pains such as muscle cramps, sprains, headaches, and similar conditions, alone or combined with chemicals like camphor, eucalyptus oil, or capsaicin—used as a gel or cream in Europe and as patches in the USA. Examples: *Tiger Balm* or knee/elbow *sleeves* or *IcyHot* patches.
- In *decongestants* for chest and sinuses (cream, patch, or nose inhaler). Examples: *Vicks VapoRub*, *Mentholatum*.
- In certain medications used to treat *sunburns* (as it provides a cooling sensation often with *Aloe*).
- As an antipruritic to reduce itching.
- Used in *oral hygiene* products and *bad-breath remedies* like mouthwashes, toothpastes, mouth and tongue-sprays and as a food flavor agent, e.g., in *chewing gum*, *candy*.
- In over-the-counter products for short-term relief of *minor sore throat* and *minor mouth irritation*.

- As an *additive* in *certain cigarette brands*, for flavor, to reduce throat and sinus irritation caused by smoking.
- Used in *perfumery* as menthyl esters to emphasize floral notes (especially rose).
- As a *pesticide against honey bees* or *tracheal mites*.
- In first aid products such as "mineral ice" *to produce a cooling effect* as a substitute for real ice in the absence of water or electricity (pouch, body patch, or cream).
- In some beauty products such as *hair-conditioners*, based on natural ingredients (e.g., St. Ives).
- In various patches for reducing fever, applied to children's foreheads, and in "foot patches" to relieve many ailments (more frequent in Asia, especially Japan).

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Chapter 7
Sesquiterpenoids (C_{15})

7.1 Introduction

The carbon content of monoterpenes has been taken as reference for the classification of terpenoids, like sesqui-, di-, sester-, and tri-, as they are formed by iterative condensation of C_5-isoprene units up to C_{25}. Two C_{15} units condense to form C_{30} compounds (cf. Fig. 5.15). "Sesqui" (Latin) means "one half more." Since monoterpene contains C_{10}, the next elongated chain will contain C_{15} (C_{10}+C_5)—thus the carbon content is one-half more compared to monoterpene and hence the prefix *sesqui* is used for C_{15}-terpenoids.

7.2 Acyclic Sesquiterpenoids: Biosynthesis

All sesquiterpenes get their skeletal carbons from *trans*, *trans*-farnesyl pyrophosphate (FPP). Thus, FPP serves as the fundamental molecule in the biosynthesis of sesquiterpenes. Like geranyl pyrophosphate (GPP, Sect. 6.1), the terminal (2–3) double bond undergoes $E \rightarrow Z$ isomerization, i.e., *trans*, *trans*-FPP (*E*, *E*) to *cis*, *trans*-FPP (*Z*, *E*) through successive ionization, conversion of 1°-carbonium ion to 3°-carbonium ion, rotation of the generated single bond, and delivery of the PP group to the carbocation (Fig. 7.1). *trans*, *trans*-FPP and *cis-trans*-FPP may also be expressed as FPP and *cis*-FPP, respectively.

Acyclic Sesquiterpene Hydrocarbons Acyclic sesquiterpene hydrocarbons, e.g., β-*farnesene*, *cis*-α-*farnesene*, and *trans*-α-*farnesene* are known to occur in nature. Their formation may be explained from the farnesyl carbocation (Fig. 7.2).

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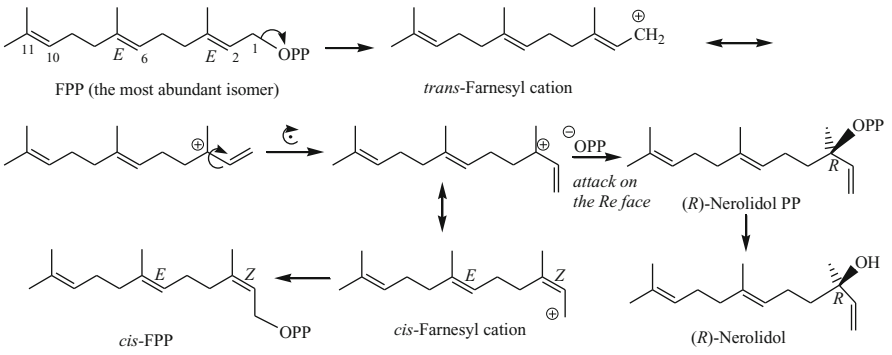


Fig. 7.1 Isomerization of FPP (*E*, *E*) to *cis*-FPP (*Z*, *E*) and formation of nerolidol

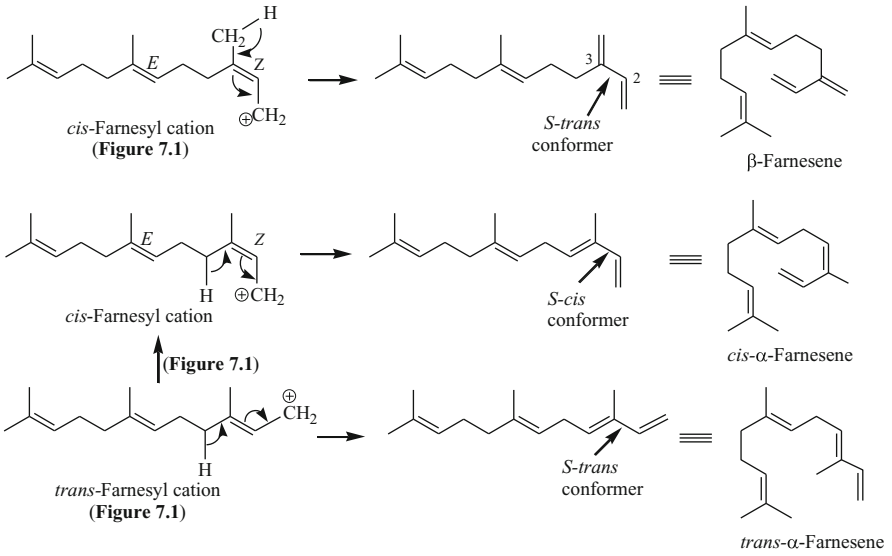
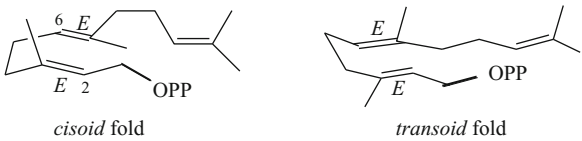


Fig. 7.2 Formation of β-farnesene, *cis*-α-farnesene and *trans*-α-farnesene

7.3 Cyclic Sesquiterpenoids

7.3.1 Biosynthesis. General Mechanistic Approach

Farnesyl pyrophosphate (FPP), being longer in chain length and richer in double bond content compared to geranyl pyrophosphate (GPP), can participate comparatively much more in c*hemical origami* resulting into the formation of more diversified cyclic (mono-, di-, tri-, and rarely tetra-) terpenoids including fused



cisoid fold *transoid* fold

Fig. 7.3 *Cisoid* and *transoid* folds of FPP

ring systems, bridge-headed ring systems, cyclopropane, and cyclobutane systems, etc. More than 200 different types of cyclic sesquiterpenes are reported in the literature. Their formation could be conceived from FPP or its isomer *cis*-FPP via their cations involving *transoid* or *cisoid* folding (Fig. 7.3). The basic chemical reactions involved during the biosynthesis of these skeletally diversified molecules are summarized in the sequel.

All sesquiterpenes are having a common progenitor, (*E*, *E*)-farnesyl pyrophosphate (FPP). *E* → *Z* isomerization of C_2–C_3 double bond of *trans*-FPP (Fig. 7.1) is needed to generate the corresponding *cis*-farnesyl carbocation (Fig. 7.1). The *trans* carbocation reacts with the distal C10–C11 double bond prior to downstream π-facial interactions leading to the products, while *cis* farnesyl carbocation can react with either C6–C7 or C10–C11 double bond depending on the nature of folding by the relevant enzyme, prior to further transformation. Generally two different classes of *cyclases* are known—one (*transoid synthase*) is required for *trans*-farnesyl carbocation generation, while the other one (*cisoid synthase*) for *cis*-farnesyl carbocation formation—the initial requirements for subsequent conversions. Two types of fold (*cisoid* and *transoid*) of *trans*-farnesyl pyrophosphate (Fig. 7.3) may also be involved with the relevant enzymes.

The stereochemical information of the processes and hence those of the products will be dictated by a limited number of the *conformational preorganizations* of FPP and their π-cation interactions with π-facial selectivity (whenever applicable). Enzymic manipulation of various biochemical processes, e.g., hydroxylation, oxidation to various stages (e.g., CH_3 → CH_2OH → CHO → COOH), peroxidation, dehydrogenation, reduction, lactonization, epoxidation, bond migration, etc., with total regio- and stereoselectivity involving pertinent enzymes gives rise to a plethora of diversified skeletal patterns in sesquiterpenes. Some examples are cited in Fig. 7.4.

7.3.2 Classification. Some Familiar Skeletal Patterns

Different terpene synthases recognize the compatible preorganized conformations of the cations from FPP or *cis*-FPP and direct them to different skeletal patterns. The classification of cyclic sesquiterpenes into some fully saturated skeletal patterns and their numberings are shown in the respective sections and figures given in the sequel.

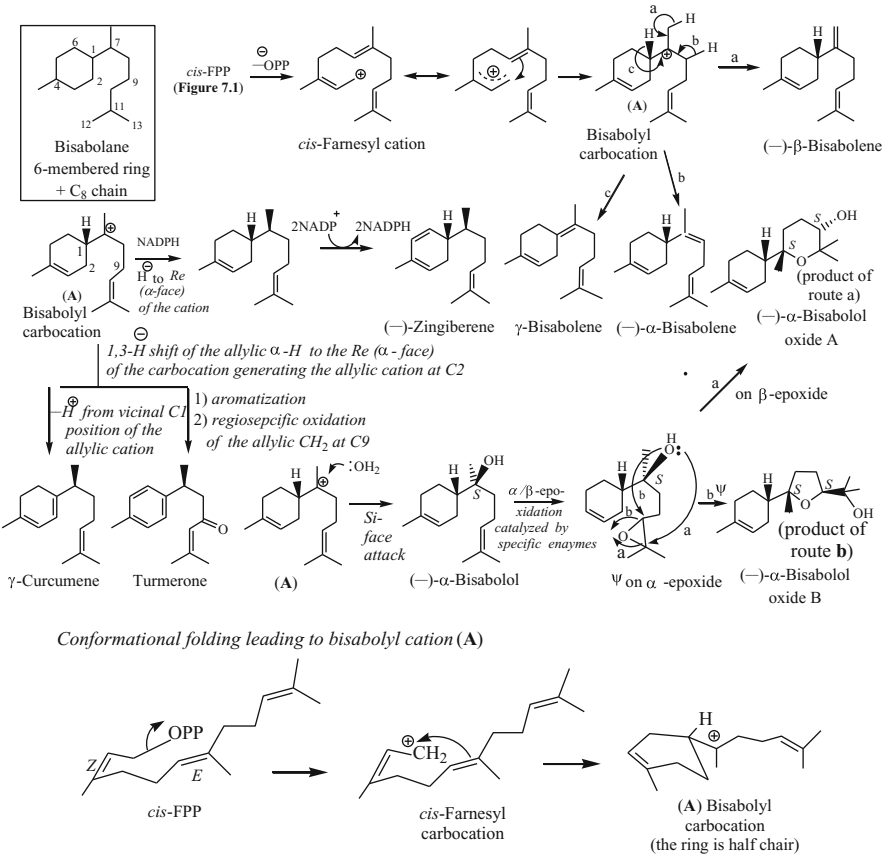


Fig. 7.4 Monocyclic sesquiterpenes derived from bisabolyl cation (**A**)

7.3.3 Monocyclic Sesquiterpenoids. Different Skeletal Patterns. Biosynthesis

Bisabolane Skeleton The plausible pathways for the biogenetic formation of some monocyclic sesquiterpenes originating from bisabolyl carbocation (**A**) are delineated in Fig. 7.4. The stereochemistry of the asymmetric centers of chiral molecules depends on the enzyme stereospecificity affecting the elimination of a particular prochiral hydrogen, or the hydride delivery on the available enantiotopic face of the olefin or carbocation.

Germacrane, Humulane, and Elemane Skeletons The probable pathways for the biosynthesis of monocyclic sesquiterpenoids possessing these skeletons are delineated in Figs. 7.5, 7.6 and 7.7 respectively.

A sesquiterpene of Humulane Skeleton. Humulene (see Sect. 7.5 also).

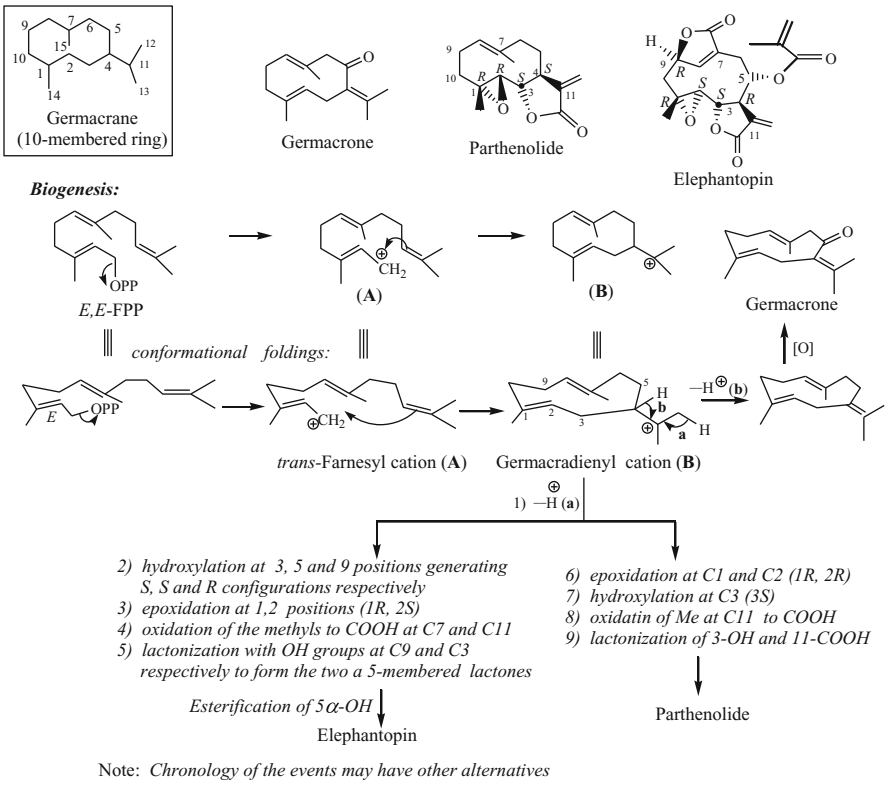
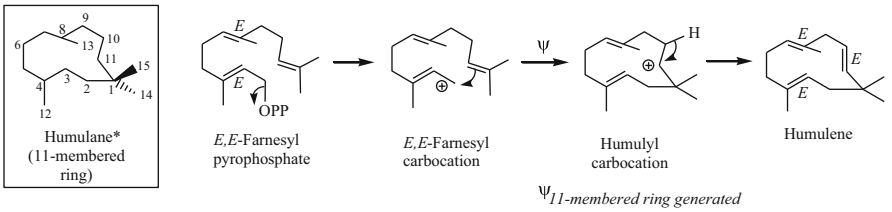


Fig. 7.5 Biosynthesis of some germacrane type monocyclic sesquiterpenes



*In this numbering system the Me groups will be bonded to carbons with minimum numbers. Its name will thus be 1,1,4,8-tetramethylcyclounedecane. In any other type of nomenclature the four methyls will be bonded to carbons with higher numbers.

Fig. 7.6 Biogenesis of humulene

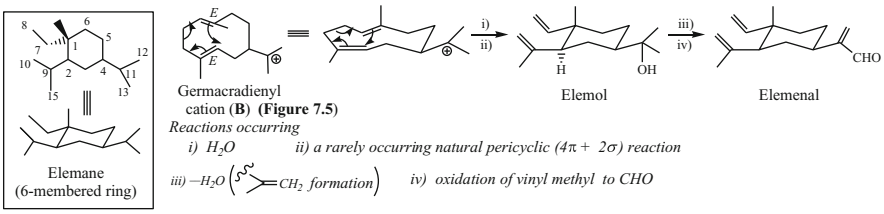


Fig. 7.7 Biogenesis of elemane type monocyclic sesquiterpenes

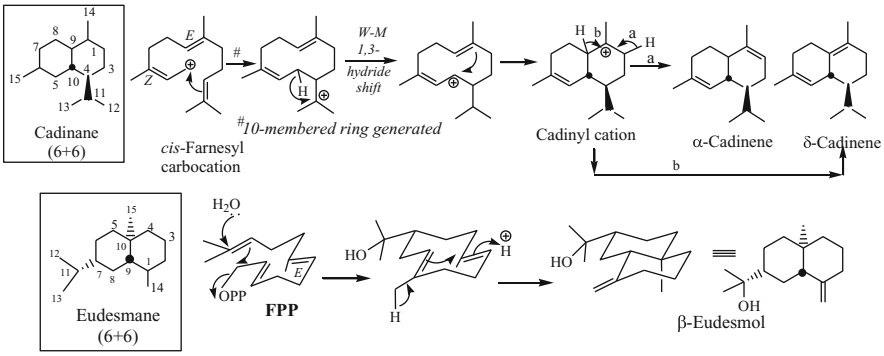
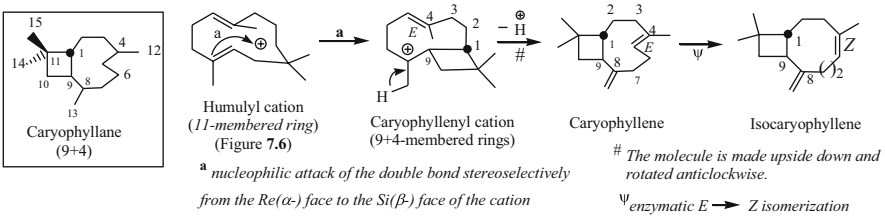


Fig. 7.8 Biosynthesis of α-cadinene, δ-cadinene, and β-eudesmol



Note *Humulene co-occurs with caryophyllene in clove oil, hence humulyl cation is supposed to be the precursor of both.*
A dot • represents a β–hydrogen at that position.

Fig. 7.9 Biosynthesis of caryophyllene and isocaryophyllene

7.3.4 Bicyclic Sesquiterpenoids

The probable biosynthetic pathways of several bicyclic sesquiterpenoids possessing different skeletons, e.g., α-*cadinene*, δ-*cadinene*, and β-*eudesmol* (Fig. 7.8), *caryophyllene* and *isocaryophyllene* (Fig. 7.9), *guaiol* and *bulnesol* (Fig. 7.10), and *carotol* (Fig. 7.11) have been devised, and are delineated in the respective figures. The basic skeletons are shown in the box in each figure.

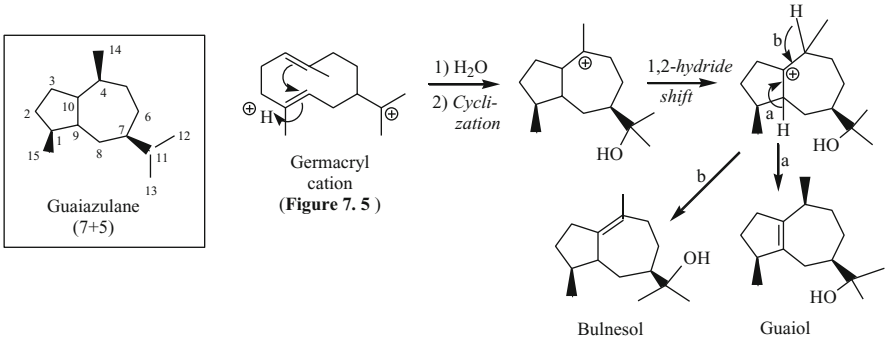


Fig. 7.10 Biosynthesis of guaiol and bulnesol

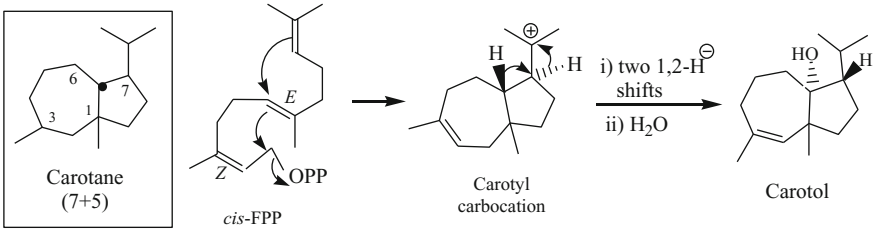


Fig. 7.11 Biosynthesis of Carotol

7.3.5 Tricyclic Sesquiterpenoids

The proposed biosynthetic sequences of sesquiterpenoids having logifolane skeleton, e.g., the longifolene enantiomers with known absolute configurations, are delineated in Fig. 7.12. Arigoni studied the biosynthesis of (+)-longifolene and achieved reasonable incorporations of activity (0.1–0.2 %) from radiolabeled 2-^{14}C-mevalonate into (+)-longifolene (Fig. 7.12), using cuttings of a young *Pinus ponderosa* tree. Applying suitable degradative methods he was able to locate the labels (Fig. 7.12). He proposed 1,3-hydride shift of the ***pro-R*** hydrogen at C5 of mevalonate in the humulyl cation (**A**) based on labeling experiment to form a more stable allylic carbocation, followed by two successive electrophilic attacks of the carbocations on the proximate double bonds, 1,2-migration (*W–M* type) of a proximate C–C bond to generate the carbocation (**B**). The specific enzyme-catalyzed pathways **a** or **b** is then followed to generate (+)-longifolene or (–)-longifolene. respectively. (–)-Longifolene may also be biosynthesized from the mirror image folding of the cation (**A**).

Another type of tricyclic sesquiterpene skeleton, cedrane, is known. The proposed biosynthetic sequences to the known sesquiterpenes α-*cedrene*, β-*cedrene*, *cedrol*, and *epi-cedrol* having cedrane skeleton are shown in Fig. 7.13. A densely

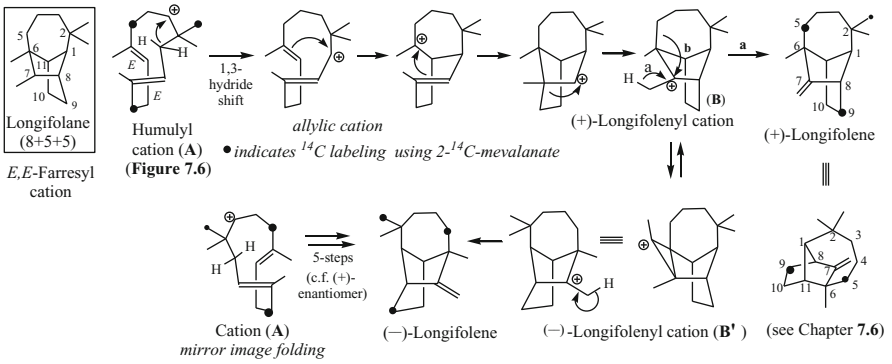


Fig. 7.12 Mechanism of the stepwise deep-seated rearrangements in the biosynthesis of (+)-longifolene and (–)-longifolene

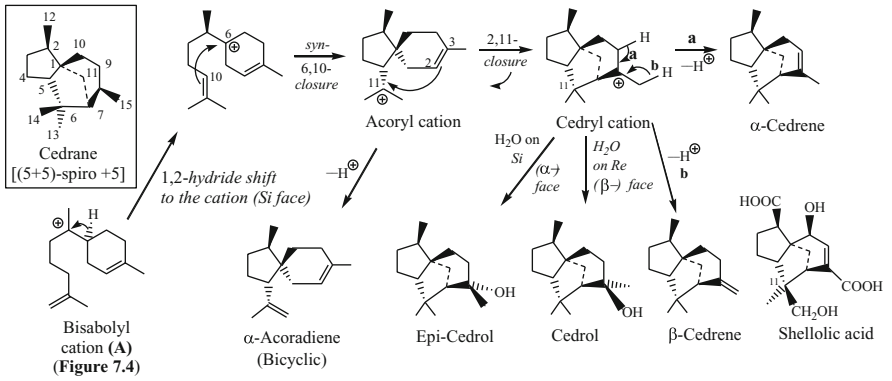


Fig. 7.13 Biosynthesis of α-cedrene, β-cedrene, cedrol, epi-cedrol, and α-acoradiene

oxygenated tricyclic sesquiterpene *shellolic acid* is formed by oxidation of the methyls to COOH or CH_2OH, formation of a double bond, and oxidation of the allylic methylene to CHOH at the positions of the cedrane skeleton as shown in Fig. 7.13 by relevant enzymes. A spiro-bicyclic sesquiterpene *α-acoradiene is formed by* the collapse of the intermediate acoryl cation by loss of a proton from one of the geminal methyls (Fig. 7.13).

7.3.6 Tetracyclic Sesquiterpenoid

One example of tetracyclic sesquiterpene is *longicyclene*, derived from the longifolenyl cation (**B**) (Fig. 7.12, as shown in Fig. 7.14. Longicyclene, being devoid of any functional group or double bond, serves as the skeletal hydrocarbon for such tetracyclic sesquiterpenes.

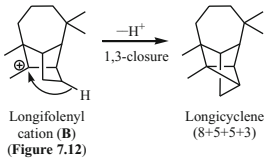


Fig. 7.14 Biosynthesis of longicycline

7.4 Farnesol, The Parent Acyclic Sesquiterpene Alcohol

7.4.1 Introduction and Structure

Farnesol, a colorless water immiscible liquid, occurs in the oil of ambrette seeds. Abelmosk, a tropical plant (*Abelmoschus moschatus*, Fam. Malvaceae), is cultivated for musky seeds (Ambrette seeds) [1]. It also occurs in many essential oils, e.g., oils of *Acacia*, *Pluchea*, *Dioscoridis*, *Pitto-sporum undulatum*, and Zea Mays, possibly protecting these plants from damages induced by parasites. It is extracted from oils of rose, cyclamen (any bulbous plant belonging to the genus *Cyclamen* and primrose family with fleshy root-stock and purple, pink, and cream rose colored early blooming flowers), etc., and from the flowers of *Cassiana* species and also of *Acacia fernesiana*. The latter has been named after a noble Italian Cardinal Odoardo Farnese (1573–1626). His family was responsible for maintaining in Rome one of the first few European botanical gardens. The plant native to Caribbean and Central America was brought to the Italian Farnese Botanical Gardens and cultivated. The compound received its name from its plant source. Farnesol possesses a mild odor of flower.

The structure has been determined by Kerschbaum in 1913. Some relevant reactions pertaining to its structure are shown in Fig. 7.15.

The above reactions are consistent with the structure (**1**) of farnesol. Finally, the structure has been confirmed by its synthesis. It is the building block of possibly all sesquiterpenoids.

7.4.2 Synthesis

(i) Farnesol has been synthesized from geranyl acetate (see Fig. 6.22).
(ii) Ruzicka [2] converted nerolidol to farnesol—a case of rotation around 2,3 single bond, followed by allylic rearrangement (Fig. 7.16).
(iii) Corey synthesized farnesol from geranyl acetone in 1967 [3] (see Fig. 6.25 of Sect. 6.2).

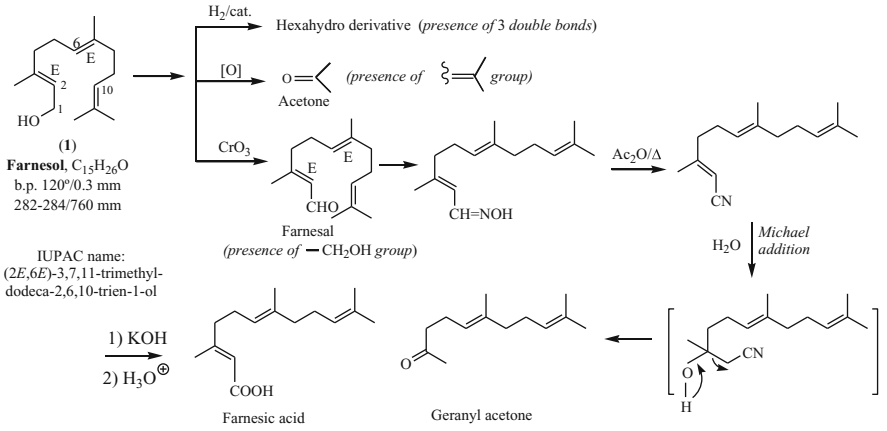


Fig. 7.15 Some reactions of farnesol

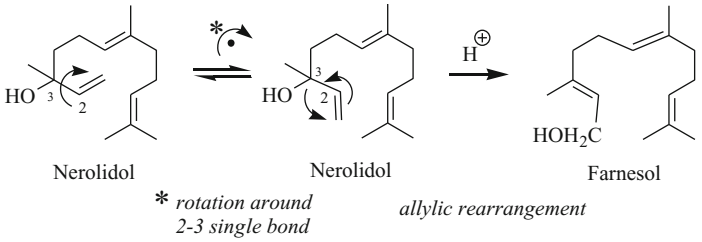


Fig. 7.16 Conversion of nerolidol to farnesol [2]

7.4.3 Biosynthesis

Farnesol pyrophosphate has been biosynthesized from one molecule of DMAPP and two molecules of IPP (Chap. 5). It undergoes hydrolysis to furnish farnesol.

7.4.4 Uses

Farnesol is a potent antimicrobial reagent. It possesses a mild sweet odor of flower and shows antibacterial properties and thus finds use in perfume industry. It helps in keeping the skin smooth and wrinkle free and in increasing skin's elasticity. It is also thought to reduce skin aging by promoting regeneration of cells and synthesis of molecules like collagen, required for healthy skin. It is, therefore, used in body lotion, shampoo, soap, etc.

7.5 Caryophyllene and Isocaryophyllene

7.5.1 Introduction

Clove oil (clove tree, *Eugenia caryophyllata, syn. Syzygium aromaticum*, Fam. Myrtaceae) serves as the major source for **caryophyllene** (**1**), in which it co-occurs with **isocaryophyllene** (**2**) (Fig. 7.18), its biogenetic relative **humulene** (**1a**) (Fig. 7.17), and few other related sesquiterpenoids. Caryophyllene has been known since 1834, although the material obtained in the initial studies on clove oil was a mixture of (**1**), (**2**), and (**1a**). It was obtained in pure form in 1892. Since then extensive degradative and structural studies continued. During the period 1945 to mid-1950s chemical work was done to establish its structure. Barton has been the major contributor to this field [6–9], and Corey accomplished its first total synthesis in 1963 in the racemate form [10, 11]. It has been shown to possess a bicyclo[7.2.0] undecane skeleton. Its medium-sized nine-membered ring being capable of undergoing several conformational equilibriums participates in cyclizations and rearrangements forming a large number of stereochemically interesting molecules, many of which are natural products. Formation of these natural products throws light on their probable biogenesis from caryophyllene in the plant cells.

7.5.2 Structure and Absolute Configuration

The structure of caryophyllene having a four-membered ring fused with a nine-membered ring has been deduced from the identification of its different degradation products (Fig. 7.17).

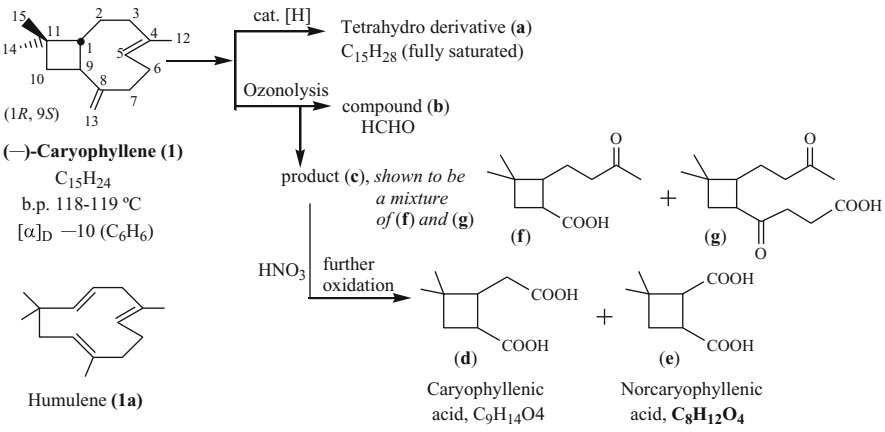
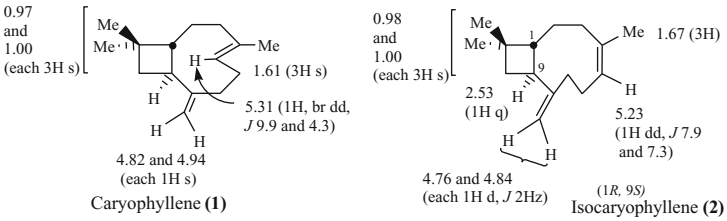


Fig. 7.17 Some degradation products of caryophyllene (**1**)

Formation of (**a**) suggests the presence of two double bonds; its hydrogen content when compared with that of the corresponding open chain saturated hydrocarbon suggests it to be a *bicyclic* system. Compound (**b**) (HCHO) comes from an exocyclic methylene group. Compounds (**d**) and (**e**) show the presence of a cyclobutane derivative carrying a geminal dimethyl group. The positive haloform tests of the compounds (**f**) and (**g**) require the presence of a $–COCH_3$ group in their molecules and of the –C(Me)=CH– system in the parent molecule (**1**). The location of the –C(Me)=CH– system in (**1**) follows from the structures of (**f**) and (**g**). By a consideration of the molecular rotations of some tricyclic derivatives of known structure and stereochemistry (derived from caryophyllene), the absolute configuration of the natural (–)-caryophyllene has been established by Barton [7]. Finally, the X-ray analysis of *caryophyllene chlorohydrin* [12] showed the presence of a *trans*-fused cyclobutane ring in a bicyclo[7.2.0]undecane skeleton, a *trans*-endocyclic double bond carrying a vinyl methyl group in a nine-membered ring, and an exocyclic methylene group and confirmed the structure of (–)-caryophyllene as (**1**) with (1*R*, 9*S*) configurations. The absolute configurations of some secondary alcohols derived from caryophyllene have been determined in 1966 by Horeau [8] by the application of his "partial resolution" method reported [9] in 1961. The results confirm the assignment of configuration of (**1**) made earlier [7].

7.5.3 ^{1}H NMR Spectral Data of Caryophyllene and Isocaryophyllene (Fig. 7.18)



MS of (**1**) (EI, 70 eV, MS 9) [25]: *m/z* (% Base peak), 205 (M^++1, 0.13), 204 (M^+, 0 .78), 161 (3.5), 150 (6.8), 149 (3.4), 148 (3.3),133 (14.7), 121 (5.1), 119 (8.8), 107 (10.6), 105 (21.4), 94 (25.1), 92 (40.2), 79 (40.9), 77 (28.0), **41 (100),** 40 (10.1), 39 (33.9).

Fig. 7.18 ^{1}H NMR [4] and Mass spectral [5] data of caryophyllene and isocaryophyllene

7.5.4 *Synthesis of (±)-Caryophyllene*

7.5.4.1 Corey's Synthesis

The first elegant total syntheses of (±)-caryophyllene (**1**) and (±)-isocaryophyllene (**2**) have been achieved by Corey and coworkers [10, 11] in 1963. The structure (**1**) represents the absolute configuration of (–)-caryophyllene, the natural enantiomer. The synthesis to be described here is not an asymmetric synthesis, and the final product is (±)-caryophyllene, i.e., racemic. For convenience, however, the structures will be drawn with the stereochemistry that will lead to (–)-caryophyllene. *At each step formation of the mirror image twin in equal amount giving the racemate is implied.*

During the chemical construction of the molecule, its following two structural features were taken care of: (i) the *trans*-fused 4-membered and 9-membered carbocyclic rings and (ii) the presence of an endocyclic *E* double bond in the 9-membered carbocyclic ring.

The endocyclic double bond of isocaryophyllene (**2**) is *Z*.

Though photochemical dimerization has long been known in the literature, Corey first realized the potential of this method of intermolecular addition with different olefinic substrates towards the synthesis of natural products. He put his imagination to test and successfully built the cyclobutane ring via [2+2] photocycloaddition between isobutene and cyclohex-2-enone in the desired orientation. This addition serves as the first step of the total synthesis of (±)-caryophyllene (**1**) as well as (±)-isocaryophyllene (**2**).

Two unsymmetrical alkenes can undergo [2+2] photocycloaddition to form the following possible regiochemical isomers and also stereoisomers (Fig. 7.19).

When cyclohexenone is irradiated in the presence of isobutene with UV at a longer wave length, >300 nm, the radiation will be absorbed by cyclohexenone only, while isobutene will remain in the ground state. The enone then attains a triplet state by intersystem crossing from n–π^* excited singlet and gets polarized; the charge distribution of the enone becomes opposite to that of its ground state; consequently the β-carbon acquires δ-charge as shown in Fig. 7.20; hence, normal HOMO/LUMO interaction does not take place, and (**3a**) + (**3b**), and not (**4a**) + (**4b**), are formed.

The triplet state of the polarized enone then interacts with isobutene which is also polarized in the ground state and forms an exciplex or a charge transfer complex. Excited enone acts as the electron acceptor and electron-rich isobutene

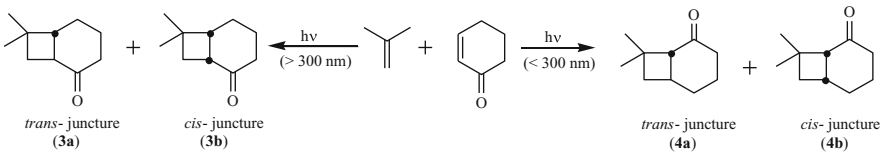


Fig. 7.19 [2+2]Photocycloaddition of isobutene and cyclohex-2-enone

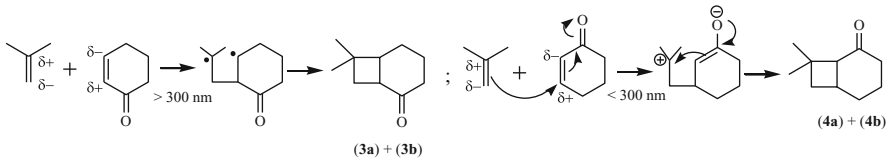
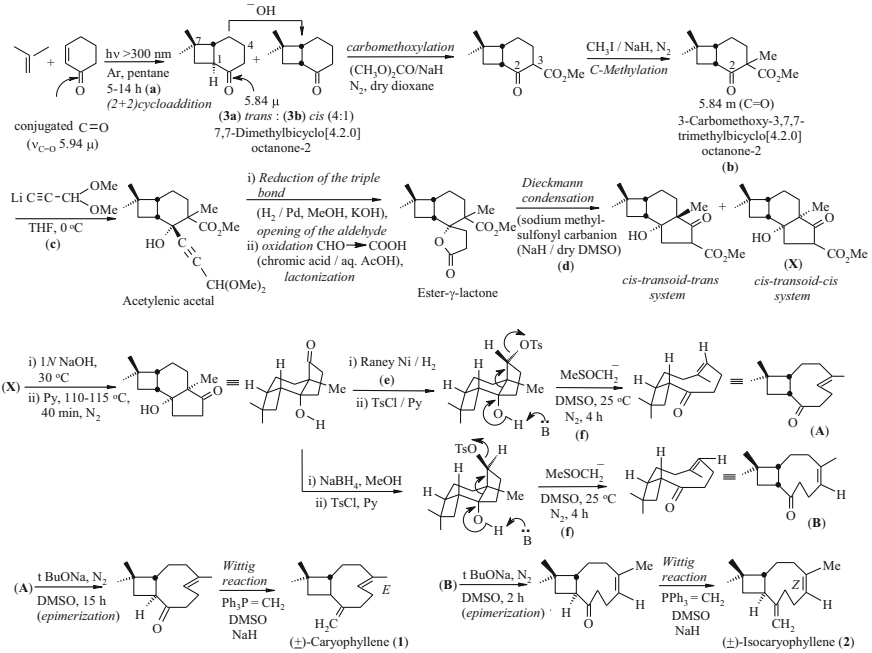


Fig. 7.20 Stereoisomers and regioisomers formed by [2+2] photocycloaddition



*(**a**) Depending on the amount of the reactants, (**b**) Diastereoisomers are formed, the stereochemistry of the C3 chiral center is not important – as it is lost later. (**c**) Nucelophilic addition at the CO from the convex side (the side of the synclinal system cis to the hydrogen of the ring juncture). (**d**) The lactone ring opens up and probably gets esterified under the reaction condition before the Dieckmann condensation. (**e**) Reduction gives epimeric alcohols almost in equal amount; the desired alcohol is separated and tosylated. (**f**) Relative stereochemistry (cis)of the 6/5-ring juncture is of no consequence in the subsequent elimination effecting the rupture of the common bond between 6/5-membered rings. The relative stereochemistry of the angular methyl and the leaving group (OTs) confers the stereochemistry of the endocyclic double bond.*

Fig. 7.21 Total Synthesis of (±)-caryophyllene and (±)-isocaryophyllene [10]

as electron donor. Finally, the nucleophilic end of isobutene attacks the electrophilic C2 of enone and then via the 1,4-diradical intermediate *cis* and *trans* isomers (**3b**) and (**3a**) are formed. The regioselectivity may be controlled by the rate of conversion of exciplex to the product. Head-to-tail regioisomers like (**3a**) and (**3b**) are the major products when electron-rich alkenes are used. In mild alkaline condition (**3a**) is converted to (**3b**).

The multistep syntheses of (±)-caryophyllene/(±)-isocaryophyllene are delineated in Fig. 7.21.

The unusual structures of caryophyllene (**1**) and isocaryophyllene (**2**), possessing fused 4- and 9-membered rings, have attracted the attention of chemists all over the world. Till 1995 five more syntheses have been reported [4, 13–18], of which we include here only two interesting syntheses.

7.5.4.2 Devaprabhakara's Synthesis

A new and improved 7-step procedure for the synthesis by Devaprabhakara et al. [14] of (±)-socaryophyllene, starting from the easily available synthon *cis–cis*-1,5-cyclooctadiene [18], is illustrated in Fig. 7.22.

7.5.4.3 Suginome's Synthesis

The newer synthesis based on a three-carbon ring expansion involving β-scission of alkoxy radicals as the key step, devised by Suginome et al. [4], is delineated in Fig. 7.23.

7.5.5 Rearrangements of Caryophyllene

The flexibility of the medium size 9-membered ring confers conformational mobility to caryophyllene. The various acid-catalyzed rearranged products of caryophyllene are competitive in their formation and are controlled by the conformation of the 9-membered ring as well as reaction condition. Two general features emerging from various rearrangements and cyclization products are the following: (i) The endocyclic *trans* 4,5 double bond of caryophyllene is more reactive than the

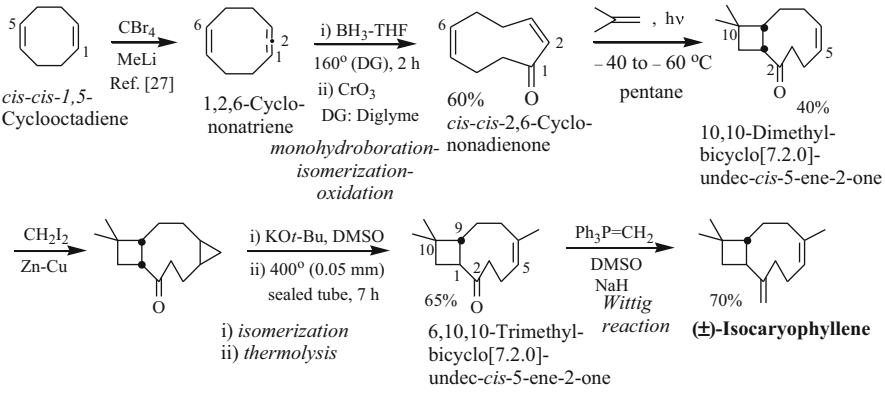


Fig. 7.22 Synthesis of (±)-isocaryophyllene by Devaprabhakara [14]

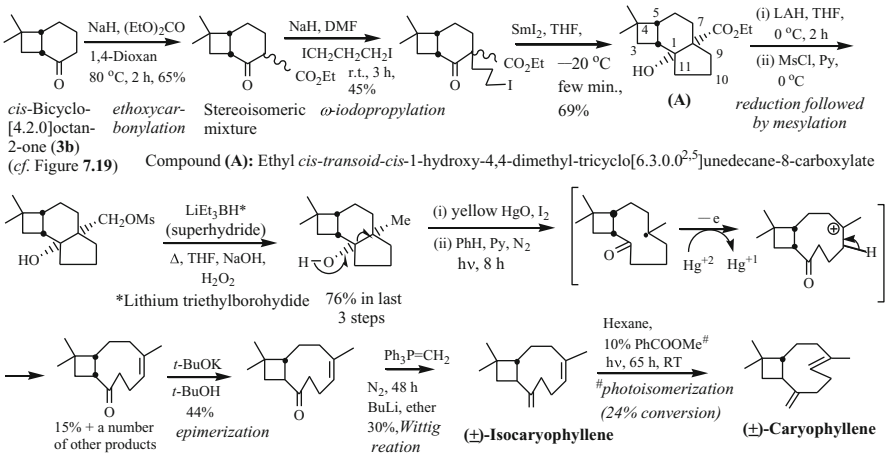


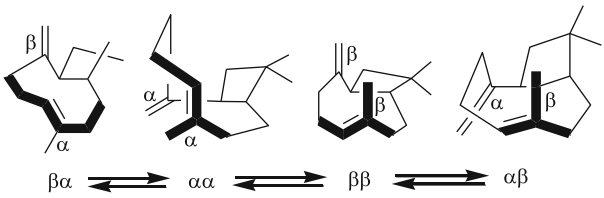
Fig. 7.23 Synthesis of (±)-isocaryophyllene and (±)-caryophyllene [4] (1995)

exocyclic 8(13) double bond. (ii) The system is susceptible to undergo rearrangements and cyclization reactions.

Extensive cyclization product profile analysis formed under manipulative conditions showed the formation of several natural and unnatural sesquiterpenes of much chemical interest, where the C–C bond formation takes place by way of characteristic cation-olefin addition/cyclization and 1,2-migration under strict stereoelectronic requirement providing an access to rare sesquiterpenes in fairly good quantities for their subsequent use, which otherwise would have been difficult to procure.

7.5.5.1 Conformations

Four possible conformations of (–)-caryophyllene, distinguished by the relative disposition of the exocyclic methylene and olefinic methyl groups, are shown by molecular mechanics (MM1) calculations and ^{13}C NMR studies. The predicted and experimentally determined populations of these conformations [19] are shown in Fig. 7.24, which also indicate a low inversion barrier ($\Delta G^1 = 16.25 \pm 0.11$ or 16.1 ± 0.3 kcal mol^{-1}) between the βα- and ββ-conformers. The relative populations of these conformers are reflected to some extent in the ratios of the products of various reactions of caryophyllene, *viz*., epoxidation, hydroboration, and photooxidation, etc. [19] (*Scheme 1, p187of* [19] *is reproduced as Fig*. 7.24 *with the permission of the Royal Society of Chemistry).*



ΔH (kcal Mol^{-1})	12.94	14.78	13.69	16.94
% (MM1)	75.33	3.27	21.22	6.09
% ^{13}C NMR %	75	—	25	—

Fig. 7.24 Relative enthalpies and populations of the (–)-caryophyllene conformers in equilibrium

7.5.5.2 Rearrangements and Cyclizations

An excellent detailed account of recent advances in the chemistry of caryophyllene, including various cyclizations of caryophyllene and isocaryophyllene under different conditions, has been provided by Hanson and his collaborators in the light of their conformational mobility [19].

(i) Caryophyllene upon treatment with 3 equivalents of conc. sulfuric acid–diethyl ether at 0–20 ºC for 30 min furnishes a mixture of as many as 14 hydrocarbons and 4 alcohols. However, on keeping for three days the mixture is simplified to 3 hydrocarbons and 3 alcohols, all of which are tricyclic. The major products are **clove-2-ene** (**6**), **caryan-1-ol** (**7**), and **α-neoclovene** (**9**). Additionally, **clove-2β-ol** (**5**) and **α-panasinsene** (**8**) are also formed in fewer amounts.

It appears to us that the α,α-conformer generates the carbocation (**A**^{2}), which has the correct geometry to cyclize on the lower face of the molecule, followed by expansion of the cyclobutane ring to form (**5**) and (**6**) having the clovane skeleton, as shown in Fig. 7.25. In the aforesaid review [19] clove-2-ene has been shown to be formed from the β,β-conformer, which cannot be rationalized because it lacks the 4-membered ring expansion step (see Scheme **11** of [19]).

(ii) We propose that the β,β-conformer undergoes acid-catalyzed cyclization on the β-face of the molecule and a bridge is formed on the β-face of the 9-membered ring between C13 and C4; no further rearrangement takes place; only the carbocation is hydroxylated from the less hindered α-face to yield caryolan-1-ol (**7**) (Fig. 7.25).

In the same Scheme 11 of the aforesaid review [19] caryolan-1-ol (**7**) has been shown to be formed from the α,α-conformer. But a close inspection of the mechanistic sequence (Fig. 7.26) indicates that the α,α-conformer, as such, is expected to lead to a diastereomer (**7a**) of caryolan-1-ol (**7**) with the bridge formed on the α-face of the 9-membered ring between C13 and C4, followed by hydroxylation of the C8 carbocation from the β-face.

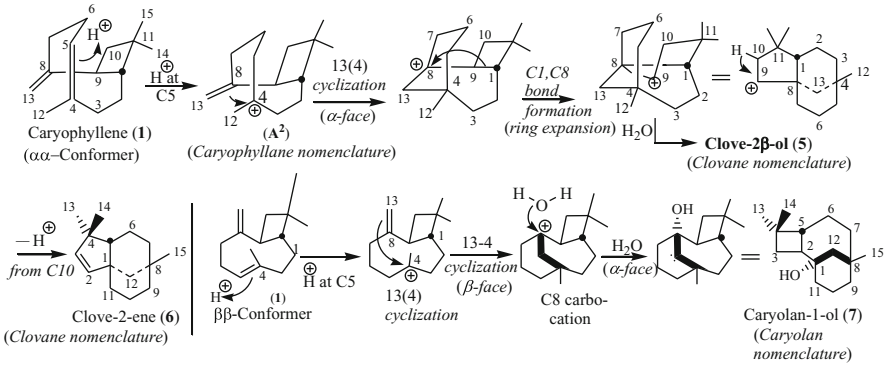


Fig. 7.25 Acid-catalyzed rearrangement of caryophyllene: formation of clove-2-ene, cloven-2β-ol, and caryolan-1-ol

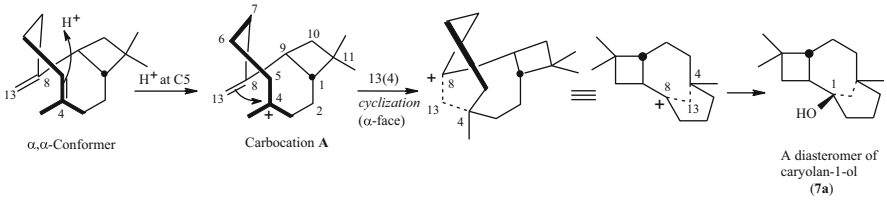


Fig. 7.26 Expected formation of the diastereomer (**7a**) of caryolan-1-ol from the α,α-conformer

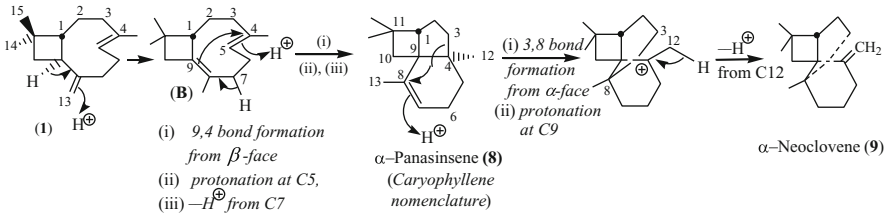


Fig. 7.27 Formation of α-panasinsene (**8**) and α-neoclovene (**9**)

The formation of the double bond isomer (**B**) of *trans*-caryophyllene (**1**) followed by cyclization yields *α-panasinsene* (**8**) (after 30 min acid treatment) which on ring expansion of the five-membered ring forms *α-neoclovene* (**9**), a product formed after 3 days (Fig. 7.27).

Solvolysis of caryophyllene derivatives leading to some tricyclic sesquiterpenes formed in a biomimetic fashion has been reported [20].

7.5.5.3 Thermal Rearrangement of (–)-Caryophyllene to (–)-Isocaryophyllene

Pyrolysis of caryophyllene under low pressure probably causes two successive [3.3] sigmatropic rearrangements leading to the formation of isocaryophyllene [19] (Fig. 7.28).

Though terpenoids are well known for undergoing carbonium ion rearrangements, caryophyllene is regarded as the super-performer on the molecular trapeze in this regard [19].

7.5.6 Conversion of Humulene into Caryophyllene (Fig. 7.29)

The structure of humulene (**1a**) was first reported by Sukh Dev in 1951 and a full paper on humulene was published [19] in 1960. Humulene when treated with *N*-bromosuccimide (NBS) in aqueous acetone gives a mixture of a monobromo hydrocarbon (**B**) and a monobromo alcohol (**C**) (Fig. 7.29). Compound (**C**) is

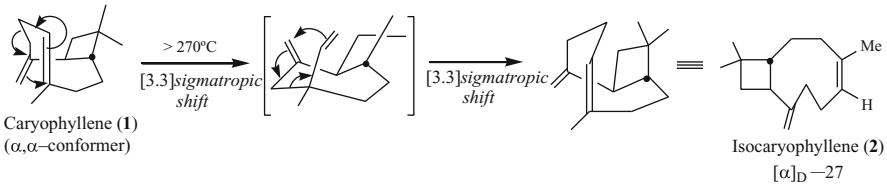


Fig. 7.28 Thermal rearrangement of caryophyllene to isocaryophyllene

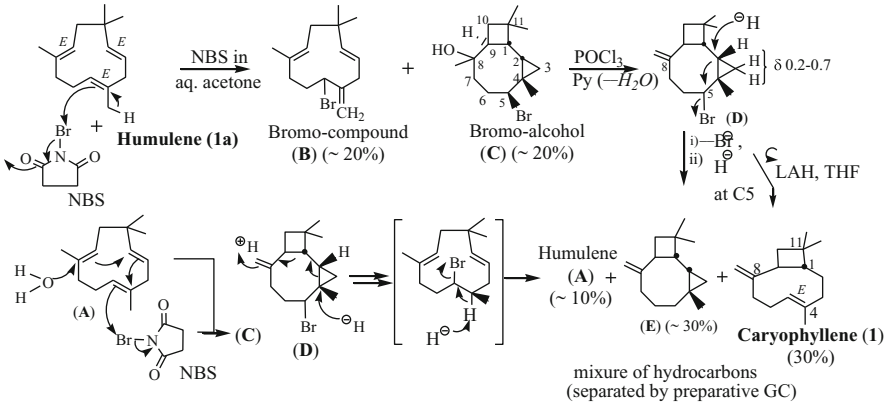


Fig. 7.29 The conversion of humulene (**1a**) into caryophyllene (**1**) [21]

separated and dehydrated with $POCl_3$ and pyridine to give compound (**D**) with a methylene moiety at C8. Compound (**D**) upon treatment with $LiAlH_4$ in THF produces a mixture of hydrocarbons caryophyllene (**1**) (30 %), the tricyclic hydrocarbon (**E**) (30, and a small amount of humulene (A) (~10 %)—isolated by preparative GC [21].

The probable mechanistic rationalizations (i), (ii), and (iii) for the formations of (**1**), (**E**), and (**1a**), respectively, from (**D**) are suggested, as shown in the Fig. 7.29.

(i) Rupture of the cyclopropane (inner bond) *anti* to C-Br in (**D**), expulsion of Br to form *trans* 4-5 double bond (*cf* E2 mechanism), and quenching of the generated C2 cation by $H^{(-)}$ give rise to caryophyllene (**1**).
(ii) Expulsion of Br^- from C5, followed by quenching of the C5 cation by $H^{(-)}$, leads to the formation of the hydrocarbon (**E**).
(iii) Ruptures of the cyclobutane (allylic) and cyclopropane inner bonds in the cyclooctane ring of (**D**), aided by the saturation of the methylene group and attack of a hydride at the generated C4 carbocation, followed by elimination of HBr (E2 type), generate humulene (**1a**) with three ***E*** double bonds.

7.5.7 Apollan-11-ol: An Interesting Acid-Catalyzed Product of Humulene

Apollan-11-ol [22, 23], an acid-catalyzed (H_2SO_4/ether) optically inactive rearranged product from commercial caryophyllene, was reported in 1922 by Asahina and Tsukamoto who temporarily tagged it as "α-caryophyllene alcohol." The structure of this compound, established by Nickon [22], turned out to be a structurally very interesting symmetrical alcohol ($\mathbf{C_s}$, plane of symmetry) and hence optically inactive. Though it was initially thought to be a product from caryophyllene, later humulene, which co-occurs in commercial caryophyllene, has been proved to be the precursor of this alcohol. This was established unambiguously by labeling experiment and spectral data of the labeled product [22]. In fact, Sukh Dev in 1951 also isolated an alcohol, $C_{15}H_{26}O$, m.p. 116° [24], suggested to be α-caryophyllene alcohol. That it is the skeletal rearranged product of humulene is supported by its formation in a much greater yield from humulene ($\mathbf{A^1}$), as observed by Parker and Roberts [23]. The X-ray crystallographic studies [23] also revealed its structure (**A**). The naming of this compound, based on some fortuitous coincidental facts, is really amazing. We quote from the extraordinarily interesting book *Organic Chemistry: The Name Game* [25]: "The molecule has appealing symmetry, and a flat drawing of the ring system strikingly resembles a rocket, with side fins and exhaust tail. So Nickon dubbed the parent alkane "apollane" in timing with the Apollo 11 moon landing. By happy coincidence, proper numbering locates the –OH at C11 and thus cemented further the onomastic link with Apollo 11. In fact, Neil Armstrong's personal memorabilia include a reprint of that chemistry publication" [25]. The nomenclature and the systematic name of apollane are given in Fig. 7.30.

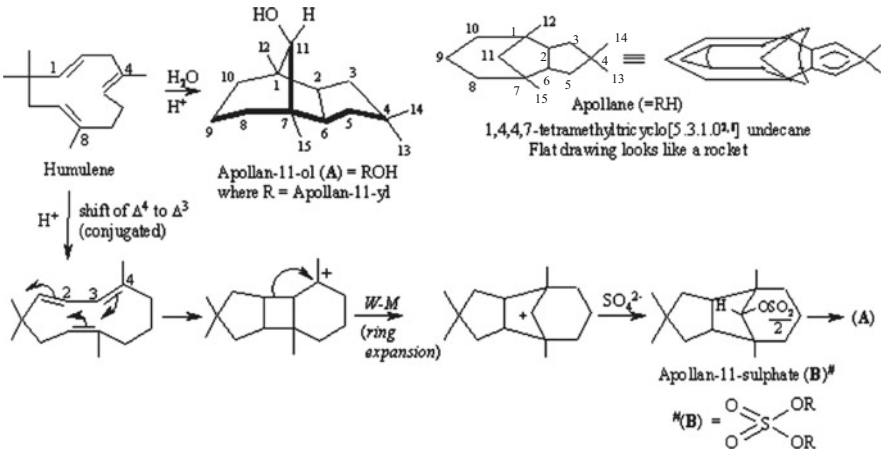


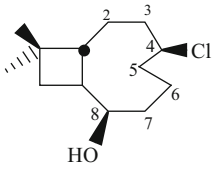
Fig. 7.30 Conversion of humulene (**1a**) into apollan-11-ol (A) via apollan-11-sulfate (**B**) [25]

A reasonable mechanistic explanation of the formation of "Apollan-11-ol" (**A**) from humulene is outlined in Fig. 7.30 [25]. Apollan-11-sulfate (**B**) can be converted to apollan-11-ol and the latter can be reconverted to the sulfate.

7.5.8 Biosynthesis of Caryophyllene

The probable biosynthetic sequences of caryophyllene (Fig. 7.9) and its precursor humulyl carbocation starting from farnesyl pyrophosphate (Fig. 7.6) have already been briefly outlined.

7.5.9 A Caryophyllan-Type Compound in a Sea Coral



Rumphellatin A

Incidentally, it may be mentioned that *rumphellatin* A, the first chlorine-containing caryophyllane-type norsequiterpenoid (=CH_2 at C8 absent), has been isolated from a Formosan soft sea coral *Rumphella antipathies* [26]. Two new hemiketal norsesquiterpenoids of the same skeleton, *rumphellatin* B (having an α-Cl at C3,

a β-OH at C4, and an α-hemiketal linkage involving C5 and C8 with an α-OH at C8) and *rumphellatin* C (having a β-Cl at C3, a β-OH at C4, and a β-hemiketal linkage involving C5 and C8 with a β-OH at C8) have also been reported [27] from the sea coral of the same species.

7.6 Longifolene: A Tricyclic Sesquiterpene

7.6.1 Occurrence

Longifolene (**1**), the major sesquiterpene of Indian turpentine oil, in which it occurs to the extent of 5–10 %, was first isolated by Simonsen in 1920. The turpentine oil was obtained from the oleoresin of *Pinus roxburghii* (syn. *P. longifolia*, Fam. Pinaceae). Longifolene [28–31] widely occurs also in various Pinaceae plants. It is commercially produced in hundred ton quantities. Several sesquiterpenes, e.g., *longipinene,* l*ongicyclene*, *caryophyllene, humulene, bisabolene* ,etc., co-occur with it though as minor constituents. Pure longifolene has $[\alpha]_D$ +54.06 ($CHCl_3$). It is pertinent to inform that though (+)-variety is prevalent in nature and occurs mainly in the higher plants (mainly Gymnospermaceae), the (–)-form co-occurs in small amounts with (−)-sativene in *Heilanthus sativum* and *H. victoriae*. Longifolene may be looked upon as the ring-expanded *sativene*. The presence of (–)-longifolene in liverwort has also been reported [32].

7.6.2 Structure: Some Reactions of Longifolene

Extensive chemical studies on longifolene have been carried out by Simonsen during 1923–1934, and much structural information was obtained from these studies [33]. However, the complete structure of longifolene (**1**) was determined by Moffett and Rogers [34, 35] from the X-ray analysis of longifolene hydrochloride, m.p. 59–60 °C, a W–M rearranged product [34, 35]. Its formation may be rationalized as shown in Fig. 7.31.

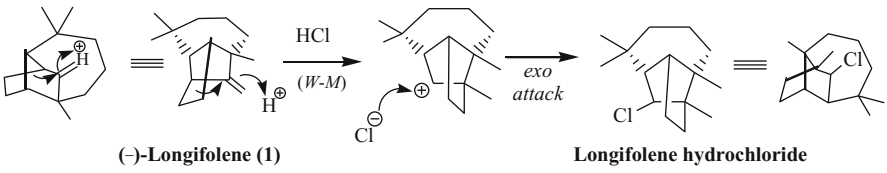


Fig. 7.31 Longifolene (**1**) to longifolene hydrochloride

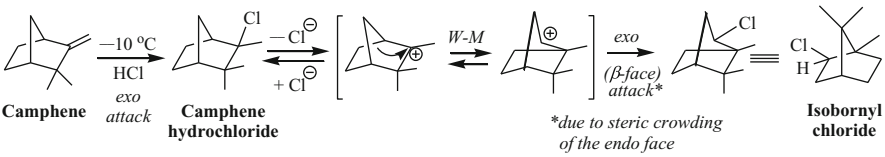


Fig. 7.32 Probable mechanism of conversion of camphene to isobornyl chloride by HCl treatment

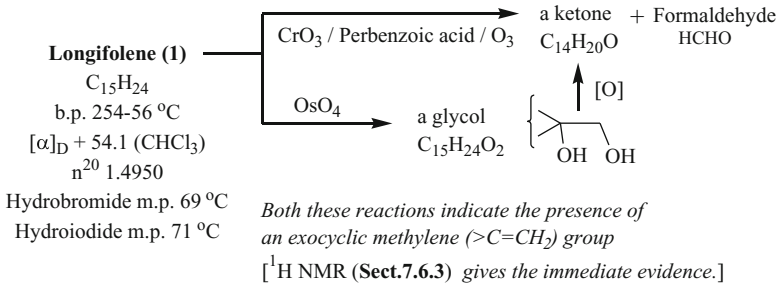
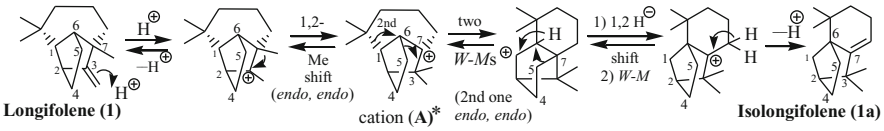


Fig. 7.33 Reactions indicating the presence of an exocyclic methylene group in longifolene (**1**)

This information is reminiscent of the conversion of camphene hydrochloride to isobornyl chloride by treatment with HCl, as shown in Fig. 7.32. The presence of an exocyclic methylene group in longifolene is indicated by the reactions shown in Fig. 7.33.

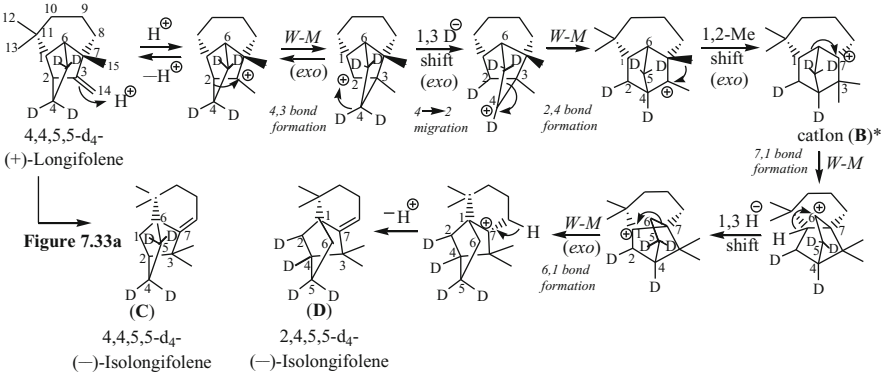
7.6.2.1 Conversion of Longifolene to Isolongifolene

Longifolene, like caryophyllene (**7.5**), is also a *molecular acrobat*, being very much prone to skeletal rearrangements. Several reports of its rearrangements are available in the literature [28–31]. (+)-Longifolene (**1**) on exposure to strong protic or Lewis acid undergoes a deep-seated molecular rearrangement to an isomeric tricyclic hydrocarbon, (−)-isolongifolene (**1a**) through a number of mechanistically explainable steps as shown in Fig. 7.34. However, a better gross mechanism of this stereospecific rearrangement has been elucidated by Sukh Dev, by using site specifically labeled longifolene-4,4,5,5-d_4 (Fig. 7.35) [28] (which was efficiently synthesized) to form (**D**) involving one D shift and thus has been shown to follow the pathway initially proposed by Berson et al. [31]. The less circuitous direct pathway (Fig. 7.34) would have resulted in the formation of the d_4-isolongifolene (**C**) (involving no deuterium shift), instead of (**D**). This pathway involves an *exo, exo* Me shift (Fig. 7.35) [in preference to the less circuitous *endo, endo* Me



* *In the carbocation* (**A**) *charge is located at the bridgehead of a bicyclo[4.2.1]nonane system (upper part, excluding the front two-carbon bridge). To accommodate the planar trigonal carbon in the cation* (**A**) *the bicyclo[2.2.1]heptane system (lower part excluding the upper four-carbon bridge) must be severely distorted, as is evident from a Dreiding model study, and hence the caion* **(A)** *is thermodynamically less stable relative to the caion* **(B)** *of* (**Fig.7.35**) [29]

Fig. 7.34 Earlier proposed pathway for the acid catalyzed conversion of longifolene into isolongifolene

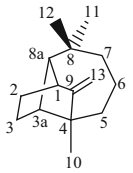


In carbocation* (B**) *charge is located at the bridgehead of a bicyclo[4.3.1]decane system, which as is observed in a Dreiding model study, can easily accommodate the planar trigonal carbon, and hence caion* (**B**) *is thermodynamically more stable than the caion* (**A**) (**Fig. 7.34a**) [29]

Fig. 7.35 Gross mechanism for the acid catalyzed rearrangement of 4,4,5,5-d_4-(+)-longifolene to 2,4,5,5,-d_4-(−)-isolongifolene (**D**) [29]

migration route (Fig. 7.34) proposed earlier], based on the intermediacy of nonclassical carbocation, which is not tenable and acceptable. The premise that an *exo, exo shift* is energetically preferred to an *endo, endo* shift may not be the only reason here. A suitable rationale for the complete preference of the more circuitous route (Fig. 7.35) [29] has been indicated in Figs. 7.34 and 7.35 (see the arguments written against the asterisks given on the ions (**A**) and (**B**)].

7.6.3 Spectral Data of Longifolene



Longifolene (1)

IR: (film): ν_{max} 3,080, 1,655 and 868 cm^{-1} (exo-methylene group)

^{1}H NMR [36] (δ, CCl_4): 0.90 (3H, s, C4 Me, angular), 0.95 (3H, s, *exo* Me, C12), 0.99 (3H, s, *endo* Me, C11), 4.45 and 4.72 (3H, *s*, each, exocyclic methylene protons, H13a and H13b).

^{13}C NMR [36] (δ, $CHCl_3$): 47.89 (C1), 29.71 (C2), 25.48 (C3), 43.93 (C4), 43.33 (C5), 21.14 (C6), 36.39 (C7), 33.55 (C8), 167.59 (C9), 30.04 (C10), 30.51 (C11), 30.50 (C12), 98.93 (C13).

MS (EI, 70 eV, M.S.9) [37]: *m*/*z* (% Base peak), 205 (2.9, P+1), 204 (M^+, 15.0), 189 (20.7) 175 (7.4), 161 (43.4), 148 (7.4), 147 (14.7), 135 (22.3), 134 (11.2), 133 (24.8), 121 (21.8), 120 (16.0), 119 (33.5), 107 (37.4), 106 (16.1), 105 (43), 95 (34.1), 94 (42.4), 93 (43.0), 92 (17.2), 91 (52.7), 81 (23.2), 79 (35.2), 77 (30.1), 69 (22.7), 67 (23.6), 65 (17.5), 55 (43.2), 53 (24.9), 43 (21.6), **41 (100)**, 39 (33.9).

MS (EI, 70 eV, M.S.9) **of Isolongifolene** [37]: *m*/*z* (% Base peak) 205 (5.44, P+1), 204 (M^+, 31.7), 189 (31.0), 175 (61.5), **161 (100)**, 148 (42.7), 147 (17.2), 135 (9.2), 134 (10.0), 133 (41.8), 121 (13.0), 120 (11.8), 119 (38.4), 107 (21.2), 105 (43.4), 95 (13.4), 93 (22.2), 91 (39.6), 81 (11.8), 79 (20.7), 77 (25.4), 69 (16.0), 65 (13.9), 55 (25.8), 53 (21.2), 41 (74.8), 39 (41.8).

Note: The base peaks of longifolene and isolongifolene appear at *m*/*z* 41 and 161, respectively. Their fragmentation patterns are found to be quite similar, varying only in the relative intensities of some peaks.

7.6.4 Synthesis of (±)-Longifolene and (+)-Longifolene by Corey's Group [38]

The strategy for the synthesis of longifolene (Fig. 7.36) was planned by Corey [38] by exhaustive analysis of the topological properties of its carbon network having complex bridged ring system. A suitable bicycle[5.4.0]undecane system (**B**) capable of undergoing *intramolecular Michael addition* (cf. formation of santonic acid from santonin served as the precedent; see Sect. 7.8) under the influence of a base appeared to be the right intermediate to construct the desired tricyclic system with

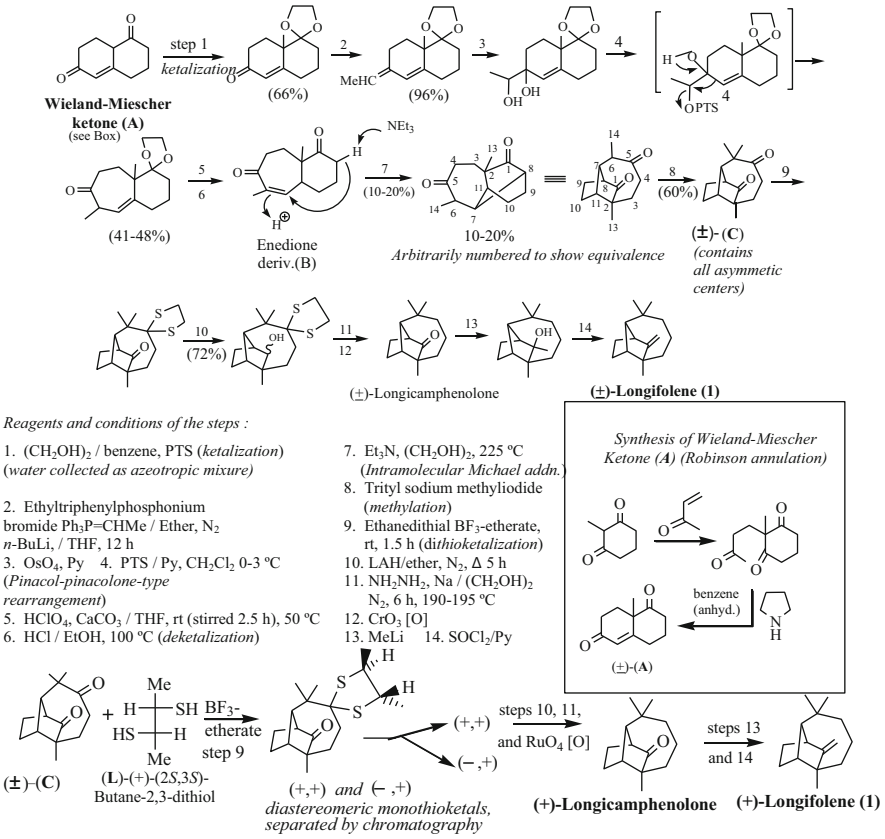


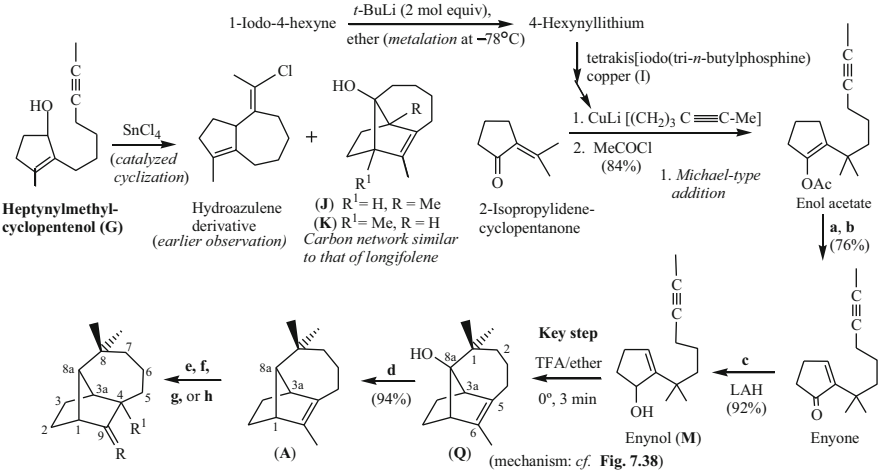
Fig. 7.36 Synthesis of (±)-and (+)-longifolene [38]

adequate functionalities and the substitution pattern of longifolene. A methyl group at the α-position of the α,β-unsaturated ketone system in the bicycle framework (**B**) is advantageous for further methylation in the subsequent step (Fig. 7.36). The Wieland–Miescher ketone (**A**) was chosen as the starting material for the synthesis of the key intermediate bicyclo[5.4.0]undecane system. The synthesis of (±)- and (+)-longifolene is delineated in Fig. 7.36. The optically active natural (+)-longifolene has been synthesized via optically active thioketal derivative using (+)-butane-2,3-dithiol and separating (+)-longicamphenolone, followed by the steps as applied to (±)-longicamphenolone with slight modification (Fig. 7.36) to obtain (+)-longifolene.

7.6.5 *Synthesis of (±)-Longifolene and Some of Its Relatives by Johnson Group [39]*

During the stannic chloride-catalyzed cyclization of heptynylmethylcyclopentenol (**G**), the expected hydroazulene system is formed along with an alcoholic compound (**J**) or (**K**), which contains the carbon network close to that of longifolene. This earlier observation [39] in Johnson's lab directed his attention to develop an appropriately substituted enynol substrate for the formation of the hydroazulene derivative, which could serve as the potential intermediate in the synthesis of longifolene (Fig. 7.37). The novel synthesis of (±)-longifolene (**1**) and (±)-longicamphenylone (**D**) (also occurring naturally) and two other relatives (**B**) and (**C**) is schematically represented in Fig. 7.37, mentioning the reaction conditions and yields [39].

Mechanism of Conversion of (M) to (Q) (Fig. 7.38) The mechanism of cyclization of the enynol (**M**) to form (**Q**) is open to question. However, it was rationalized



Reaction Products from (A) under different conditions:

* (**B**): R^1=H, R=CH_2 (91%)

$^{\Delta}$(**C**): R^1=H, R=O (72%)

$^{\Psi}$(**D**): R^1=Me, R=O, (±)-Longicamphenolone (84%), the (+)-isomer occurs naturally.

$^{\mu}$(**1**): R^1=Me, R=CH_2, (±)-Longifolene (80%) Overall yield (21%)

Note: *Reagents and corresponding products are marked with superscripts*

d *hydrogenolysis of the C-OH bond*

Reaction conditions :

a MeLi (2.1 mol equiv) Et_2O, 0 °C; Br_2 (1 mol equiv)/CH_2Cl_2, -78 °C

b 2,4,6-$(Me)_3$ $C_6H_2CON(Me)_2$ (4 mol equiv) in MeCOMe, 1 h, 25 °C

c LAH (1 mol equiv)/Et_2O, 0 °C 1h

d $ZnBr_2$ (2 mol equiv), $NaBH_3CN$ (3 mol equiv)/Et_2O, 2 h, 25 °C

***e** PTS (small excess),

$^{\Delta}$**f** RuO_4, large excess of H_5IO_6-$NaIO_4$ (per iodate [O]), H_2O-*t*-BuOH, 18 h, 25°

$^{\Psi}$**g** $LiN(iPr)_2$ (10 mole equiv)/THF, MeI (10 mol equiv), -78 °C

$^{\mu}$**h** MeLi, $SOCl_2$/Py

Fig. 7.37 Synthesis of (±)-longifolene (**1**) and some of its relatives [39]

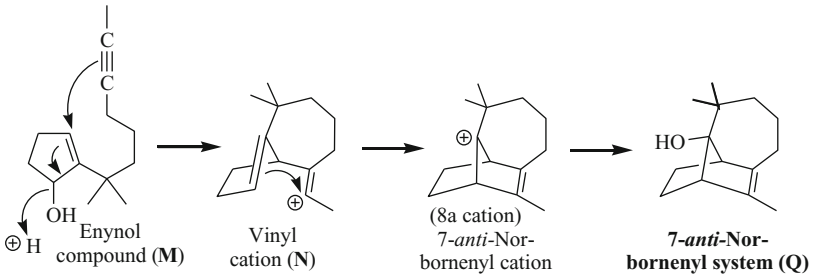


Fig. 7.38 Mechanistic rationale for the conversion of (**M**) to (**Q**) of Fig. 7.37

as follows [39]. The allylic alcohol (**M**) would first cyclize to give the vinyl cation (**N**). Intramolecular nucleophilic attack at the vinyl cation by the olefinic bond in the five-membered ring would result in further cyclization to yield the interesting cation (**8a**), which apparently may be destabilized due to violation of Bredt's rule, but it embodies the potential stabilizing characteristic of the 7-*anti*-norbonenyl cation. Nucleophilic attack of the **8a** cation (Fig. 7.38) by H_2O would yield the norbornenyl system (**Q**).

Other total syntheses of (±)-longifolene and (+)-longifolene have been reported by McMurry [36] and Oppolzer [40].

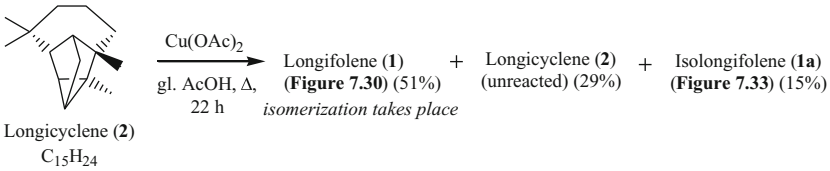
7.6.6 Biosynthesis

Biosynthesis of longifolene [32] has been discussed in Sect. 7.3 (Figs. 7.6 and 7.12).

7.7 Longicyclene: The First Reported Tetracyclic Sesquiterpene

7.7.1 Occurrence and Structure

Longicyclene, $C_{15}H_{24}$, b.p. 82 ºC/2 mm, $[\alpha]_D$+33.6 (neat), the first reported tetracyclic sesquiterpene, has been detected as a minor congener hydrocarbon of longifolene during the vapor phase chromatography (VPC/GLC) of the higher boiling portion of Indian turpentine oil obtained from the oleoresin of *Pinus longifolia* [41, 42]. This observation led to its systematic study of isolation and structure elucidation. The data in Fig. 7.39 and the spectral data for longicylene are consistent with its structure (**2**) which is confirmed by the total stereoselective synthesis of (±)-longicyclene (Fig. 7.41).



***Note**: The constituents of the mixture of the hydrocarbons were identified by GLC*

Fig. 7.39 Interconversions of longicyclene and longifolene

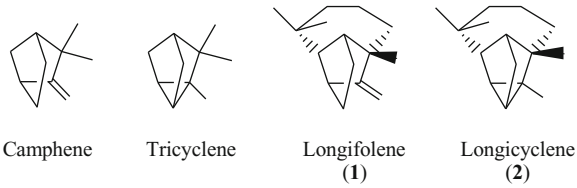


Fig. 7.40 Relationship of longifolene to longicyclene is like camphene to tricyclene

Longicyclene when subjected to catalytic hydrogenation or perbenzoic acid treatment remains unreacted indicating its fully saturated character. With tetranitromethane it develops a faint but distinct color indicating the presence of a cyclopropane ring [44] in its molecule. The chemical shifts of its upfield cyclopropane protons (see Sect. 7.7.2) provide definite evidence of the presence of a cyclopropane ring. The hydrogen content of its molecule, $C_{15}H_{32}$, indicates it to be a tetracyclic. Logicyclene upon prolonged refluxing with glacial acetic acid undergoes isomerization to longifolene and isologifolene (Fig. 7.39).

Interconversions of longifolene and longicyclene support the structure of longicyclene as well as constitute a formal total synthesis of (±)-longicyclene in view of the total synthesis of (±)-longifolene (Figs. 7.36 and 7.37).This new tetracyclic sesquiterpene has been named longicyclene which holds the same relationship to longifolene as tricyclene to camphene (Fig. 7.40) [42].

7.7.2 Spectral Properties [43]

IR: (CCl_4): ν_{max} 3,085 (cyclopropane type C–H stretching), 1,385; 1,370 cm^{-1} (gem-CH_3), a strong peak at 840 cm^{-1} (characteristic of a tricyclene system). In the near IR (CCl_4) it shows band at 1.640 μ, just like the absorption of tricyclene (CCl_4, 1.650 μ) assignable to the first overtone of cyclopropane ring C-H stretching [45].

^{1}H NMR (CCl_4): δ 1.04 (*s*, 3H, CH_2), 0.98 (*s*, 3H, CH_3), 0.92 (*s*, 3H, CH_3), 0.88 (*s*, 3H, CH_3). The ^{1}H NMR contains no olefinic proton; it showed the presence of

four unsplit methyls. This upfield region of methyls integrated for 14H; hence 2H may be attributed to the two upfield cyclopropane protons. In fact, the spectrum also shows a 1H signal at δ 0.77, together with another minor signal at δ 0.67; thus in all probability two cyclopropane protons are indicated [42].

7.7.3 *Synthesis of Longicyclene [43]*

A stereoselective total synthesis of longicyclene (Fig. 7.41) has been achieved in which (–)-carvone has been converted to tetrahydroeucarvone through ring expansion. To get a 2-carboxyethyl side chain α- to the carbonyl, tetrahydroeucarvone is alkylated with 2-chloro-pent-3-ene and then trunked via oxidation. The keto acid is induced to form enol lactone. The latter on reduction with iBuAlH followed by acid rearrangement produces a bicycle[4.2.1]nonan-ketol system. The compound being unstable is immediately esterified with methane sulfonyl chloride and finally eliminates to form a bicyclic nonane compound. Sequential treatment of this compound with usual reagents led to the formation of a bicyclo-olefenic acid which is converted to the tetracyclic cyclopropyl ketone via diazoketone intermediate derived from the acid. The ketone has been reduced with diisobutylaluminium hydride and the stereochemistry of the hydroxyl group as shown in the structure is due to steric approach control—the bulky reagent should approach from the less hindered face of the molecule. Finally the OH group is removed in the conventional way.

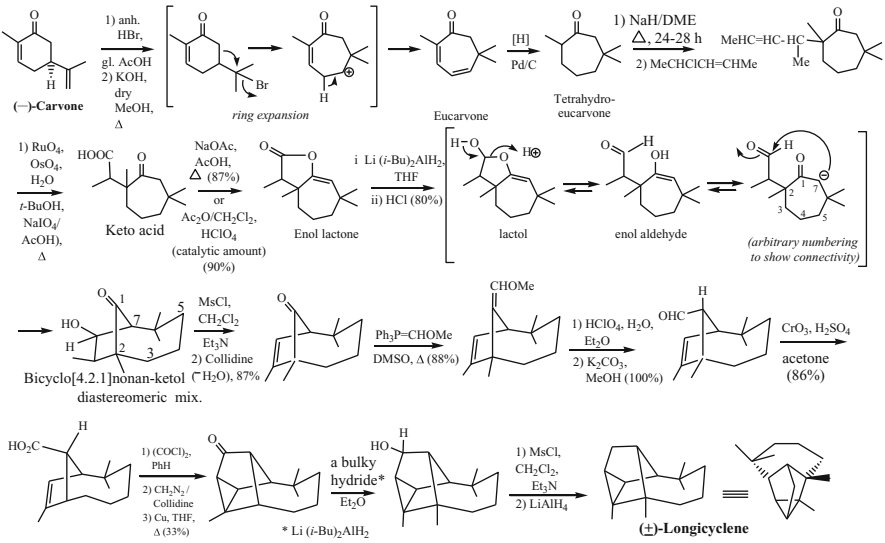


Fig. 7.41 Total synthesis of (±)-longicyclene starting from (−)-carvone [43]

7.7.4 *Biosynthesis of Longicyclene*

This has been discussed earlier along with that of longifolene (see Figs. 7.6, 7.12, and 7.14).

7.8 Santonin

7.8.1 *Occurrence and Structure*

Santonin is the principal anthelmintic component of various *Artemisia* species. It was isolated first by Kahler in 1830 from *Artemisia santonica* (Fam. Compositae/Asteraceae) and later on from various other *Artemisia* species (e.g., *A. maritima*, *A. cinica*, etc.), as well as from other related plants. *A. santonica* belongs to the family of plants known for centuries as "worm seed" [46], as it was useful in the treatment of intestinal worms.

Santonin is a very well-studied sesquiterpene lactone. Its structure (without stereochemistry) has been elucidated from its degradative studies and reactions. Some are presented here (Fig. 7.42).

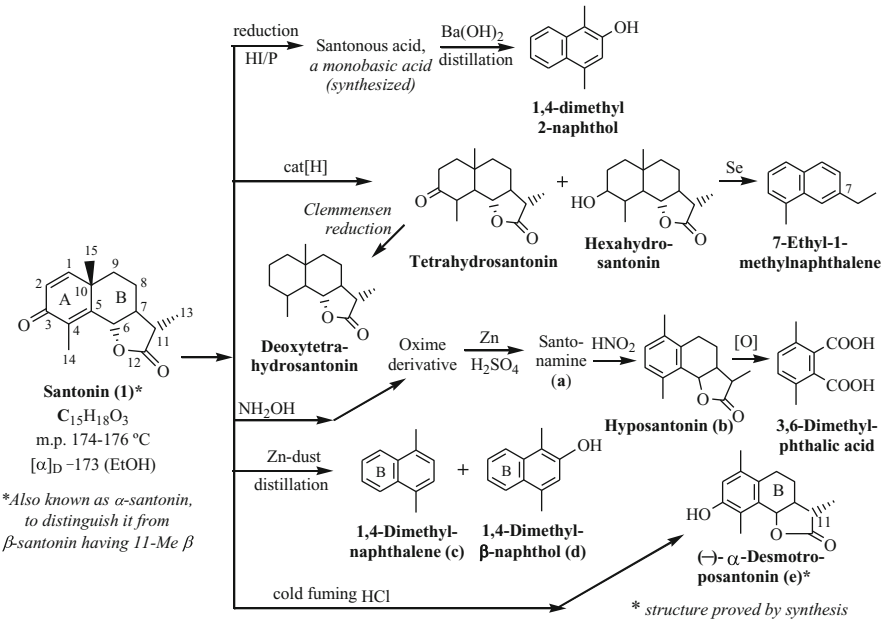


Fig. 7.42 Reactions and degradations of santonin

Earlier studies [47] on the structural elucidation involving the formation of the products (**b**), (**c**), (**d**), and (**e**) (Fig. 7.42) led to the placement of the two methyl groups at the 1 and 4 positions of the same ring in santonin molecule. However, George Clemo first proposed its correct structure (1929–1930) [48–50] with an angular methyl group on the basis of the following reactions and identification of the products (Fig. 7.43).

These reactions suggest the structure (**1**) for santonin without stereochemical description. On the basis of this structure, the formation of desmotroposantonin (**e**) and hyposantonin (**b**) (Fig. 7.42) can be explained as shown in Fig. 7.44.

The first reaction (Fig. 7.44) is known as dienone–phenol rearrangement, which finds wide application (Chap. 31) in cases of such organic molecules. Similarly, santonamine (**a**) on treatment with HNO_2 undergoes 1,2-methyl shift with the expulsion of N_2 to form hyposantonin (**b**). Likewise, 1,4-dimethyl-β-naphthol (**d**) is formed by a dienone–phenol rearrangement, and 1,4-dimethylnaphthalene (**c**) is formed by a dienol (formed by zinc dust reduction)–benzene rearrangement involving in each case a 1,2-methyl migration (Fig. 7.42). In both cases ring B gets aromatized.

The structure of santonin has been established by its synthesis (Fig. 7.45).

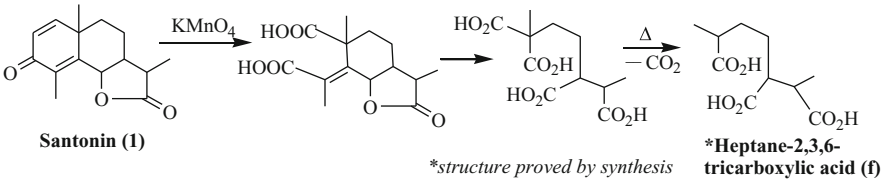


Fig. 7.43 Degradation of santonin to heptane-2,3,6-tricarboxylic acid (**f**)

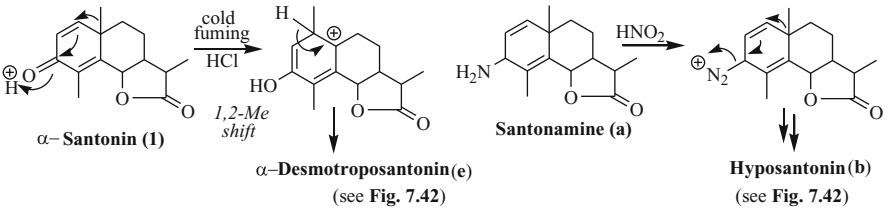


Fig. 7.44 Formation of desmotroposantonin (**e**) from santonin (**1**) and hyposantonin (**b**) from santonamine (**a**)

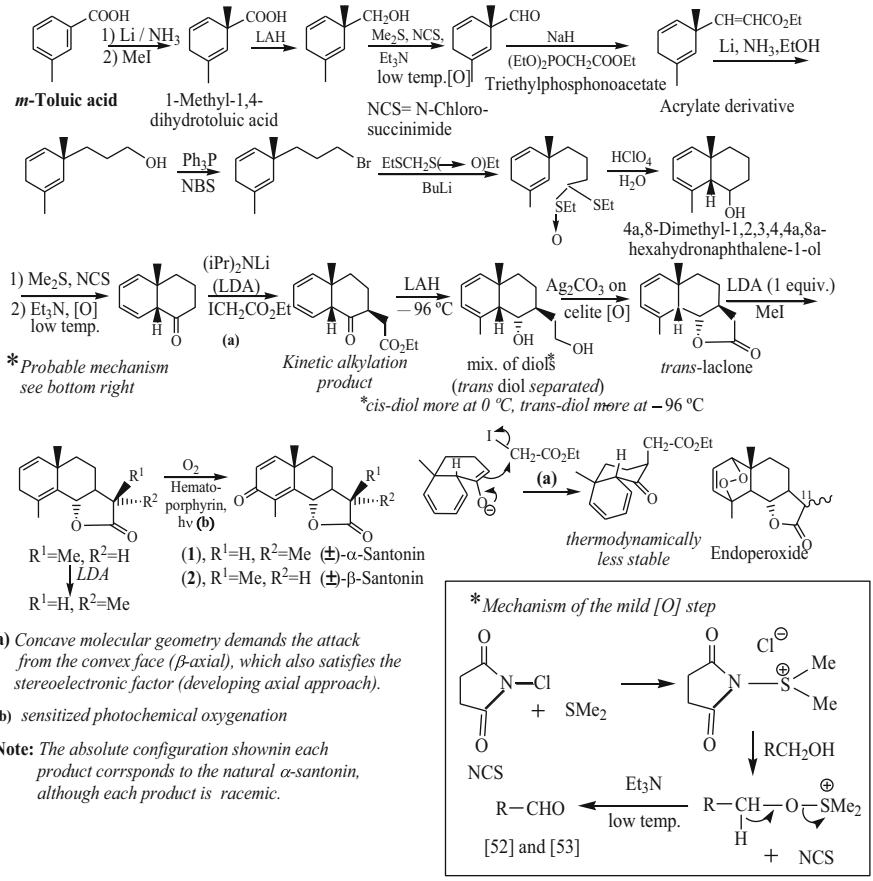


Fig. 7.45 Stereocontrolled total synthesis of (±)-α-santonin and (±)-β-santonin (1978) [51]

7.8.2 *Stereocontrolled Total Synthesis of Racemic α-Santonin and β-Santonin [51]*

The stereocontrolled total syntheses of α-santonin and β-santonin are delineated in Fig. 7.45. *m*-Toluic acid is reduced with Li in liquid ammonia (Birch reduction) followed by alkylation to obtain 1-methyl-1,4-dihydro-*m*-toluic acid. The COOH group is then expanded to a C_3-side chain in five steps forming *n*-propylbromide-substituted 1,4-dihydro-m-xylene. The latter has been alkylated with the lithium salt of the monosulfoxide of formaldehyde diethylthioacetal. Acid-catalyzed cyclization *via* the butanal derivative afforded 4a,8-dimethyl-1,2,3,4,4a,8a-hexahydronaphthalen-1-ol. Oxidation, alkylation, reduction, and subsequent treatment with silver carbonate impregnated celite on the separated *trans*-diol yields a lactone. The lactone undergoes methylation from the convex β-face leading to the

thermodynamically less stable 11-β-methyl compound. The latter could be epimerized to the more stable α-isomer. The β-isomer and the α-isomer are converted through sensitized photochemical oxygenation to β-santonin and α-santonin, respectively (Fig. 7.45). In each case, a significant amount of corresponding endoperoxide (11β-methyl derivative isolated, 11α-methyl derivative presumed to be formed also) was produced.

7.8.3 *Biogenetic-Type Synthesis of Santonin [54]*

The starting material in this biogenetic-type synthesis of santonin is its possible biogenetic precursor, the readily available santamarine (obtained in ample amounts from bay leaves). Its conversion to santonin [54] is shown in Fig. 7.46.

7.8.4 *Absolute Configuration of α-Santonin and Related Compounds at C_{11}. Full Stereostructures by X-ray Studies*

The absolute configuration at C11 of α-santonin has been determined to be (*S*) by its multistep transformation into a secondary alcohol (**A**) without affecting the stereochemical integrity of C11 and subsequent application of the Prelog atrolactic acid method (Sect. 2.11.5) to this compound [55, 56] as delineated in Fig. 7.47. Later, this absolute stereochemistry at C11 has also been deduced [57].

X-Ray analysis of α-santonin has firmly established (*s*)-configuration at both C10 and C11 and *trans* stereochemistry of the B/C ring juncture (**1**) [58]. Earlier, the X-ray single crystal structures of 2-bromo-α-santonin [59–61] and 2-bromo-β-santonin [62], elucidated by the usual phase-determining heavy atom

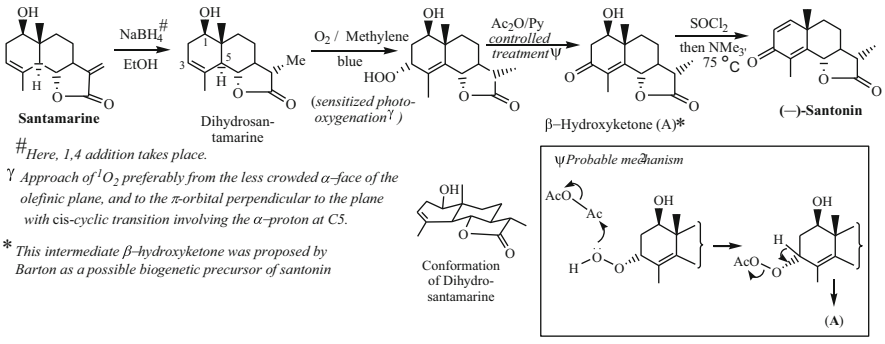


Fig. 7.46 Biogenetic-type synthesis of (–)-santonin (1983) [54] from santamarin

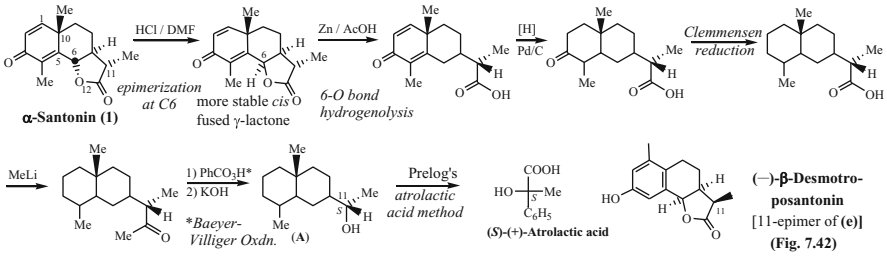


Fig. 7.47 Absolute configuration of α-santonin at C11 as (*S*) by Prelog atrolactic acid method [55]

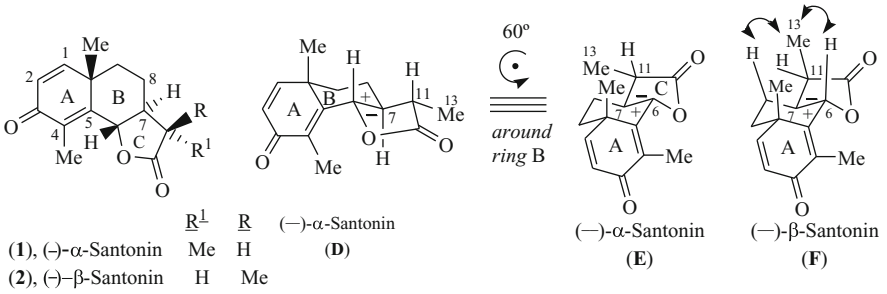


Fig. 7.48 Molecular conformations of α-santonin and β-santonin

method, revealed the full stereostructures of (−)-α-santonin and (−)-β-santonin as (**1**) and (**2**), respectively (see Fig. 7.48). The absolute stereochemistry of (−)-β-desmotroposantonin (Fig. 7.47) was established by X-ray analysis of its 2-bromoderivative [61]. It follows that (−)-α-desmotroposantonin [compound (**e**) of Fig. 7.42], the 11-epimer of the β-isomer, must have (*S*)-configuration at C11.

7.8.5 *Molecular Conformations of α-Santonin and β-Santonin*

The conformational features revealed from the stereostructure drawing as well as from a careful examination of a molecular model (Fischer/Dreiding) of α-santonin (**1**) are as follows : [cf. (**D**) or a different projection (**E**) of the same rigid conformation] (Fig. 7.48).

1. Ring A is more or less planar.
2. Ring B is in a slightly distorted chair form (cf. methylenecyclohexane moiety).
3. The γ-lactone ring C has an envelope conformation with C7 the out-of-plane atom, as has also been revealed from its X-ray crystallographic study [59–61].

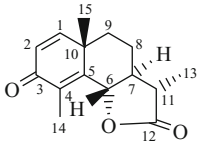
4. The ring juncture B/C is *trans* with H6 and H7 in *trans* diaxial conformation with respect to the ring B, and the Me at C11 (or C13 methyl) is pseudo-equatorial (evident from X-ray studies [59–61]).
5. The torsion angle signs at the B/C ring junction is (+/–), i.e., (+) in ring B and (–) in ring C, and not (–/+) – as evident from the conformation (**D**) or (**E**) (cf. Chap. 2).

The conformation (**E**) (another representation of the molecular conformation of α-santonin) can be derived from conformation (**D**) by 60° anticlockwise (from the top) rotation of the model around an axis, passing through the center of ring **B** and vertical to it.

In the lactone ring **C** in the conformation (**D**) C6 seems to be the nonplanar atom, at a little higher position but actually—as is evident from the X-ray study of 2-bromo-α-santonin [59–61] and the molecular model analysis as well as from the conformation (**E**), C7 is the nonplanar (lower) atom. Thus it should be noted that conformational drawings do not always give the correct three-dimensional positions of all the atoms in space in a molecule. The absolute stereochemistry of β-santonin has been elucidated from the X-ray study of 2-bromo-β-santonin [62], establishing it to be C11-epimer of santonin (or α-santonin).

The less stability of β-santonin [(**2**), C11-epimer of α-santonin, (**E**)] is attributed to the steric interactions of the β-pseudo-axial C13 methyl group with the syn-axial H6 and with syn-axial-like proximate H8 [cf. conformation (**F**)] and some resulting deformations of rings B and C. ^{13}C NMR Study of β-santonin also supports the pseudo-axial orientation of its C13 methyl group (Fig. 7.48).

7.8.6 *Spectral Properties*



α–Santonin (**I**)

UV: $\lambda_{max}^{(EtOH)}$ 236 nm (ε 12,000) unsaturated conjugated ketone.

IR: ν_{max} 1,165, 1,615, 1,645 (unsaturated ketone), 1,660, 1,780 cm^{-1} (saturated γ-lactone).

^{1}H NMR: δ (CDCl3) 1.25 (3H, *d*, C11–Me), 1.35 (3H, *s*, C10–Me), 2.10 (3H, *s*, C4–Me), 4.83 (1H, *d*, C6–H), 6.16 (1H, *d*, *J* 8.5 Hz, H2), 6.72 (1H, d, *J* 8.5 Hz, H1).

^{13}C NMR [63]: 155.1 (C1), 125.9 (C2), 186.0 (C3), 128.4 (C4), 151.5 (C5), 81.5 (C6), 54.0 (C7), 23.8 (C8), 39.3 (C9), 41.7 (C10), 41.2 (C11), 177.4 (C12), 12.5 (C13), 10.9 (C14), 25.3 (C15).

It has been observed [63] that in case of β-santonin (**2**), the C7. C8, C9, C11, and C13 are shielded by 4.5, 3.0, 1.1, 3.0, and 2.6 p.p.m., respectively, relative to those

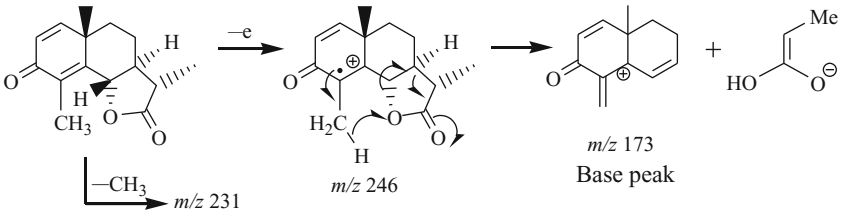


Fig. 7.49 Fragmentation of santonin to generate the base peak

of α-santonin, due to the pseudo-axial β-orientation of C13 methyl in the former, causing some distortion of the conformation of its B and C rings. All other carbons of β-santonin, excepting C12 (which is deshielded by 0.9 p.p.m.), have almost the same chemical shifts as those of α-santonin [63]. The configurational dependence of the carbon-13 chemical shifts of 6-epi-α-santonin and 6-epi-β-santonin (both having B/C rings *cis* fused) has also been discussed in detail by Randall group [63]. Inversion of C6 (*trans* to *cis* fusion) causes 5.0 p.p.m. upfield shift for the α-cases, and 3.9 p.p.m. upfield shift for the β-cases. Thus, 13C NMR studies provides with a sure method of assigning the stereochemistry of the ring juncture and the orientation of C11 methyl in santonin derivatives.

MS: The most intense peak that appeared in the mass spectrum of santonin (**1**) is at *m/z* 173 (M^+-73). The fragmentation is initiated by the removal of one electron from C4–C5 double bond when a resonance stabilized ion at *m/z* 173 is formed [64] (Fig. 7.49).

7.8.7 *Conversion of Santonin to Santonic Acid [46, 65]*

Conversion of santonin to santonic acid by prolonged vigorous boiling with concentrated alkali, discovered by Hvoslev in 1863 and studied later by Cannizzaro, remained a riddle for many decades. Woodward elucidated the structure of santonic acid [65] in 1948. His approach was based on the mechanistic way of thinking this conversion leading to its, till then, unidentifiable structure. In course of this study he discovered *an intramolecular Michael addition* as a means of a bridgehead formation via carbanion leading to a tricyclic skeleton (Fig. 7.50). Later, this type of intramolecular Michael addition has been employed during longifolene synthesis (Fig. 7.36) by Corey. Mechanistically the above conversion is explained stepwise in Fig. 7.50. The first step is the hydrolysis of the lactone to form the hydroxycarboxylate anion (**A**). The latter after loss of H^+ from C6, and the 3,6-diketone (**B**) formation, as shown, is converted to a resonance stabilized C7 carbanion (**C**). In the last step the carbanion (**C**) undergoes an intramolecular Michael addition to the proximate β-carbon (C1) of the α,β-unsaturated carbonyl system, in the conformation shown, followed by acidification to give santonic acid. The absolute

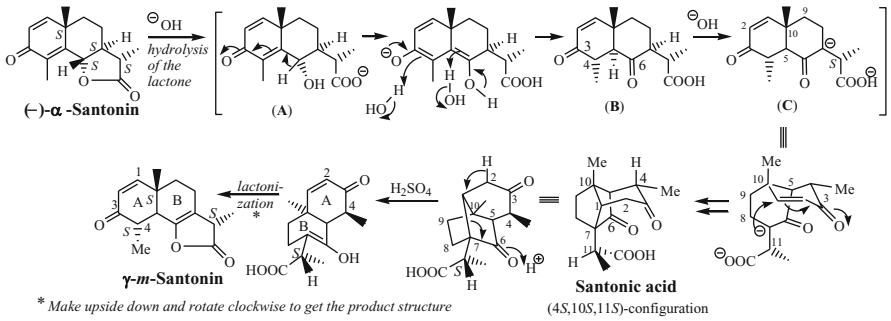


Fig. 7.50 Mechanistic rationales for the conversions of santonin to santonic acid and of the latter to γ−*m*-santonin

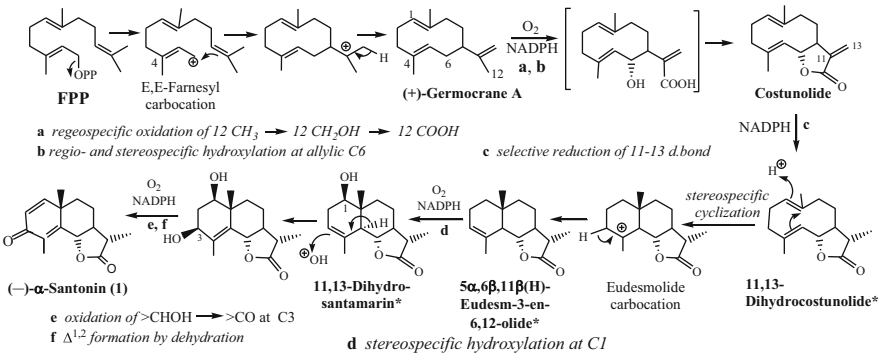
configuration of santonic acid has been firmly established in 1999 by single crystal X-ray analysis [58].

Santonic acid, on treatment with sulfuric acid, yields γ-*m*-santonin and its interesting mechanistic rationale is also shown in Fig. 7.50.

Thus, the structure and absolute configuration of santonin and santonic acid could be determined by single crystal X-ray analysis in a matter of days, rather than decades of chemical investigation. *At present, NMR spectroscopy and X-ray analyses are used as ultimate weapons for complicated structure determination leaving little or no room for important chemical information or intuition. However the chemical intuition and mechanistic way of thinking are indispensable for development of synthetic chemistry.*

7.8.8 Biosynthesis of Santonin

A preliminary study of the biosynthesis of santonin [66] in *A. maritime* has been carried out (Fig. 7.51). Santonin, a member of the eudesmol family of sesquiterpenoids, is derived from *E*,*E*-farnesyl carbocation. The latter cyclizes to a ten-membered carbocation (germacranyl carbocation) having two ethylenic linkages ideally suitable for cyclization to eudesmol. However, it has been shown through intermediate feeding in the biosynthetic study that prior to cyclization the ten-membered cation is converted into constunolide and then to dihydrocostunolide, both bearing the angular lactone and the latter a C11–Me instead of C11–C13–methylene. It then undergoes π-cation cyclization to build the eudesmol cation which quenches through the loss of a proton into 5α,6β,11β(H)-eudesm-3-en-6,12-olide. Experiments on the sequence of lactone formation suggest that the lactonization precedes the construction of the dienone group. Evidence that the ten-membered ring compound dihydrocostunolide is an intermediate has been secured. The probable sequence of the biogenesis of santonin is delineated in Fig. 7.51. It was demonstrated that the tritium-labeled compounds (marked with



Note •*Tritium labeled (at C11) compounds marked with an asterisk are those whose incorporation in santonin was demonstrated.*
• *Each step takes place, as usual, being catalyzed by a specific enzyme.*

Fig. 7.51 Probable biosynthetic route to santonin (Barton) [66]

asterisks) were incorporated in santonin [66] though in small percentages (3–8 %). Such low incorporations even in alkaloid biosynthesis have often been reported. 1,2-Dihydrosantonin, a major lactonic component of *A. stellariana*, quite unexpectedly failed to act as a precursor of santonin. Zero incorporation of 1-^{3}H-FPP and minute incorporation (2.25 %) of 1-^{3}H-farnesol may be due to the difficulty of translocation of these compounds to the site of biosynthesis [66].

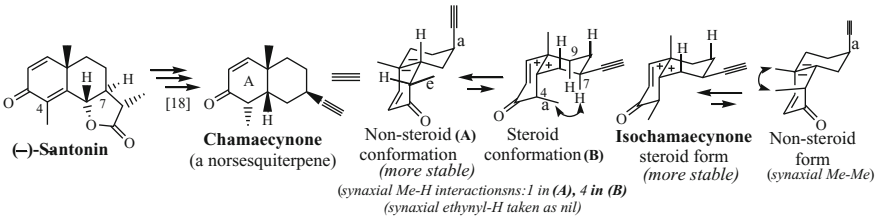
7.8.9 Santonin as a Synthon

Santonin has been employed as a synthon for the synthesis of quite a big number of sesquiterpenes ans their intermediates and derivatives [67–71].

Natural Norsesquiterpenes Having Nonsteroid and Steroid *cis*-Decalin Conformations Santonin has been used for the synthesis of *chamaecynone* having a novel nonsteroid *cis*-decalin conformation (–/– torsion angles at the ring junction) (see Sects. 2.15.1.2 and 2.15.1.3). It is one of the first few examples of natural acetylenic compounds of terpenoid origin [71, 72] (Fig. 7.52). It was isolated from the essential oil of the Benchi tree (*Chamaecyparis formosensis*, Fam. Cupressaceae).

The corresponding steroid conformation (+/+) torsion angles at the ring junction) of chamaecynone is highly unstable due to the steric interactions of the 4α-methyl group with 7α- and 9α-axial hydrogens (Fig. 7.52).

In this connection it is interesting to note that another natural norsesquiterpene, *isochamaecynone* [72], a C4-epimer of chamaecynone, possesses a steroid *cis*-decalin conformation (+/+) torsion angles at the ring juncture) in which 4β-methyl



Note: *The ring* A *which should be of somewhat flattened half chair conformation has been drawn as chair for convenience of drawing.*

Fig. 7.52 Structure and nonsteroid conformation of chamaecynone

group being equatorial entails no destabilizing interaction with 7α- and 9α-axial hydrogens (Fig. 7.52). On the contrary, its flipped nonsteroid conformation imposes strong *syn* axial interaction between 4-CH_3 and 10-CH_3 groups, and hence it does not exist (for more details, see Sect. 2.15.1.2 and 2.15.1.3). For convenience of reproduction the ring A in both these norsesquiterpenes has been presented as chair conformation [72], although because of the α,β-unsaturated moiety ring A will exist in a flattened half-chair conformation.

7.8.10 Photochemical Transformations of Santonin

Santonin (**1**) containing a cross-conjugated ketone, as expected, should be sensitive towards light. In fact, the photochemistry of santonin has an illustrious history of the formation of various photolysis products in manifold media or conditions. The mechanisms of these photochemical transformations have been studied by many leading organic chemists [73–77]. The formation of isophotosantonic acid lactone (**E**) (having a guaianolide skeleton) as a product of photolysis of santonin in aqueous acetic acid is best rationalized in terms of Zimmerman's explanation (Fig. 7.53) [78, 79]. An initial $n \rightarrow \pi^*$ transition produces a diradical (**A**) which isomerizes to (**B**), followed by collapse (intersystem crossover, ISC) to a dipolar species (**C**). In aqueous acetic acid the anion is protonated, and the resulting carbonium ion (**D**) rearranges to the lactone (**E**). Barton showed this rearrangement to be general, and consequently it has been used in the synthesis of several perhydroazulenes of the guaianolide series [80]. This photolysis if performed in glacial acetic acid yields the corresponding acetate (**F**).

The photochemical conversion of santonin in absolute ethanol to lumisantonin (**G**) (~13 % yield) and that of lumisantonin in cold aq. AcOH at –5 to 5 °C to photosantonic acid (**J**) via an intermediate dienone (**H**) (isolated and characterized) [76, 77] are delineated in Fig. 7.54. Plausible stepwise mechanism based on the

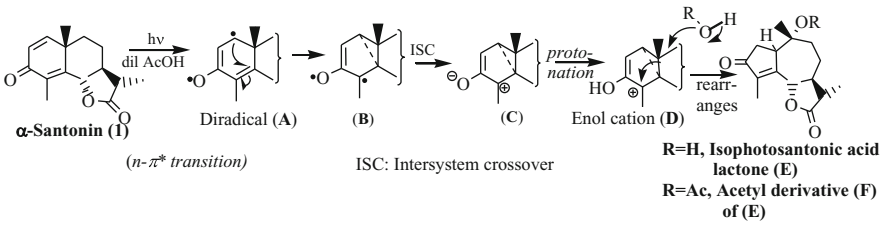


Fig. 7.53 Mechanistic rationale for the photoconversion of santonin to isophotosantonic acid

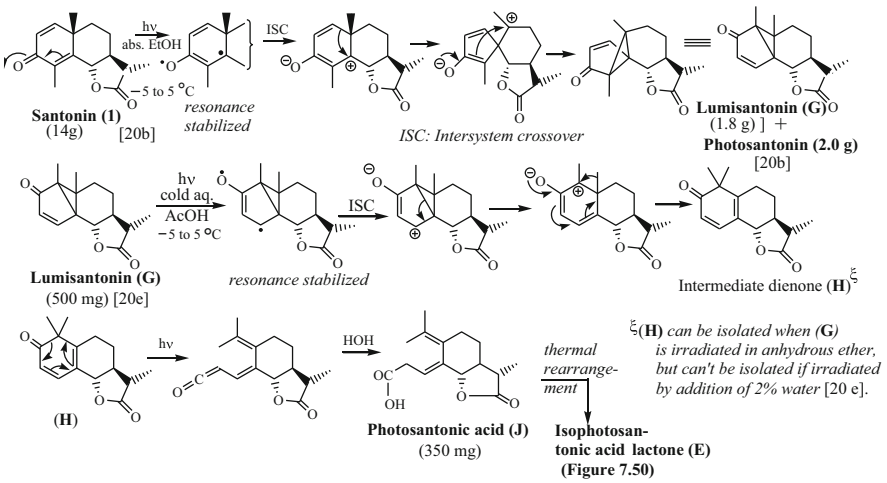


Fig. 7.54 Mechanistic rationale for the photoconversion of santonin to lumisantonin (**G**) and of (**G**) to (**J**) via (**H**)

intensive studies by Barton, Chapman, Richards, and others is shown in Figs. 7.53 and 7.54. Lumisantonin upon thermal rearrangement forms isophotosantonic acid (**E**). The intermediate dienone (**H**) is rapidly converted to photosantonic acid (**J**) in presence of light and water. Irradiation of lumisantonin in anhydrous ether requires 2.5 h to achieve maximum concentration of (**H**). Addition of 2 % water and continued irradiation under identical condition leads to complete disappearance of (**H**) in less than 45 min. Thus, for correct interpretation of the photochemical processes the nature and number of discrete photochemical reactions involved should be known [76].

7.9 Artemisinin: *A Sesquiterpene Lactone with an Endoperoxide Linkage and Profound Antimalarial Activity*

7.9.1 Introduction. Occurrence. Structure

Qing hao meaning "green herb" and pronounced as Ching how (*Artemisia annua*, Fam. Compositae) has been used in Chinese traditional medicine as a treatment for fever and malaria for many centuries. Its uses have appeared in several Chinese medicinal texts. The *Book of Fifty Two Prescriptions* discovered in the tomb of Mawangdui Han dynasty, dating from 168 B.C. contains the use of *qing hao* in the treatment of hemorrhoids. The antimalarial property of this drug was first described in 340 A.D. in *Zhou Hou Bei Ji Fang*, (Handbook of Prescriptions for Emergency Treatments) [81]. In 1596 Li Shizhen mentioned in the treatise *Ben Cao Gung Mu* (Compendium of Treatments) the use of *qing hao* for the treatment of shivering and fever of malaria [81–84]. In 1971 it was observed that crude ether extracts of *A. annua* produced encouraging results in mice infected with the malaria parasite *Plasmodium berghei*. In 1972 the active principle responsible for the profound antimalarial activity of *Artemisia annua* (*qing hao*) was isolated by a Chinese group of workers [81–83] as a white crystalline compound, $C_{15}H_{22}O_5$, colorless needles (from hexane), m.p. 156–157 ºC, $[\alpha]_D$ + 66.3 ($CHCl_3$) from the leafy part of the plant.

7.9.2 Absolute Stereochemistry and Conformation

The compound named artemisinin (*qinghaosu*) was characterized as a sesquiterpene lactone with an unusual trioxane structure (Fig. 7.55) from its X-ray crystallographic studies by Qinghaosu Research Group of China. It was shown to possess seven stereogenic centers (*R/S designation of all chiral centers is shown in the*

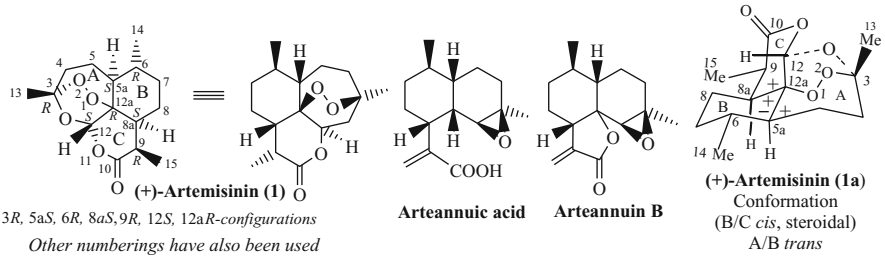


Fig. 7.55 Stereostructures of artemisinin and arteannuin B

Figure) on a tetracyclic framework (**1**). The absolute configuration and A/B *trans* and B/C *cis* ring junctures have also been settled by comparing its ORD curve with that of arteannuin B of known stereochemistry. Its rigid conformation (1a) with rings B/C in chair/chair *cis*-decalin steroidal conformation (Sect. 2.15.1.3) and A/B *trans*-decalin (Sect. 2.15.1.2)-type conformation is depicted in Fig. 7.55. The absolute configurations at all the seven stereogenic centers (Sects. 2.6.2 and 2.7.5) are shown. Chemical support to this structure was also provided [82, 83]. Artemisinin is a timely discovery since malaria caused by the protozoa of the genus *Plasmodium* (strain *Plasmodium falciparum*) became resistant to chloroquine and many other parallel synthetic drugs (Chap. 33).

7.9.3 Synthesis

The overwhelming antimalarial property of *artemisinin* with no side effect (unlike the synthetic antimalarials) and its interesting unusual structure claimed vigorous studies on it. Consequently, a number of its total as well as semi-syntheses appeared in the literature. The first total synthesis, achieved by Schmid and Hofheinz [85] of Hoffman-La Roche in Basel, displayed remarkable stereoselectivity. However, semi-syntheses [86, 87] (Figs. 7.56 and 7.57) and a recent (2010) shortest synthesis of artemisinin by Yadav et al. [88] will be presented here. Artemisinin and its derivatives have become attractive target molecules for their semisyntheses [89] and total syntheses [90]. A recent report [91, 92] on the semisynthesis of artemisinin employing biologically synthesized artemisinic acid through engineered yeast cell culture and its subsequent conversion to artemisinin using modified commercially viable chemical methodology will also be discussed briefly.

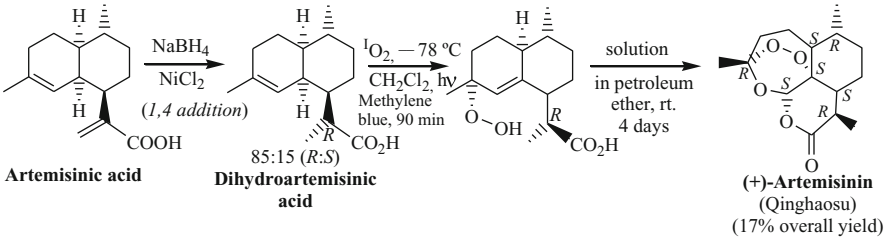
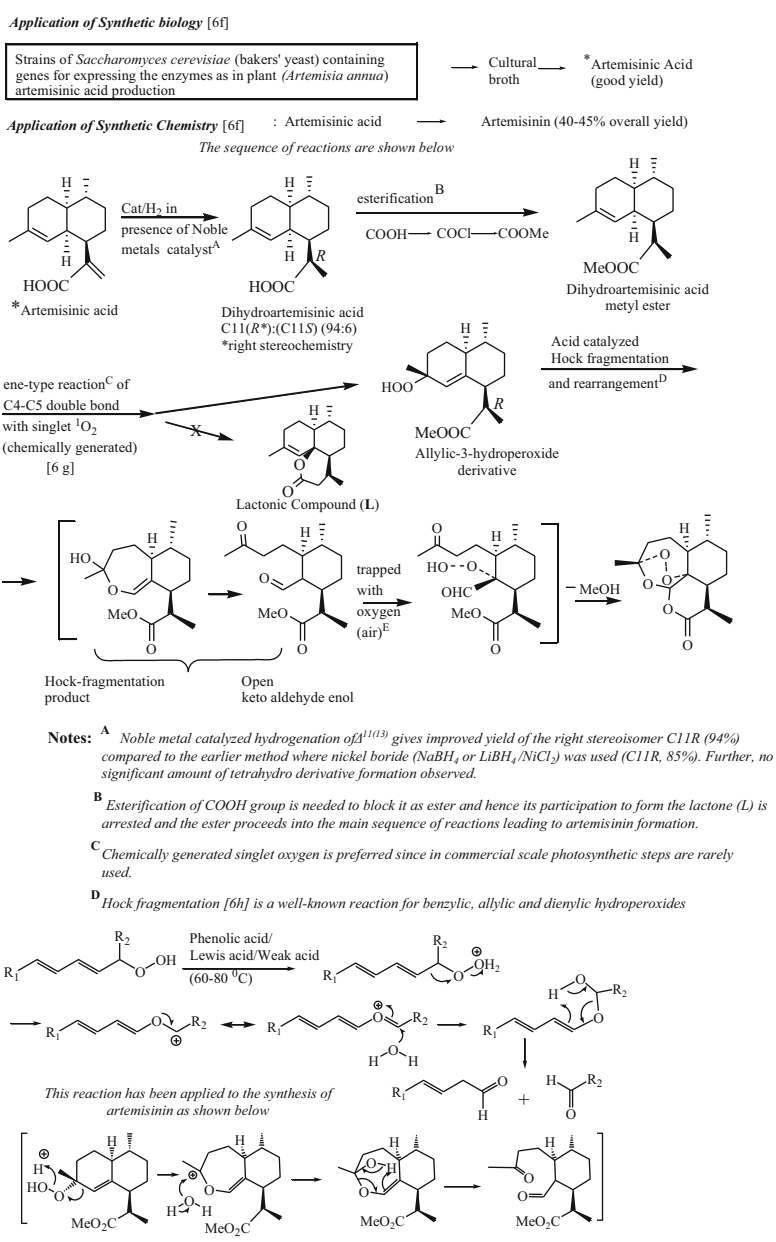


Fig. 7.56 Semisynthesis of artemisinin from artemisinic acid [86]



Notes: **A** *Noble metal catalyzed hydrogenation of $\Delta^{11(13)}$ gives improved yield of the right stereoisomer C11R (94%) compared to the earlier method where nickel boride ($NaBH_4$ or $LiBH_4/NiCl_2$) was used (C11R, 85%). Further, no significant amount of tetrahydro derivative formation observed.*

B *Esterification of COOH group is needed to block it as ester and hence its participation to form the lactone (L) is arrested and the ester proceeds into the main sequence of reactions leading to artemisinin formation.*

C *Chemically generated singlet oxygen is preferred since in commercial scale photosynthetic steps are rarely used.*

D *Hock fragmentation [6h] is a well-known reaction for benzylic, allylic and dienylic hydroperoxides*

In this step less expensive reagent benzenesulphonic acid/sulphonate Cu(II) – Dowex resin has been used in place of copper triflate.

E *Pure oxygen has been replaced by air for safely reason*

HPLC studies show that artemisinin prepared by this method is purer than commercial artemisinin (plant source). Thus the entire chemical conversion of artemisinic acid to artemisinin showed a significant efficiency.

Fig. 7.57 Biological synthesis of artemisinic acid in yeast cell culture and its chemical conversion to artemisinin

7.9.3.1 Semisynthesis

(1a) A semisynthesis of artemisinin starting from *artemisinic acid*, a relatively abundant constituent of *Artemisia annua*, has been reported by Roth and Acton [86, 87, 89–94]. Artemisinic acid is converted to dihydroartemisinic acid with $NaBH_4$ in the presence of $NiCl_2$. Allylic hydroperoxidation is carried with singlet oxygen. On standing in air in the presence of a little acid and in a hydrocarbon solution the hydroperoxide facilitates the introduction of a second molecule of oxygen and is converted into artemisinin in 17 % overall yield (Fig. 7.56). It is a biomimetic synthesis, as artemisinic acid co-occurs with artemisinin in the same plant and acts as the precursor of artemisinin in the biosynthetic pathway (Fig. 7.60). The conversion of the hydroperoxide to artemisinin is a remarkable reaction. The mechanism of such conversion is not obvious. It involves an oxidation, ring expansion, an intramolecular nucleophilic attack, and lactonization with the competence of enzymatic reactions of its biosynthetic process [87] (*cf*. Fig. 7.60). The importance of artemisinin has also led to its semi-synthesis [89] from another naturally occurring biogenetic precursor *arteannuic acid* (Fig. 7.55).

The systematic name of artemisinin is 3,6,9-trimethyl-9,10b-epidioxyperhydropyrano[4,3,2-*ik*]benzoxepin-2-one, but this is hardly convenient. *Chemical Abstracts* adapted the name artemisinin [84].

(1b) "Yeast makes artemisinin on demand" [91]. This title refers to a research note appearing in the May 2013 issue of *Chemistry World*. It includes a summarized report on the reconstruction of the biosynthetic path of plant artemisinin in yeast cells. Jay Keasling's group at the Lawrence Berkeley National Laboratory in California, Berkeley, reported in *Nature* in 2006 about the genomically engineered yeast cells and their functions in the biosynthesis of artemisinic acid, a biogenetic precursor of artemisinin which can be chemically converted to the drug. However, genes necessary to produce the two particular biosynthetic enzymes made the yeast ill and the entire process was not competitive with plant source extraction, Recently, Chris Paddon of Amyris, Emeryville, California, developed strains of yeast cells (*Saccharomyces cerevisiae*, baker's yeast) using synthetic biology that can encode two more dehydrogenase enzymes necessary to synthesize plant artemisinic acid. Thus with full complement of five key enzymes artemisinic acid is biosynthesized in yeast cell culture in increased yield. The yields are 10 times higher than the previous reports and the yeast cells remain healthy. Chris Paddon, his group, and his collaborators reported their findings in *Nature* 2013 [92] and the title of their paper reads as "High-level semi-synthetic production of the potent antimalarial artemisinin." This method with slight difference has already been commercially used and semi-synthesized 35 metric tons of artemisinin—roughly equivalent to 70 million malaria cures [91]. The conversion of artemisinic acid to artemisinin (40–45 % overall yield) has been done in a scalable and practical way to offer a stable and affordable source for the drug [92] (Fig. 7.57).

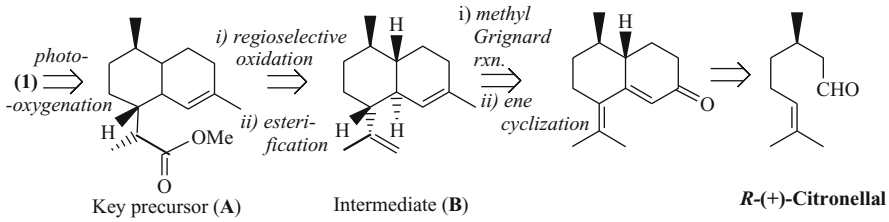


Fig. 7.58 Retrosynthetic plan for artemisinin [88]

7.9.3.2 Total Synthesis of (+)-Artemisinin by Yadav et al. [88]

A protective group-free, concise, streoselective total synthesis of (+)-artemisinin has been reported in which *R*-(+)-citronellal, a commercially available monoterpene, serves as the starting material. The retrosynthetic plan for this synthesis is delineated in Fig. 7.58 [88].

In the present synthesis (Fig. 7.59) R(+)-citronellal is alkylated with methyl vinyl ketone (MVK) by asymmetric 1,4-addition (Michael reaction) in the presence of a chiral proline-derived catalyst, when the product was obtained in 70 % yield with 83 % *de*. Intramolecular aldol condensation yielded an enone. The latter on Grignard reaction formed a mixture of diastereisomeric alcohols. The stereochemistry at C4 will be absent in subsequent steps. The mixture underwent $SnCl_4$-catalyzed cyclization to give the ene intermediate (**B**), a *trans*-decalin derivative. Regioselective hydroboration of (B) with 9-BBN formed the primary alcohol in 85 % yield. Swern oxidation of the latter formed the aldehyde (94 %), which upon oxidation with $NaClO_2/NaH_2PO_4$ produced the acid in 80 % yield. The corresponding methyl ester was subjected to photooxidation following Haynes protocol (DCM, rose Bengal, copper triflate) [95] to yield (+)-artemisinin (**1**) (Fig. 7.59) in 13.0 % overall yield.

7.9.4 Spectral Properties [88]

IR (KBr): ν 2,921, 2,854, 1,738, 1,115, 994 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 Mz): δ 1.00 (d, 3H, J = 5.8 Hz), 1.02–1.13 (m, 1H), 1.21 (d, 3H, J = 7.3 Hz), 1.45 (*s*, 3H), 1.33–1.54 (m, 4H), 1.69–1.83 (m, 2H), 1.84–1.94 (m, 1H), 1.95–2.11 (m, 2H), 2.36–2.53 (m, 1H), 3.34–3.46 (m, 1H), 5.86 (s, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.5, 19.8, 23.4, 24.8, 25.2, 32.9, 33.6, 35.9, 37.5, 45.0, 50.0, 79.5, 93.7, 105.4, 172.0.

Mass: **APCI**: *m*/*z* 283 $(M+H)^+$.

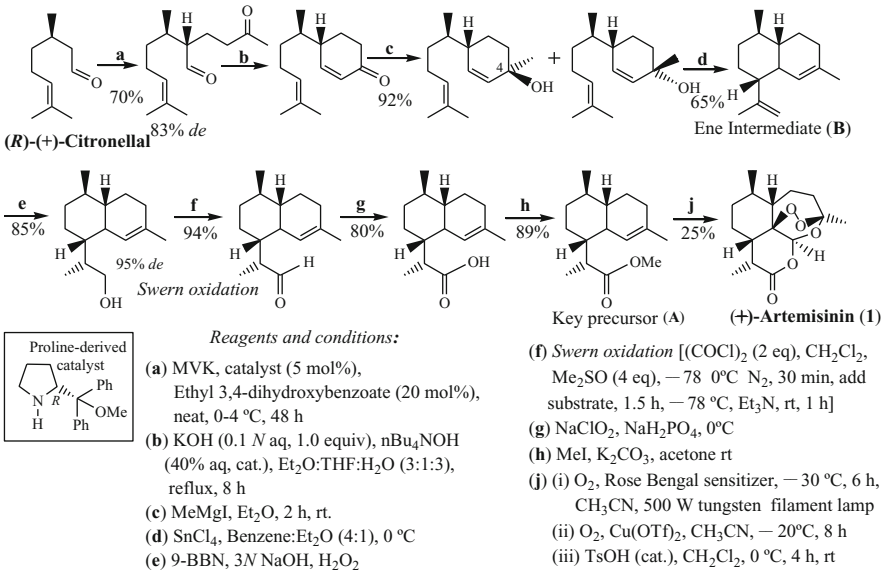


Fig. 7.59 Total synthesis of (+)-artemisinin [88]

7.9.5 Biosynthesis of Artemisinin (qinghaosu) [84, 96]

A number of enzymes exhibiting sesquiterpene synthase properties have been found to occur in the leaves of *Artemisia annua*. One such enzyme, a sesquiterpene cyclase, has been isolated and sequenced and has been found to cyclize FPP to a number of products, of which amorpha-3,11-diene predominates. The latter has been shown to be the precursor of artemisinic acid which is subsequently converted to artemisinin (Fig. 7.60).

7.9.6 Uses

Uses of artemisinin and some of its bioactive derivatives have been discussed in Chap. 33.

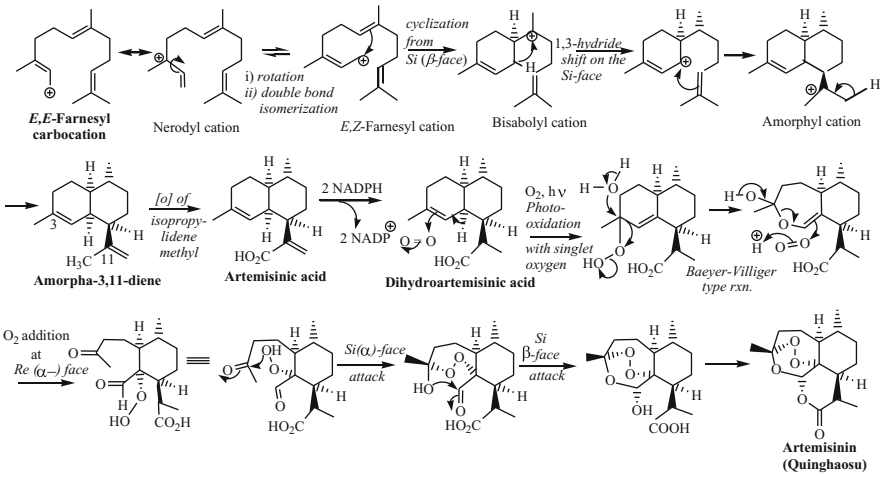


Fig. 7.60 Biosynthesis of artemisinin *via* amorpha-3,11-diene, artemisinic acid, and dihydroartemisinic acid

7.10 Abscisic Acid: A Sesquiterpene Phytohormone

7.10.1 Introduction. Occurrence

There are five major classes of phytohormones having diverse structures and functions (Fig. 7.61). One of them is *abscisic acid*, a sesquiterpene in composition with cyclofarnesane-type skeletal pattern. It regulates the water balance in plants (stomata opening and closing) and causes abscission (shedding of leaves) acceleration, bud dormancy, and growth inhibition. Such growth-inhibitory compounds are also known as "dormins." On the other hand, there are *gibberellins* (*e.g.*, GA_3), C_{19}-diterpenes (Chap. 8), which induce elongation growth of internodes, *cytokinin*—a prenylated adenine which stimulates cell division, *auxin*—an indole-derived plant hormone causing stimulation to cell elongation, and *ethylene*—derived from the methionyl moiety of *S*-adenosylmethionine, which helps in ripening the fruits. Plant hormones are structurally quite different from animal hormones.

Abscisic acid mp 160–161 °C, $[\alpha]_D$ + 430 has been isolated in extremely low yield (0.000004 %) from the young fruits of *Gossypium hirsutum* (Fam. Malvaceae). It also occurs in "sycamore" (*Acer pseudoplatanus,* Aceraceae) [97] and birch (*Betula pubescens,* Betulaceae) leaves, rose (*Rosa arvensis*, Rosaceae), cabbage (*Brassica oleraceae*, Crucifarae), potato (*Solanum tuberosum*, Solanaceae), avocado (*Persea grantissima*), and lemon (*Citrus medica*, Rutaceae). Its structure has been deduced from degradation products, synthesis, and spectral properties. The absolute configuration of the only asymmetric center has been determined to be *S*. These are briefly discussed in the sequel.

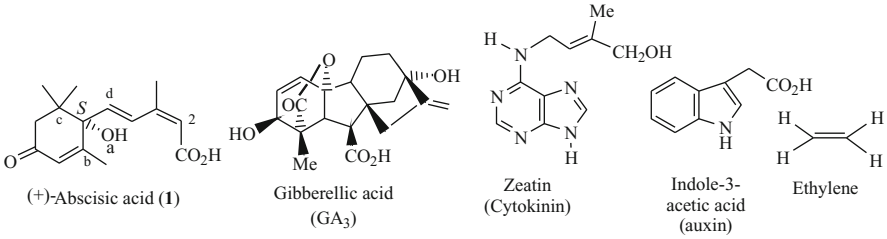


Fig. 7.61 Some phytohormones including (+)-abscisic acid

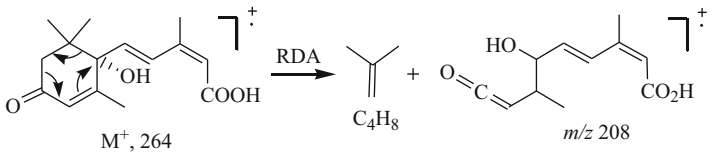


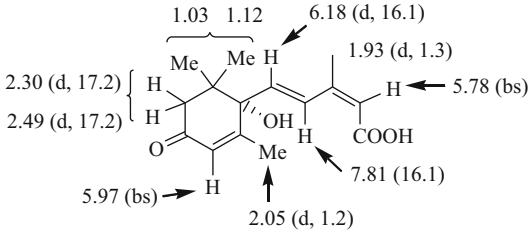
Fig. 7.62 Spectral properties of abscisic acid

7.10.2 Spectral Properties (Fig. 7.62)

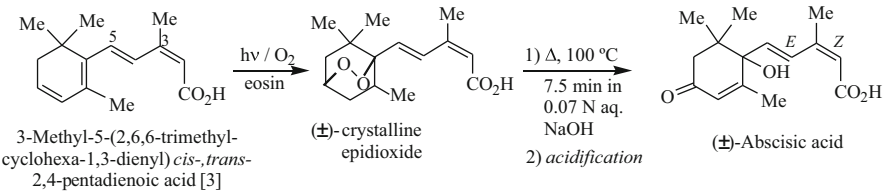
UV (EtOH): λ_{max} 246 nm (ε 25,00).

IR (KBr): ν in cm^{-1} 3,405 (OH), 1,674 (acid > =O), 1,650 (α,β-unsaturated > =O), 978 (trisubstituted double bond).

PMR ($CDCl_3$): δ (J Hz) [98].



MS [98]: *m/z* 264 (M^+), 246 ($M^+ - H_2O$), 208 ($M^+ - C_4H_8$).



3-Methyl-5-(2,6,6-trimethyl-cyclohexa-1,3-dienyl) *cis-,trans*-2,4-pentadienoic acid [3]

Fig. 7.63 Cornforth's synthesis of (±)-abscisic acid (1965) [99]

7.10.3 Synthesis

7.10.3.1 Cornforth's Synthesis of (±)-Abscisic Acid

The photosensitized 1,4-addition of oxygen to homoannular 1,3-diene (*epidioxidation*), a well-known reaction, has been used as the key step in the synthesis of abscisic acid by Cornforth [99]. The synthesis is outlined in Fig. 7.63.

It should be mentioned that the m.p. of the synthetic product is 188–190 °C, is around 30 °C more than the natural one and is with half activity (being *dl*) compared to the natural one, the (+)-enantiomer.

7.10.3.2 Synthesis of Optically Active Abscisic Acid

In a number of syntheses of abscisic acid (ABA) *Reformatsky reaction* has been employed conveniently for the stereospecific formation of (2*Z*,4*E*)-diene side chain responsible for its hormonal activity. Its isomerization to (2*E*,4*E*) causes the loss of the hormonal activity [98]. The orientation of the carboxylic group is thus significantly important for its activity. More than 100 abscisic acid derivatives have been synthesized to find out ABA-like strong activity and the molecular conformation needed for ABA action [100].

An ordinary synthon with an epoxy aldehyde group could not be tried for the (2*Z*,4*E*)-diene side-chain formation using Reformatsky reaction since the latter affects the epoxide also. However, Sakai et al. [98] employed a suitable epoxyaldehyde synthon, in which the expoxy group is guarded by substitution from the attack of the Reformatsky reagent, which then only reacts with the aldehyde function generating the (2*Z*, 4*E*)-diene side chain (Fig. 7.64).

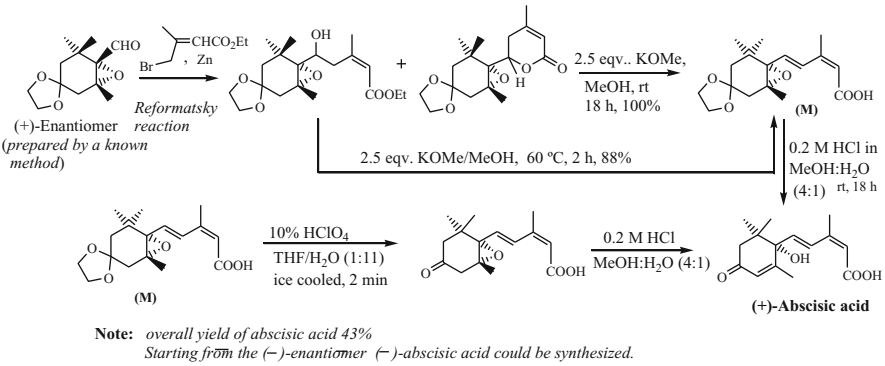


Fig. 7.64 Synthesis of (+)-abscisic acid [98]

7.10.4 Absolute Configuration

7.10.4.1 By Chemical Correlation

Violaxanthin, derived from carotenoid (see Fig. 7.69) on photooxidation, gave a number of products of which *xanthoxin*, a widespread carotenoid-derived natural growth inhibitor, is one. The latter could be converted to (+)-*trans*-abscisic acid (Fig. 7.65). Since the stereochemistry of the chiral centers of violaxanthin is known, this conversion shows (*S*) (new CIP convention, Sect. 2.4) configuration of the only chiral center of (+)-abscisic acid [103]. The reversal of configuration during this chemical conversion is extremely unlikely. Thus the absolute configuration *(S)* of C6 of (–)-*cis*-xanthoxin, which has been converted to (+)-abscisic acid, is also established.

The absolute configuration for natural (+)-abscisic acid (ABA) {6β-OH in place of 6α-OH in (**1**) or (6*R*)-configuration according to the new CIP convention)} was proposed by Cornforth et al. [104] on the basis of $[M]_D$-values of *cis*- and *trans*-3,6-diol esters [obtained from the (+)- and (–)-abscisic acid)] by application of Mill's empirical rule. The absolute configuration has been revised by other authors [105, 106] as (6*S*). Perhaps the diol esters are not suitable for the application of Mills' empirical rule. According to old (1956) CIP convention of (+)-abscisic acid was designated (*S*) by Cornforth [104].

7.10.4.2 By CD Studies (Exciton Chirality Method) (Sect. 2.19.6.5)

The configuration (6*S*) has been established by Nakanishi et al. [107] by CD studies (exciton chirality method) of ABA and some of it structural relatives having similarly substituted cyclohexenone ring with C6 (bearing OH) having both (*R*)-

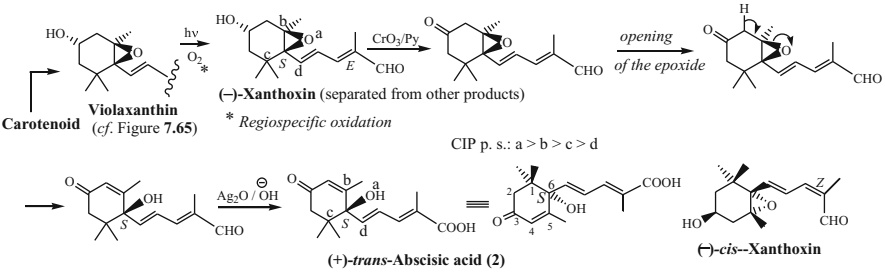
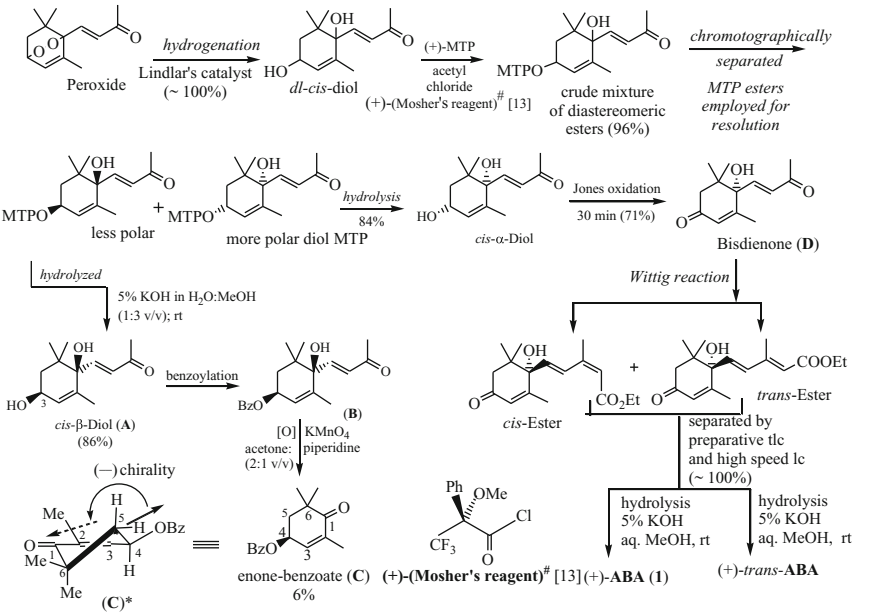


Fig. 7.65 The absolute configuration of (+)-abscisic acid



*The direction of the front electric transition moment is parallel to the C4-OBz bond, and that of the back is passing through the mid-point of C3 and the carbonyl oxygen. The chirality between the two chromophores is negative, exhibiting (—)-CE. The absolute configuration of the benzoate is hence represented by (**C**) and that of the diol by (**A**).

(+)-MTP Acetyl chloride = (+)-1-Methoxy-1-trifluoromethyl-1-phenylacetyl chloride [(+)-**Mosher's reagent**]#

Fig. 7.66 Preparation of (**A**), (**B**), and (**C**) exhibiting (–)-CE, and of (**D**), (+)-**ABA,** and (+)-*trans*-**ABA** exhibiting (+)-CE in CD studies

and (*S*)-configurations. The preparation of the compounds (**A**) and (**B**), having (6*R*)-configuration, and also compound (**C**), and that of the compounds (**D**), (+)-ABA, and (+)-*trans*-ABA are outlined in Fig. 7.66.

The CD of the benzoate (**C**) (Table 7.1) showed a split Cotton effect due to interaction between the benzoate and the enone chromophores. The negative first Cotton effect as par the exciton chirality method indicates a negative chirality (**C1**)

Table 7.1 The CD, $\Delta\varepsilon$ (nm) in MeOH

Compound	$\pi - \pi^*$	Compound	$\pi - \pi^*$
(**A**)	–6.0 (232)	(**D**)	+ 38.4 (242)[a] –30.2 (208)[a]
(**B**)	–5.3 (230)	(+)-ABA	+34.5 (261)[a] –28.0 (229)[a]
(**C**)	–19.0 (238)[a] +3.21 (219)[a]	(+)-*trans*-ABA	+25.5 (254)[a] –12.6 (221)[a]

[a]Davidov split Cotton effects centered around UV maxima

between the two axes of electric transition moments. The absolute configuration of the benzoate is hence represented by (**C**) and that of the diol by (**A**) [109].

The positive first Cotton effects of the bisdienone (**D**) and of (+)-ABA and (+)-*trans*-ABA each one showing Davidov splitting (Table 7.1) support the absolute configuration (**S**) at C6 of these compounds, as depicted which is consistent with the (+)-chirality of the two interacting chromophores.

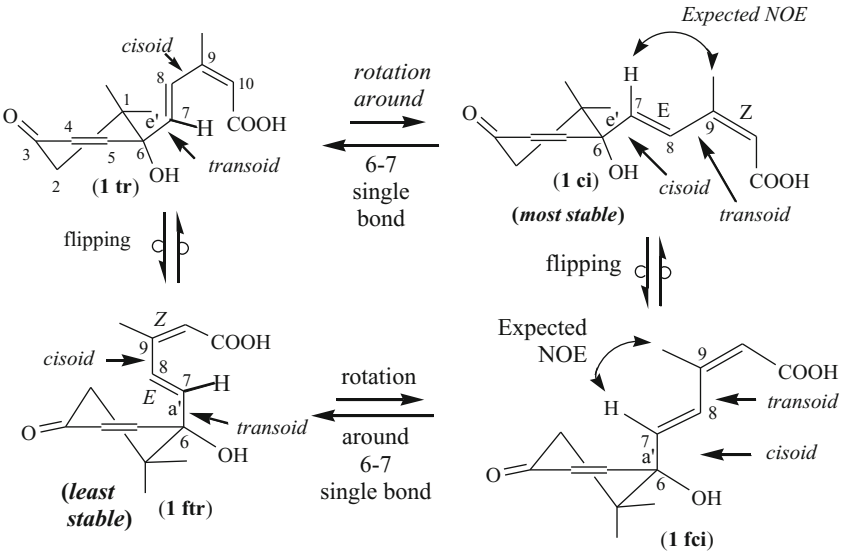
The enantiomers (–)-ABA and (–)-*trans*-ABA were similarly prepared from the *cis*-β-diol and their CD data were also measured and found to exhibit (–)-Cotton effect (CE), like compounds (**A**), (**B**), and (**C**).

The absolute configuration (6*S*) for (+)-*trans*-abscisic acid has been established by Harada [110] by quantitative application of the exciton chirality method.

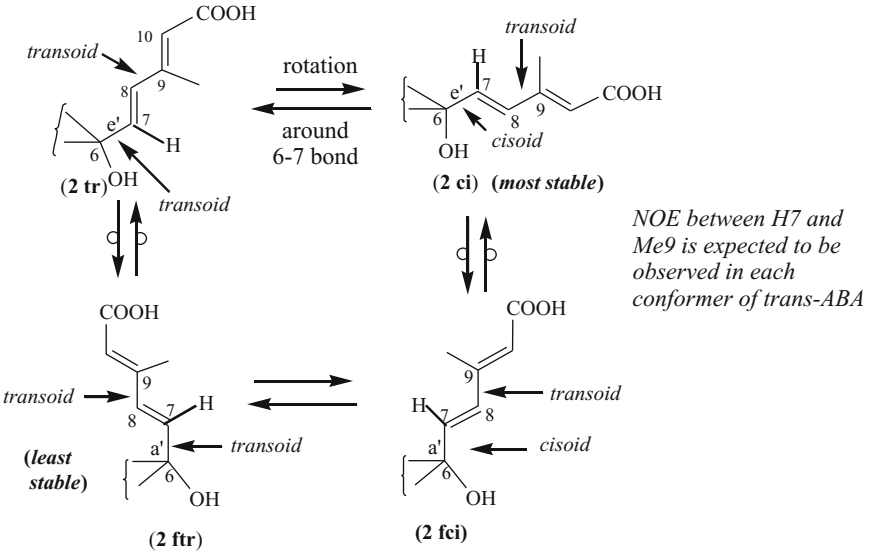
Natural (+)-abscisic acid has also been correlated with (*S*)-malic acid (*S*-COOH. CHOH.CH2.COOH), leading to the same absolute configuration (*S*) at C6 [111].

7.10.5 Molecular Conformations of (+)-ABA and (+)-trans-ABA

The cyclohexenone ring of (+)-ABA or (+)-*trans*-ABA adopts a half-chair conformation as shown in Fig. 7.67. Both types of molecules are quite flexible. The half-chair undergoes ring inversion giving rise to two possible half-chair conformers in each case, *viz*., (**1tr**) and (**1ftr**) and (**1ci**) and (**1fci**) for ABA (**1**). Moreover, the side-chain conformation at C8 is expected to be mainly *cisoid* or *5-cis* (C8 and OH on the same side of the 6–7 single bond), since the side chain goes away from the cyclohexenone ring. Of the two flipped *cisoid* conformers in each case, (**1Ci**) and **2Ci** having pseudoequatonal (e′) side chain will be more stable and hence the most stable conformer of (+)-ABA and (+)-*trans*-ABA, respectively. Moreover, the 7,9-diene in (**1ci**) is *S-trans* and hence contributes to its stability, while it is *S-cis* in (**1tr**). The above arguments point out the conformers (**1ftr**) and the (**2ftr**) as the least stable ones for ABA and *trans*-ABA, respectively. Harada [110] determined the absolute configuration of (+)-*trans*-ABA by a quantitative application of the exciton chirality method for all the four conformers (**2tr**), (**2ftr**), (**2ci**), and (**2fci**). Thus quantitative application of the exciton chirality method [110] may provide a useful tool for conformational analyses of natural products.



Conformers of (+)-ABA (1)



*The conformations of the ring are same as the corresponding conformers of (**1**)*

Conformers of (+)- *trans*-ABA (2)

Fig. 7.67 Conformers of (+)-ABA (**1**) and (+)-*trans*-ABA (**2**)

7.10.6 Biosynthesis [101, 102, 112]

As a fungal metabolite abscisic acid is formed from farnesyl pyrophosphate [112] (Fig. 7.68) while in higher plants it is formed by the oxidative cleavage of the carotenoid violaxanthin.

Abscisic acid is formally a sesquiterpene. However, it is also very similar to the end portion of certain carotenoids. The absolute configuration of abscisic acid is same as that of violaxanthin (isolated from orange peel) (Sect. 7.10.4.1), the pigment precursor of ABA. A reversal of configuration during the biosynthesis would be extremely unlikely.

In plants abscisic acid is derived from carotenoids (Fig. 7.69). The first committed precursor is zeaxanthin which undergoes a series of enzymatic reactions

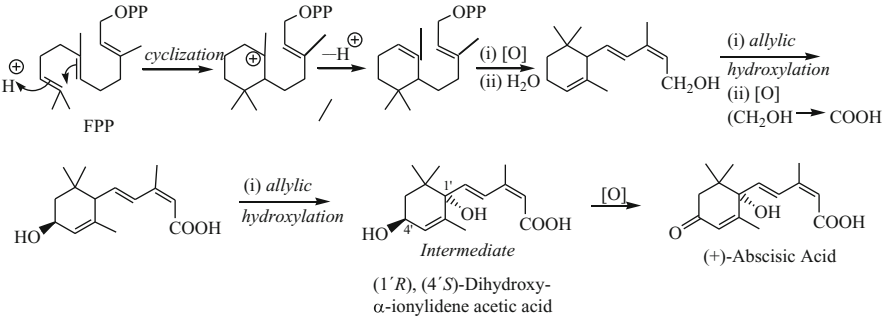


Fig. 7.68 Probable biosynthesis of abscisic acid in fungus [112]

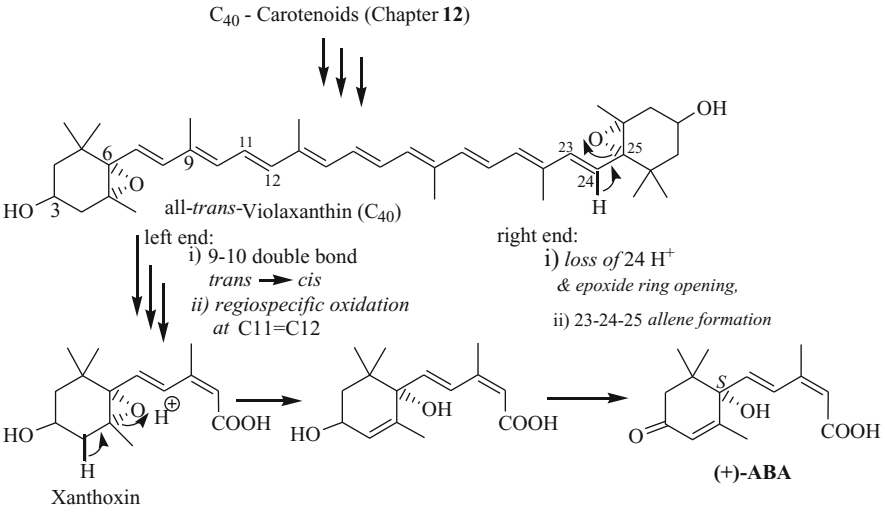


Fig. 7.69 Biosynthesis of abscisic acid in higher plants [101, 102]

involving epoxidation and cleavage of C_{40} violaxanthin to xanthoxin [101], a C_{15} precursor of abscisic acid. When [2-^{14}C]-xanthoxin was fed to cut shoots of bean and tomato it was converted to (+)-abscisic acid in high yield [111]. However, it was shown that [2-^{3}H]-MVA when fed to avocado pears ^{3}H-labeled ABA was found. Most ABA in plants might be produced directly from MVA. However, it appears that under different metabolism the above two different routes may be operative. The biosynthesis of (+)-abscisic acid in plants is outlined in Fig. 7.69.

7.11 Gossypol: An Interesting Dinaphthyl Bis-Sesquiterpene with Cadinane Skeletal Pattern

7.11.1 Introduction. Occurrence, Biological Activity

Gossypol, an interesting and unusual example of a bis-sesquiterpene, is a yellow-colored highly functionalized binaphthyl crystalline compound possessing axial chirality [113] (Sect. 2.16) arising out of restricted rotation around the aryl–aryl bond. Biogenetically it belongs to sesquiterpenoids with cadinane skeletal pattern. Its polyphenolic character apparently does not attest its association with the sesquiterpene class; rather it seems to be biogenetically associated with the polyketide pathway which is not true. However, the presence of isopropyl group, carbon content, and the methyls at right intervals of the basic C_{15} chain certainly point to it being a dimeric sesquiterpene (*cf.* Sect. 7.11.5). Some monomeric naphthol and naphthaldehyde derivatives with cadinane skeletal pattern of plant origin are known. Gossypol was first isolated in 1899 from the seeds of cotton (*Gossypium hirsutum*, Fam. Malvaceae) in chemically pure but stereochemically mixture forms. It occurs in seeds of most of the cotton plants (*Gossypium* species) (*G. herbaceum*, *G. barbadense*, *G. arboretum*) (0.1–0.6 %).

Gossypol occurs in *Gossypium* species mainly as a dextrorotatory atropisomer (Sect. 2.16.5), $[\alpha]_D$ +445 ($CHCl_3$)], and also as its (±)-form. In these species (+)-form predominates over the (−)-form. The (±)-variety may be resolved into active forms via diastereomeric Schiff's bases with various amino acid esters (*e.g.*, methyl L-phenylalanate), followed by silica gel column chromatography [114]. (±)-Gossypol complexes with acetic acid, but neither enantiomer does so. Thus (±)-gossypol can be separated from the excess of (+)-isomer by suitable treatment of cotton seed extracts. Cotton being a major plant crop, a large amount of cotton seed is obtained as a by-product, which is mainly used as a food for cattle. Cotton seed is rich in protein, but it is not used as food due to the hepatotoxic and cardiotoxic effects of gossypol in humans and animals other than ruminants. Gossypol acts as a male contraceptive; it alters sperm maturation and spermatozoid motility and inactivates the sperm enzymes necessary for fertilization. Although the antifertility effect is reversible, irreversible infertility results from the prolonged use of the drug for longer periods [115]. Some other plants of Malvaceae family, *viz.*, *Thespesia*

populnea (3.3 %) and *Montezuma speciosissima* (6.1 %), are especially rich sources of almost entirely the (+)-form that does not possess contraceptive property. However, *Thespesia danis* stands as an exception; its aerial parts contain 72 % (−)-gossypol (yield ~ 0.2 %). Gossypol has also shown antimalarial property as it inhibits the enzyme essential for the parasite *Plasmodium falciferum*.

7.11.2 Absolute Configuration of Gossypol

In the mid-1980s, the resolution of enantiomeric mixture into the active forms of gossypol has been achieved in the preparative scale.

The absolute stereostructures of the enantiomers (Fig. 7.70) were elucidated [116] by Snatzke in collaboration with Huang's group in 1988 by the application of circular dichroism and exciton chirality theory (Sect. 2.18 and 2.19.6.5).

7.11.3 Synthesis

The first stereoselective synthesis of (*P*)-(+)-gossypol has been achieved by Meyer et al. using Ullmann coupling of the appropriate bromo monomer (Fig. 7.71). The (*P*),(*S*)-atropdiastereomer formed in 94.6 % diastereomeric excess is converted to (*P*)-(+)-gossypol through several appropriate steps.

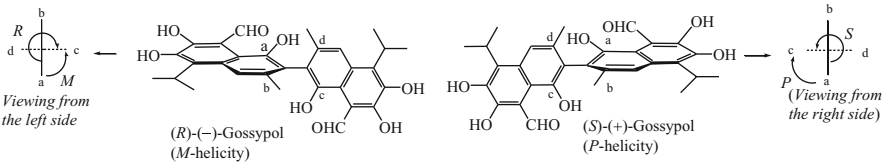


Fig. 7.70 Absolute configuration of (−)-gossypol and (+)-gossypol

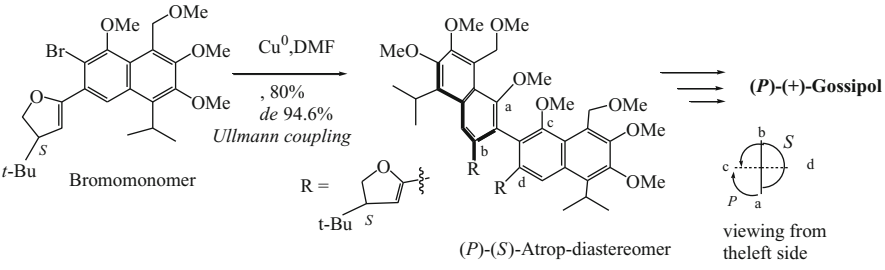
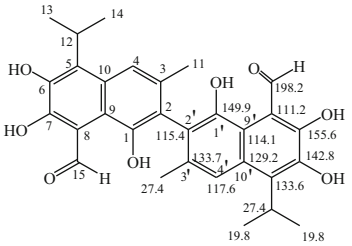


Fig. 7.71 First stereoselective synthesis of (*P*)-(+)-gossypol



Note: *The C2 axis of the molecule is orthogonal to the pivotal C2-C2' bond and to the projection plane, and passes through its midpoint. The homotopic pairs of carbon atoms , viz., 1 & 1', 2 & 2', 3 & 3', and so on, exchangeable by the C2 operation are isotropic , and have same chemical shifts.*

Fig. 7.72 ^{13}C NMR spectral chemical shifts of gossypol

7.11.4 ^{13}C NMR Spectral Data of Gossypol [117]

^{13}C NMR spectral chemical shifts of gossypol having $\mathbf{C_2}$ symmetry are shown in Fig. 7.72.

7.11.5 Biosynthesis of Gossypol [118]

From the biosynthetic experiments for gossypol [118] with three different radioactive precursors (4-^{14}C-mevalonate, 2-^{14}C-mevalonate, and 5-^{3}H-mevalonate) labeled gossypol was isolated from the incubation flask and purified as dianilinogossypol (2-CHO → 2-CH=N-Ph). The degradative study of the latter in search of incorporation (radioactivity measurements) and locations suggested that three molecules of mevalonate are involved in the formation of the C_{15}-linear precursor and the compatible folding pattern (**A**) (Fig. 7.73). The formation of the hemigossypol component occurs as with other sesquiterpenes of cadinane skeleton *via* a ten-membered ring cation, formed by 1,3-hydride shift [(**B**) → (**C**)], and various oxidative stages. During hemigossypol biosynthesis *via* deoxyhemigossypol ^{3}H-equivalent from C5 is preserved in the isopropyl chain (5-^{3}H-mevalonic acid precursor) (radioactivity measurements). Diradical coupling takes place in both possible ways to form *P*-(+)-gossypol in larger amount in the said *Gossypium* species and also *M*-(−)-gossypol, depending upon the specific enzyme present.

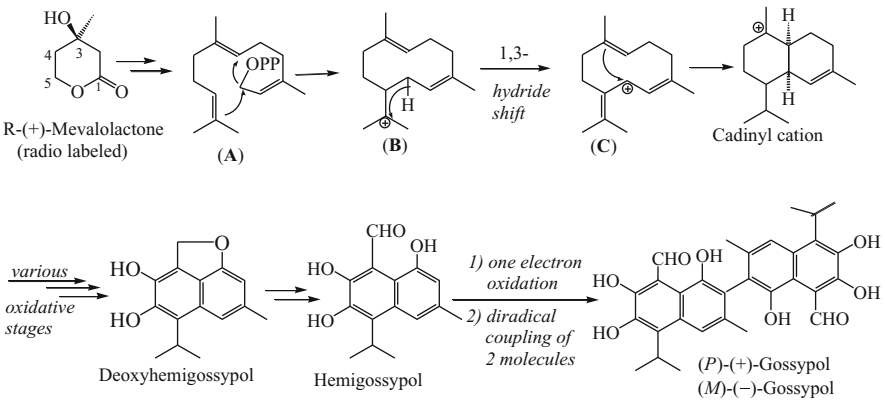


Fig. 7.73 A scheme for the biosynthesis of gossypol

7.12 Ainsliadimer A. A Novel Sesquiterpene Lactone Dimer with a Cyclopentane Ring

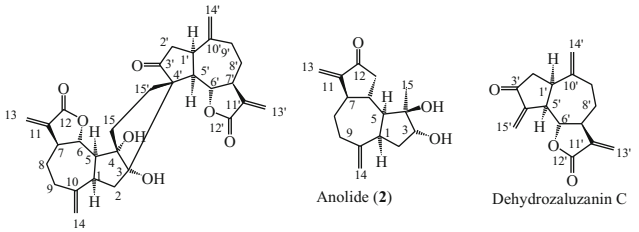
7.12.1 Occurrence. Structure. Biogenesis. Bioactivity

The genus *Ainslisea* (Compositae) comprises 70 species of which 48 are indigenous to China. Some of the species are used in folk medicine. *Ainslisea* species are well known for elaborating sesquiterpenes, and recently (2008) [119] a novel sequiterpene lactone dimer ainsliadimer A (**1**) has been isolated from a chemically virgin Southwestern Chinese *Ainslisea* species (*A. macrocephala*). Its structure has been elucidated from its extensive NMR studies and single crystal X-ray analysis (ORTEP). The dimer is generated biogenetically (Fig. 7.74) from two molecules of dehydrozaluzanin C which is chemically close to the known compound anolide (**2**), a guanolide derivative.

Ainsliadimer (1), $C_{30}H_{34}O_7$ was isolated as colorless prism, $[\alpha]_D$ +47 ($CHCl_3$); (m.p. was not reported).

7.12.2 Spectral Data [119] (Fig. 7.74)

IR (KBr): $_{max}$ 3471 (OH), 1773 (five-membered keto carbonyl), 1750, 1719 (ester carbonyl), 1262 (-O-CO stretching) cm^{-1}.



^{1}H NMR ($CDCl_3$) δ**:** *for left hand part* H1 3.15 (dd, 11.4, 12.0), H2 2.38 (dd 12.0, 13.8), H2 (1.88-1.92*), H5 2.55 (t, 11.4), H6 4.07 (dd, 9.0, 11.4), H7 2.72 (m), H8 2.22-2.24 (m), H8 1.37 (dq 6.0, 12.6) H9 2.67 (m), H9 1.88-1.92*, H-13a 6.19 (d, 3.0), H13b 5.46 (d, 3.0), H14a 5.15 (s), H14b 5.03 (s), H15a, 2.25-2.30#, H15b 2.16-2.21# * / # overlapping

For right hand part: H1' 3.04-3.06*, H2' 2.91 (dd, 16.8, 9.6) H2' 2.45 (d 16.8), H5' 3.04-3.06* H6' 4.12 (dd 8.4, 10.2), H7' 3.04-3.06*, H8' 2.25-2.30, H8'β 1.50 (dq 3.6, 12.6) H9' 2.16-2.21, H9' 2.57 (m), H13'a 6.31 (d, 3.0), H13'b 5.61 (d, 3.0), H14'a 4.96(s), H14'b 4.59(s), H15'a 2.05 (dd, 5.4, 14.4), H15'b 2.01 (dd, 7.8, 14.4) * overlapping.

^{13}C NMR ($CDCl_3$) δ : *for left hand part:* C1 41.8, C2 38.1, C3, 90.5, C4 89.1, C5 53.1, C6 82.9, C7 49.0,C8 31.3, C9 37.8, C10 147.5, C11 139.5, C12 170.0, C13 119.7,C14 113.8, C15 33.1.

For right hand part: C1' 50.1, C2' 46.9, C3' 224.9,C4' 62.2, C5' 38.9,C6' 84.0,C7' 43.7, C8' 32.8, C9' 38.9, C10' 150.0, C11' 138.6, C12' 169.0, C13' 122.0, C14' 113.7, C15' 26.3.

The assignments have been made on the basis of extensive ^{13}C NMR (including HMBC correlation) and comparison of some data with those reported in the literature for the monomers anolide and dehydrozaluzanin..

MS : m/z 505 2170 (calc. for $C_{30}H_{34}O_7$ 505.2226) based on HRESIMS (negative mode) [M–1].

Fig. 7.74 Spectral data of ainsliadimer A

7.12.3 Biogenesis

The biogenetic dimerization through hetro Diels–Alder reaction of dehydrozaluzanin C leading to the formation of ainsliadimer A might have taken place as shown in Fig. 7.75. The steps following the first one are different from the pathway shown in Scheme 1 of [119].

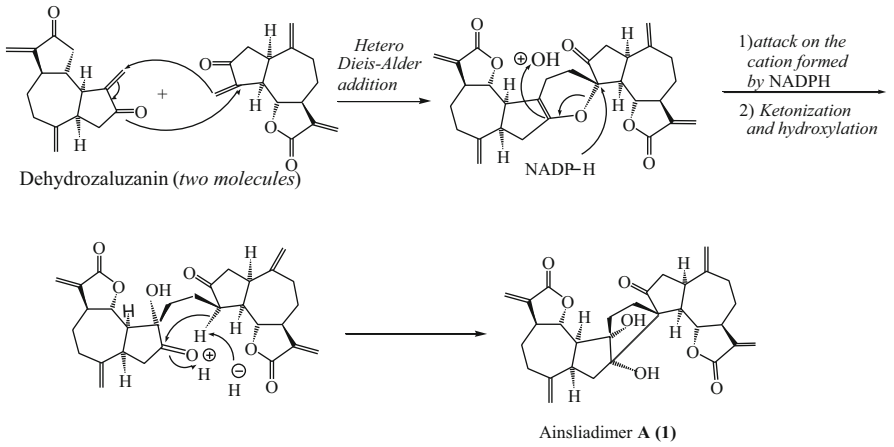


Fig. 7.75 Probable biogenetic pathway of ainsliadimer A

7.12.4 Bioactivity

Ainsliadimer A shows remarkable inhibitory effect against NO production. NO plays an important role in the inflammatory process and hence ainsliadimer A might find therapeutic use in the inflammatory diseases.

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Chapter 8
Diterpenoids (C_{20})

8.1 Occurrence. Biosynthesis

Geranylgeranyl pyrophosphate, $C_{20}H_{33}OPP$ (GGPP) (Chap. 5), an allylic isoprene tetramer diphosphate, serves as the universal precursor of diterpenoids. Acyclic diterpenes are of rare occurrence because of the participation of its rightly disposed olefinic bonds in the cation–olefin cyclization under the influence of diterpene cyclases to yield diverse skeletal patterns. Four different types of GGPP cyclases (synthases) were known till 1999 and their functional proteins have been sequenced [1–3]. They are casbene synthase, *ent*-copalyl disphosphate synthase, taxadiene synthase, and abietadiene synthase. The basic building blocks (IPP and DMAPP) of (*E,E,E)*-GGPP are of non-mevalonate origin (deoxyxylulose pathway, Chap. 5) and are biosynthesized most probably in the plastids where mevalonate or acetate precursors for IPP are not accepted [4], while the precursors for deoxyxylulose pathway are utilized.

Plant cells thus have two distinguishable compartments where IPP could be synthesized independently. Location and precursors based generated isoprenes have been earmarked for the biosynthesis of monoterpenoids, diterpenoids, and carotenes (plastids) and for sesquiterpenes, triterpenes, and steroids (cytoplasm) [4]. Though this compartmentalization is supported by labeling experiments, some controversial experimental observations of labels in products related to precursors and use of inhibitors debated the use of exclusive compartmentalization for IPP synthesis of triterpenoids of different classes [4].

A few examples of diterpenoids, both acyclic and cyclic (mono-, di-, tri-, and tetracyclic diterpenes), and their biosynthesis are schematically presented in the Figures in the sequel.

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8.1.1 Acyclic Diterpenes

Structures of some acyclic diterpenes and acyclic diterpene derivatives are shown in Fig. 8.1.

Phytol is universally distributed in green plants as a lipophilic chain (linked as an ester) component of chlorophyll (Chap. 3). It is also present as a structural part of both vitamin E and vitamin K_1. The sequence of reduction of all the double bonds save the allylic one of geranylgeraniol is not well known. However, it has been shown by labeling experiment that both geranylgeraniol and geranyllinalool are converted to the phytol part of chlorophyll in corn seedling.

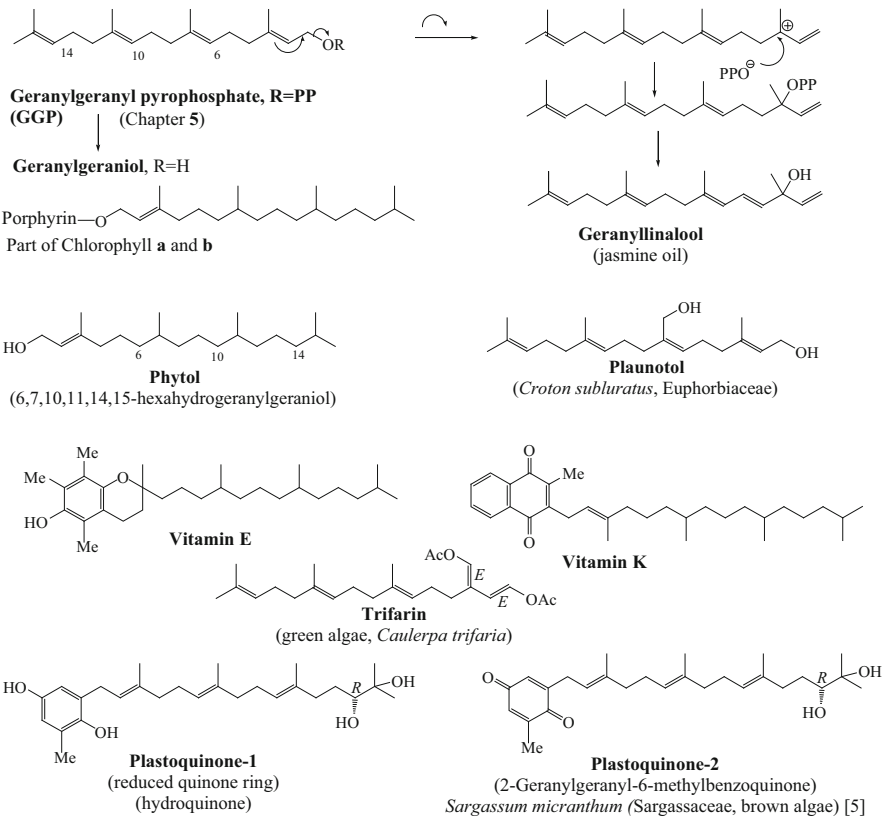
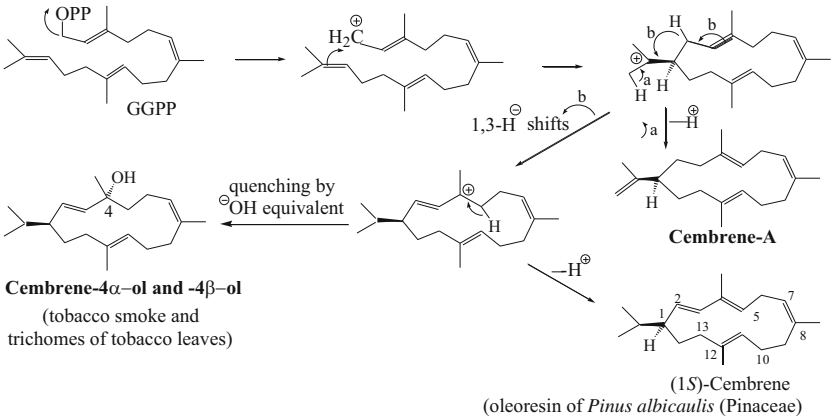


Fig. 8.1 Acyclic diterpenes and some of their derivatives



Casbene (a 14-membered monocyclic diterpene containing a cyclopropane ring):

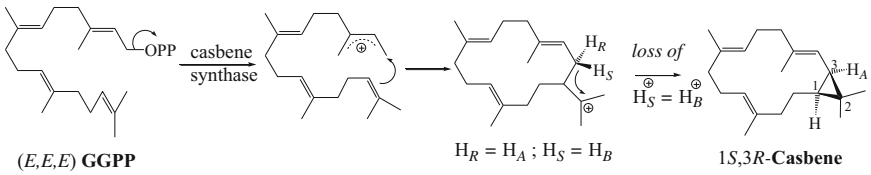


Fig. 8.2 Biosynthesis of some monocyclic diterpenes

8.1.2 Monocyclic Diterpenes

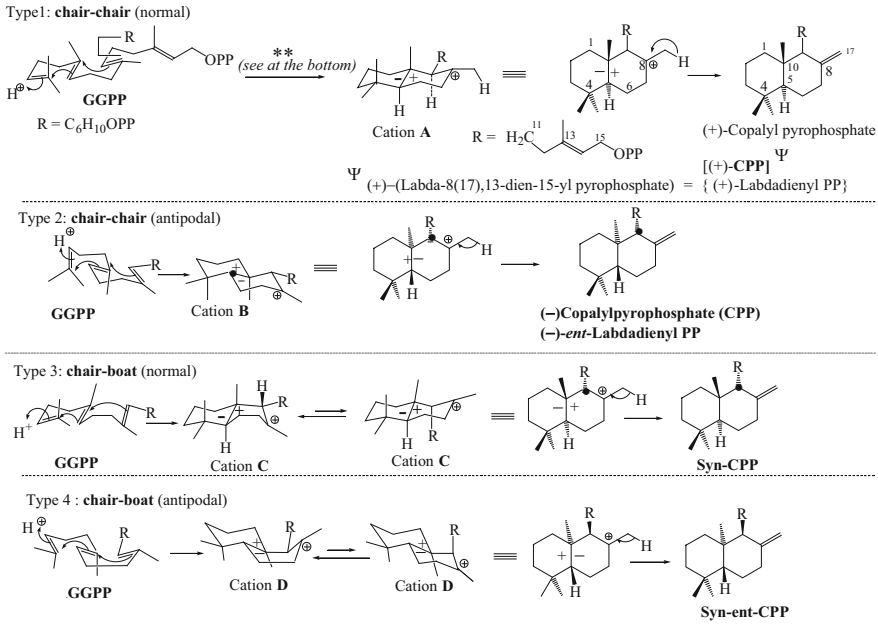
Monocyclic diterpenes occur comparatively rarely; a few of them are *cembrene* and *casbene* (14-membered carbocyclic compounds) [6] (Fig. 8.2). Casbene is a *phytoalexin*, produced by the fungus *Rhizopus stolonifer,* which grows on *Ricinus communis* (Euphorbiaceae).

8.1.3 Bicyclic Diterpenes

In case of bicyclic diterpenes decalin derivatives are formed in different stereochemical routes *via* preorganized chair–chair (normal and antipodal) and chair–boat (normal and antipodal) conformations of the substrate (*E,E,E*)-GGPP (Fig. 8.3). Thus four types of cyclizations give rise to four possible products, depending on the conformation of the prochiral substrate, as shown in Fig. 8.3.

Some Examples of Bicyclic Diterpenes: Biosyntheses of some bicyclic diterpenes are outlined in Figs. 8.4 and 8.5.

It is interesting to note that normal and enantiomeric products do occur sometimes in the same plant, e.g., sclarene and ent-sclarene are found in different specimens of *Dacrydium intermedium* [8].



** *May be cyclized in steps, mono→ bicycle since monocyclic diterpenes, retinones and labdones are reported to occur in Bellardia trixago* [7]

Note *: Torsion angle signs at both sides of the A/B ring junctions of* **A, B, C** *and* **D** *are shown* [*cf.* **Sections 2.15.1.1 & 2.15.1.2**]. *Syn-CPP and syn-ent-CPP are in equilibrium with the conformers having B rings also in chair forms, like their cations.*

Fig. 8.3 Four types of cyclizations to form two pairs of enantiomers of two diastereomeric 6–6 bicyclic diterpenes

8.1.4 Tricyclic Diterpenes

In tricyclic diterpenes of perhydrophenanthrene-type ring orientation, A and B rings could be formed by any of the types of cyclizations presented in Fig. 8.3 . This is followed by further cation–olefin cyclization to form ring C. During ring C formation the attack on the *Re* or *Si* face of 13-14 olefinic plane will leave the stereochemical information about the chirality generated. In many cases of tetracyclic diterpenes (kaurens), the unused part of the chain undergoes further intramolecular cation–olefin cyclization of the right rotamer [caused by bringing the side chain in bonding proximity of the cation by its slow movement at angles required for the purpose clockwise/anticlockwise, preferably avoiding nonbonding steric interactions (Fig. 8.6)]. 1,2-Migration, though prevalent in triterpenes, also takes place during diterpene skeletal biosynthesis [9]. Regio- and stereospecific hydroxylation, oxidations (e.g., $-OH \rightarrow > = O$, $-CH_3 \rightarrow -CH_2OH \rightarrow -CHO \rightarrow COOH$), lactonization, etc., are the common biological events that take place at some defined/undefined steps (sequence of which may not be defined) during their biosynthesis. Through some common examples these biogenetic concepts of cyclization of tri- and tetracyclic diterpenes will be illustrated in subsequent figures (Figs. 8.6 and 8.8).

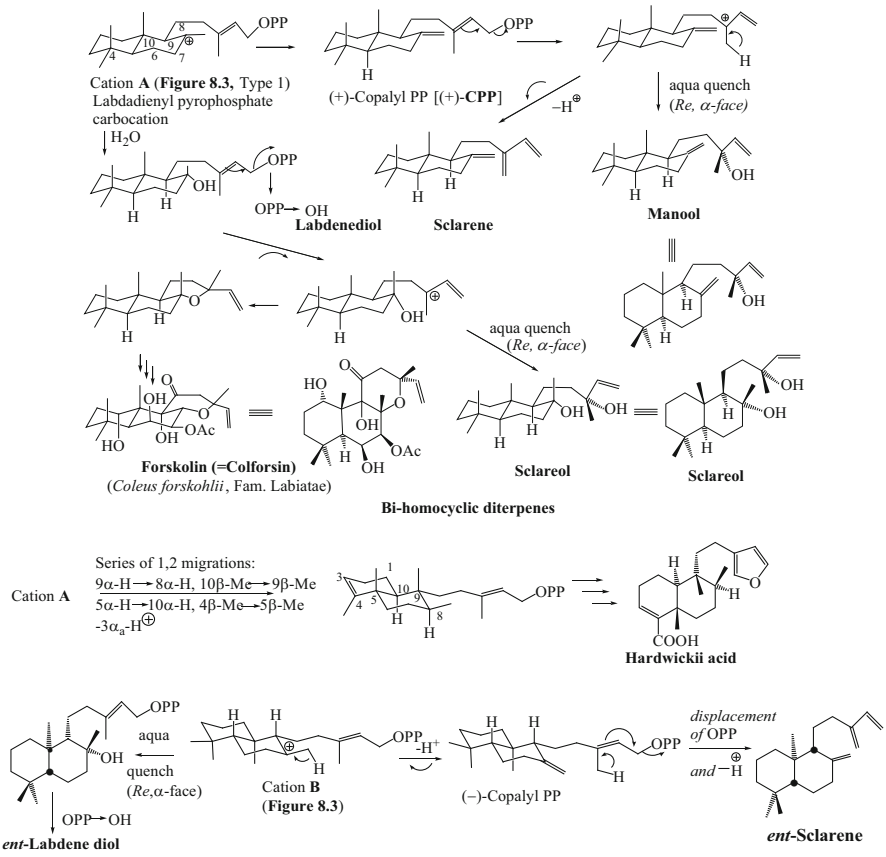


Fig. 8.4 Biosynthetic pathways of some bicyclic diterpenes

The structures of rosenonolactone and rosololactone (Fig. 8.6) have been defined by extensive chemical degradation [10]. Their absolute stereochemistry has been settled by CD and ORD studies [11, 12], as well as by X-ray crystallographic analysis [9] of the dibromo derivative of rosololactone, a close relative of rosenonolactone. Its biosynthesis, studied in detail by Arigoni [9], Birch [10], and Hanson [13, 14], is outlined in Fig. 8.6. The hydride and methyl migrations which accompany ring C formation have been documented by careful labeling studies.

Biosynthesis of abietic acid, the most important diterpene possessing abietane skeleton and abietadiene, and biosynthesis of taxol, the diterpene with unique antitumor activity, have been discussed in Sects. 8.3 and 8.4, respectively.

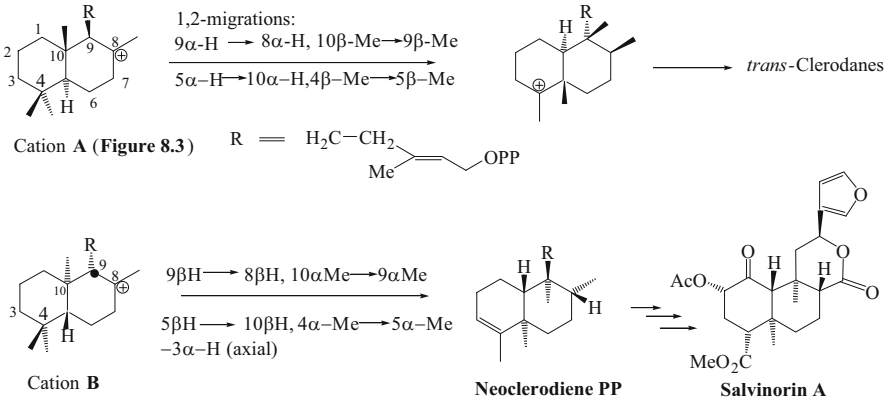


Fig. 8.5 Biosynthesis of some diterpenes derived from cations A and B

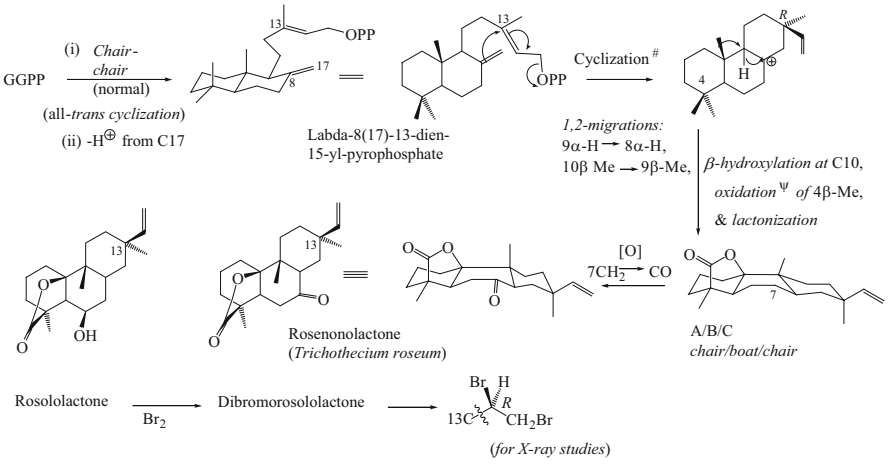
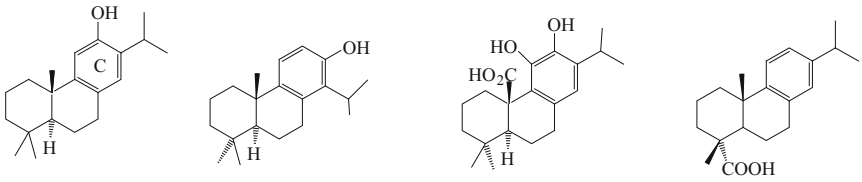


Fig. 8.6 Biosynthesis of rosenonolactone, a tricyclic diterpene lactone

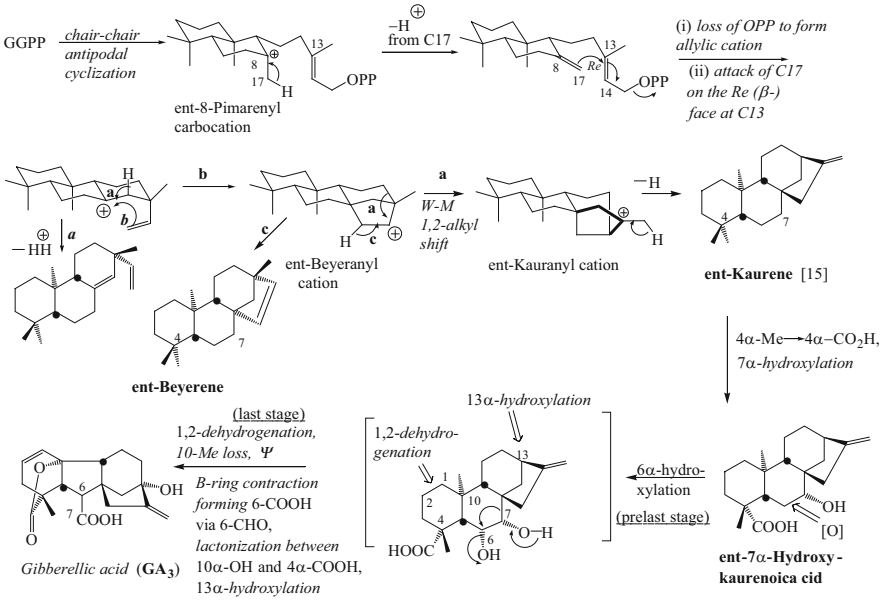
8.1.4.1 Ring C Aromatized Diterpenes

A number of ring C aromatized diterpenes of abietane skeleton are known to occur in nature (Fig. 8.7).



Ferruginol (*Podocarpus ferruginea*) **Totarol** (*Podocarpus ferruginea*) **Carnosic acid** (*Rosmarinus officinalis*) **Dehydroabietic acid**

Fig. 8.7 Some natural diterpenes with ring C aromatized



Ψ 10α-Me undergoes oxidation to 10 α-CHO followed by **B.V.** *oxidation to generate 10 α-OH. The sequence of the steps involved in the last two stages cannot be defined.*

Fig. 8.8 Biosynthesis of gibberellic acid and some common tetracyclic diterpenes

8.1.4.2 Sandarocopimaradiene

The absolute configuration of the angular methyl and vinyl group at C13 depends on the face of attack on 13–14 double bond. In case of kaurene and sandarocopimaradiene cyclization takes place by *Re*(β-face) attack of C17 on C13 of 13–14 double bond generating (*S*)-configuration of C13 (Fig. 8.8).

8.1.5 Tetracyclic Diterpenes

The probable biosynthetic pathways to sandaracopimaradiene, ent-7-hydroxykaurenoic acid, and gibberellic acid are delineated in Fig. 8.8. See Sect. 8.6.1 for some other information on gibberellins.

8.1.6 Ginkgolide Biosynthesis [4]

Ginkgolides are diterpene trilactones isolated from *Ginkgo biloba*. During their biosynthesis GGPP is first converted into an abietane-type tricyclic hydrocarbon intermediate which is subsequently converted to ginkgolides. The *t*-butyl group has been generated by ring A cleavage (Fig. 8.9). The compounds being lactones, oxidations take place densely and the gross carbon skeleton is a *W-M* rearranged product of abietane-type tricyclic skeleton.

By labeling experiment it has been shown that the C2–C3 bond of the dehydroabietane skeleton is preserved throughout the biosynthesis of ginkgolides. The C3-H_2 radical generated during C3–C2 bond cleavage picks up a neighboring hydrogen to form a methyl of the tertiary butyl group of ginkgolides.

The probable sequence of steps for the biosynthesis of the ginkgolides starting from (+)-dehydroabietane has been delineated in Fig. 8.10.

The formation of ginkgolides from primary carbocation via dehydroabietane involves a number of oxidation steps and *W-M* rearrangements (1,2-shifts) until it ends up into a densely oxygenated lactonized spiro skeleton (Fig. 8.10). (For detail [4] should be consulted.)

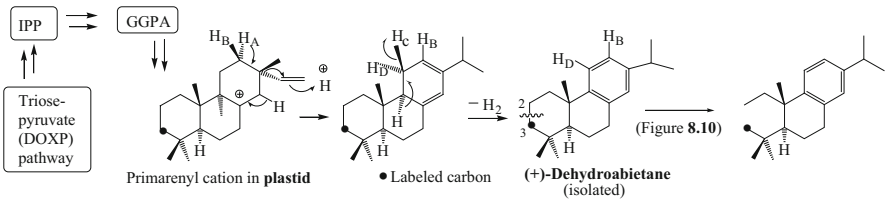
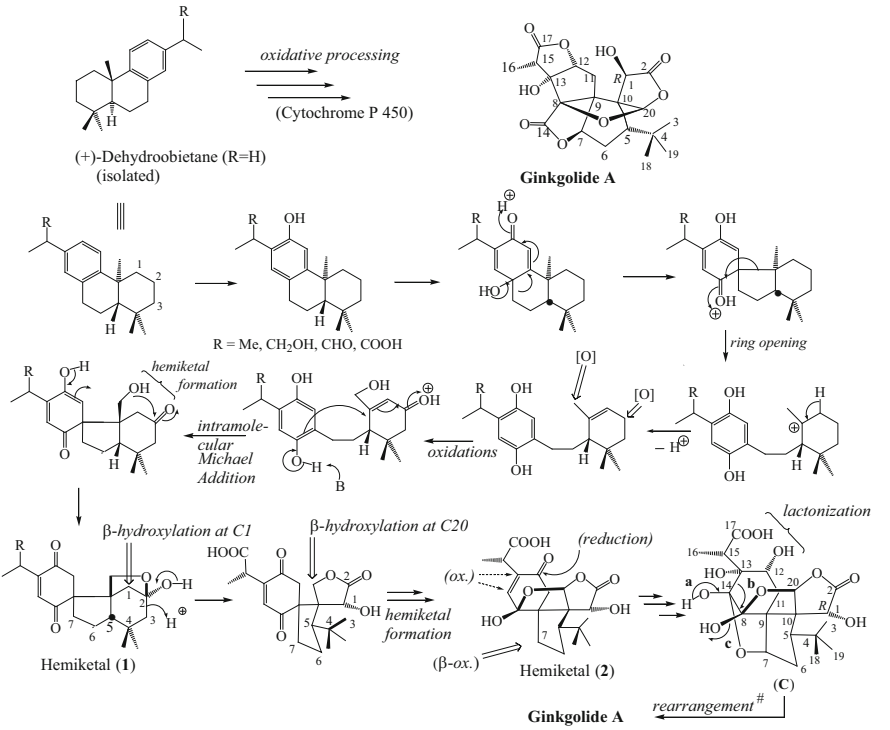


Fig. 8.9 Bioformation of the *t*-butyl group of ginkgolides



*# Mechanism of rearrangement involving lactol OH at C14 in (**C**) (step **a**), ring contraction (step **b**) and explusion of OH from C8 (step **c**) has been shown in the precursor (**C**) of ginkgolide **A***

Note : *Starting from the hemiketal (**1**) several redox steps and a well precedented rearrangement gives ginkgolide A. Most other ginkgolides (**Figure 8.12**) are accessible from ginkgolide A by hydroxylation at the appropriate positions*

Fig. 8.10 Biosynthesis of Ginkgolides

8.2 Geranylgeraniol

8.2.1 Introduction

Geranylgeraniol, $C_{20}H_{34}O$, b.p. 145 °C/0.35 mm, has been isolated and characterized in 1967 from the wood of *Cedrela toona* (Meliaceae) [16]. It has also been isolated in 1966 from linseed oil *Linum usitatissimum* (Linaceae) [17]. In *Cedrela toona*, it occurs in the free state and also as esters of fatty acids. The pleasant aroma of the wood is due to geranylgeraniol. Its structure has been deduced in the conventional way and from spectral properties [1] of the alcohol, its acetate, and oxidized product, the aldehyde. A number of syntheses have been reported in the literature. The synthesis by Trost [18] is delineated in Fig. 8.11.

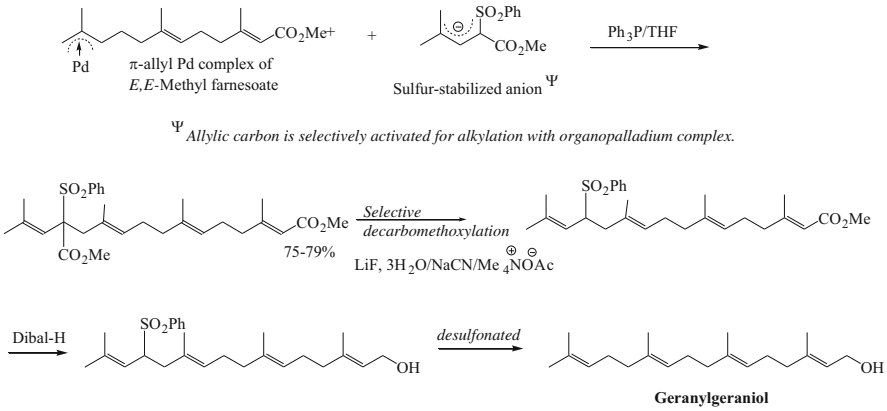


Fig. 8.11 Synthesis of Geranylgeraniol (1) [18]

8.2.2 Synthesis of Geranylgeraniol

In this synthesis [18] (Fig. 8.11) π-allylpalladium complex, which requires a soft nucleophile, is alkylated with a quite versatile sulfur-stabilized allylic carbon anion capable of undergoing selective alkylation. This is followed by selective decarboxylation, reduction of COOMe to CH_2OH, and subsequent desulfonation to yield the desired product. This strategy serves as a method for the synthesis of higher isoprenoids from the lower ones.

Spectral data [16]

IR: 3,200, 1,000 cm^{-1}, acetate, 1,745, 1,232 cm^{-1} oxidation product (aldehyde H > =O, 1,682 cm^{-1}).

^{1}H NMR (60 MHz): 9H singlet at 97 c/s, 8H singlet at 101 c/s, 6 vinylic methylenes (2 signals at 120 and 123 c/s total 12H), 2 hydroxymethine protons at 240 and 247 c/s shifted at 267 and 275 c/s in acetate, 4 vinylic protons (overlapping triplets centered ~ 307 and 324 c/s).

Mass : *m/z* 290 (M^+ 2), 217 (7), 132 (77), 104 (100), 91 (70), 69 (36).

The biosynthesis of geranylgeraniol (**1**) has been discussed in Chap. 5.

8.3 Abietic Acid and Other Resin Acids

8.3.1 Introduction. Occurrence

Abietic acid was obtained from "firs" which belongs to the genus *Abies* and hence the name. Abietic acid occurs mainly in the exudates of the pine tree bark (*Pinus pulustris, P. sylvestris* (Fam. Pinaceae)) and also in other *Pinus* species (e.g., Portuguese *Pinus pinester*, etc.). In ancient time the nonvolatile part of the exudates

(*oleorosin*) was traded in Colophon [19], one of the 12 ancient Ionian cities of Asia Minor. The oleorosin (rosin) perhaps was named colophony (rosin) after the city of its trade.

Rosin is a complex mixture of resin acids of general formula $C_{19}H_{29}COOH$. Abietic acid, the best known resin acid, is a secondary acid, since it is formed from its precursor diterpene, levopimaric acid (see Fig. 8.22), by acid treatment of colophony during the process of its isolation, when double bond isomerization of levopimaric acid takes place. Abietic acid is purified as its sodium salt.

8.3.2 Structure

Its structure has been deduced from the reactions shown in Fig. 8.12 and its synthesis (see Fig. 8.15) [20–22].

The corresponding decarboxylated hydrocarbon of the saturated tetrahydroabietic acid ($C_{19}H_{33}CO_2H$) will have molecular formula $C_{19}H_{34}$ which possesses 6H less than the corresponding open chain saturated hydrocarbon (C_nH_{2n+2}, $C_{19}H_{40}$); hence this hydrocarbon and abietic acid are tricyclic. The probable part structures based on the above reactions are shown in Fig. 8.13.

Formation of (**a**), (**b**), and (**c**) and other data from Fig. 8.12 suggest (2) as a part structure for abietic acid in which two double bonds are to be placed. Formation of (**b**) claims the placement of isopropyl group on a double bond. Since a Diels–Alder product is formed under comparatively drastic condition, the double bond must be conjugated but in *S-transoid* configuration and not in Diels–Alder friendly *S-cisoid*

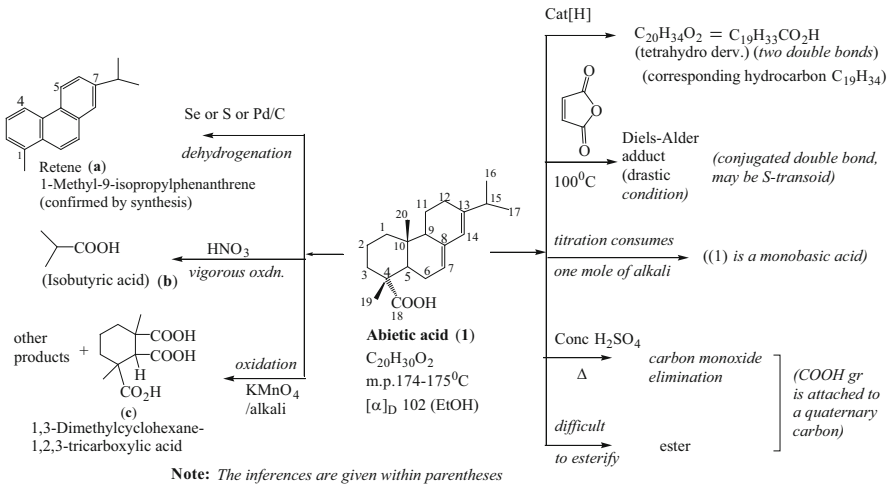


Fig. 8.12 Some reaction products of abietic acid

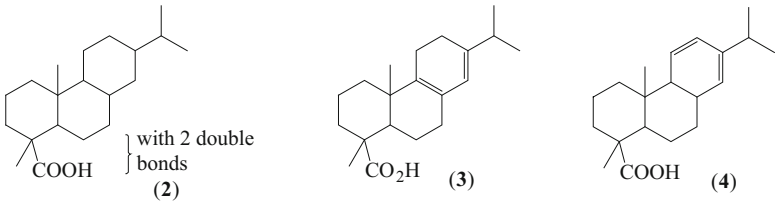


Fig. 8.13 Probable part structures for abietic acid

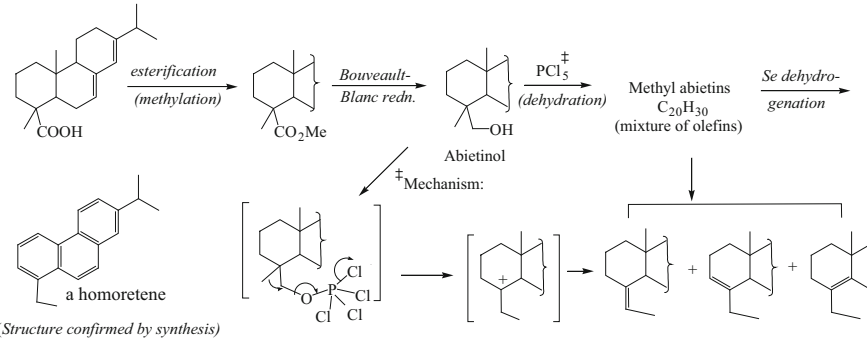


Fig. 8.14 Location of the COOH group in abietic acid

configuration. This observation excludes structures (**3**) and (**4**) and points (**1**) as the structure for abietic acid without the stereochemical features. The configuration of the double bonds is supported by the closeness of the calculated λ_{max} based on structure **1** and the observed value. λ_{max} calcd. [214 nm (heteroannular diene) + 5 nm × 4 (5 nm for each of the four alkyl substituents) + 5 nm (one exocyclic double bond) = 239 nm; λ_{max} observed = 241 nm] (cf. Sect. 4.2). Thus the configuration of the double bonds and their locations have been proved by some chemical reactions and are supported by UV and also by NMR spectral (see Fig. 8.17) data.

However, though the germinal disposition of the $-CH_3$ and –COOH groups at C4 of abietic acid is known from the formation of compound (**c**) (Fig. 8.12), it has been elegantly demonstrated through the formation of a homoretene (Fig. 8.14), the structure of which has been confirmed by synthesis to eliminate other isomeric homoretenes.

8.3.3 Synthesis

The structure of abietic acid was confirmed by its synthesis. The total synthesis has been carried out in two parts, as delineated in Fig. 8.15. The first part, the synthesis of (±)-dehydroabietic acid, was reported by Stork [20, 21] in 1956. The synthesis of abietic acid from dehydroabietic acid (the second part) was achieved by Burgstahler [22] in 1961.

Mechanisms with stereochemical features (when necessary) of the formation of the products in stages (**a**) to (**g**) in the synthesis of (±)-abietic acid (Fig. 8.15), involving several well-known and synthetically very useful reactions, are shown in Fig. 8.16, for immediate understanding of the students.

(±)-Dehydroabietic acid and its methyl ester have also been synthesized by Ireland [23] and Meyer [24] respectively.

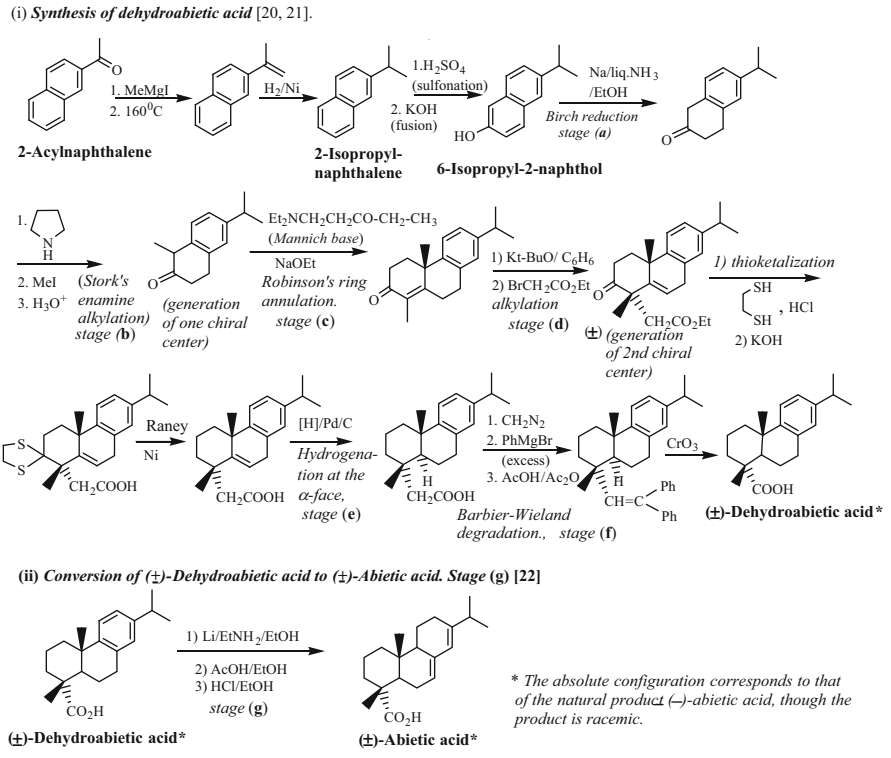
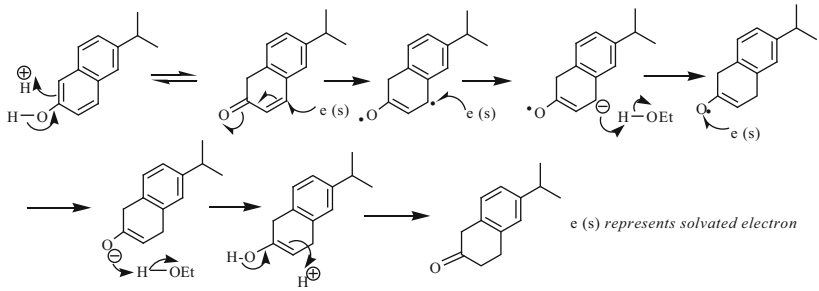
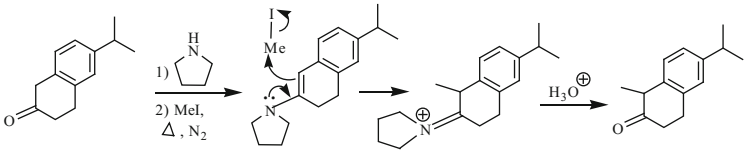


Fig. 8.15 Synthesis of (±)-dehydroabietic acid and (±)-abietic acid

Stage* (a). *Birch reduction

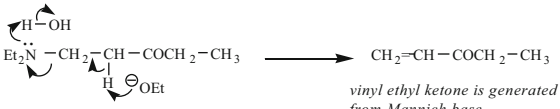


Stage* (b). *Stork enamine alkylation



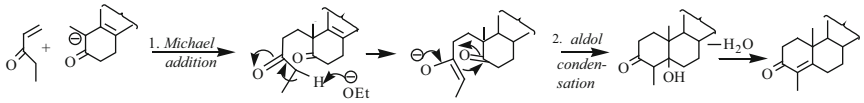
the direction of dehydration is governed by the aromatic ring (extended conjugation)

Stage* (c). *Robinson annulation (*a* [4+2] – *condensation approach*)



Mannich base

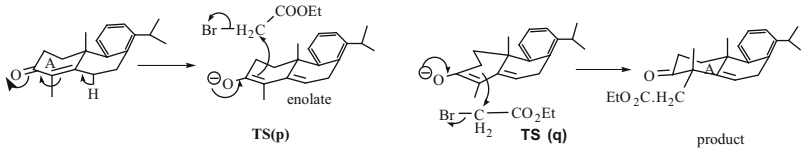
vinyl ethyl ketone is generated in silu from Mannich base



(Michael addition and aldol condensation are operative)

In step 2 the base removes the proton from the ketomethylene group. The enolate thus formed is trapped by the carbonyl of the tetrahydronaphthalone moiety resulting into the formation of a new six-membered ring; dehydration of the tertiary alcohol is extremely smooth and usually spontaneous leading to the formation of an α,β–unsaturated ketone; this conjugation is the driving force of the elimination.

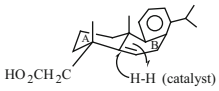
Stage* (d). *Stereochemistry of alkylation



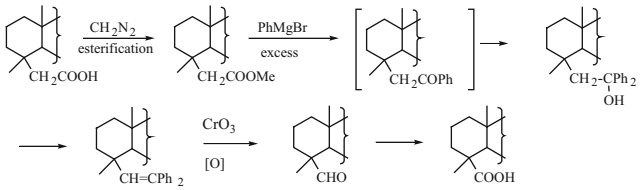
The approach of the electrophile from the β-face, orthogonal to the double bond, for head-on π-bond overlap at the location of the attack (C-4) is sterically hindered by non-bonded 1,3-diaxial type interaction with the β–methyl of the enolate in the **TS (p)**. *However, α-axial approach in the buckled A ring attaining a half-boat type conformation will suffer no such interaction in the* **TS (q),** and will allow π-bond overlap**.** *Immediately after the attack the ring of the product buckles back to the chair conformation, bringing the* CH_2COOEt *group in the equatorial orientation.*

Fig. 8.16 (continued)

Stage* (e). *Stereochemistry of hydrogen delivery: *β-face of the molecule being crowded in this conformation, the less hindered α-(Re-Si) face at C5=C6 (vide sects.* **2.9.6** & **2.9.7, Figure 2.64**) *of the molecule would be adsorbed on the catalyst surface, and thus hydrogenation would take place on the α-face making A/B ring fusion trans.*

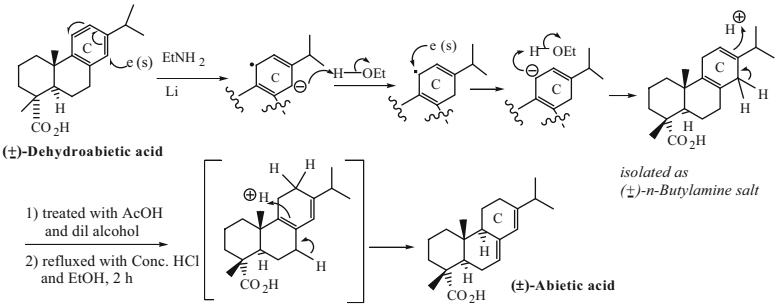


Stage* (f). *Barbier-Wieland degradation from higher to lower homologue of an acid



Stage* (g). *Conversion of (±)-Dehydroabietic acid to (±)-Abietic acid

Burgstahler and Worden [22] employed Benkeser lithium-ethylamine reduction in converting (±)-dehydroabietic acid to (±)-abietic acid.



This conversion has been achieved in good over-all yield under carefully defined conditions. One such condition is to add finely-divided lithium over a period of 15 min to a rapidly stirred solution of dehydroabietic acid taken in redistilled ethylamine [22].

Fig. 8.16 Mechanistic rationalization of some stages in the formation of (±)-dehydroabietic acid and of its conversion to (±)-abietic acid

8.3.4 Spectral Properties of Abietic Acid Methyl Ester (Fig. 8.17)

UV: [22] (for abietic acid) λmax in nm (ε) 235 (21,500), and 241.5 (23,000)

IR: [25] λmax in cm^{-1} 1735 (ester carbonyl), 1250 (-O-CO-stretching).

^{1}H NMR: (δ) [25] (300 MH_3) ($CDCl_3$) δ = 0.82 (3H, *s*, H_3-20), 1.00 (*d*, J = 6.9 Hz, 3H, H_3-16 or H_3-17), 1.01 (*d,* J = 6.9 Hz, 3H, H_3-17 or H_3-16), 1.25 (*s*, 3H, H_3 -18), 2.16-2.29 (*m*, 1H, H15), 3.63 (*s*, 3H, ester C H_3), 5.33-5.41 (*m,* 1H, H7), 5.77 (*s,* 1H, H14).

^{13}C NMR [25]**:** (75.6 MHz, $CDCl_3$) : δ = 14.03 (C20), 17.01 (C19), 20.85 (C16 or C17), 21.42 (C17 or C16), 22.46 (C11), 25.68 (C6), 27.48 (C12), 34.53 (C10), 34.88 (C15), 37.12 (C3), 38.33 (C1), 45.10 (C5), 46.59 (C4), 50.94 (C9), 85 (C21 ester methyl), 120.62 (C14), 122.36 (C7), 135.53 (C8), 145.33 (C13), 179.01 (C1 8) (for numbering see structure 1).

(APC1-MS [25]**:** m/z (%) 317.2 (100) $(M+1)^+$, 315.2 (77), 313.2 (47), 257.2 (25, (M+1) –$HCOOCH_3$).

Fig. 8.17 Spectral properties of abietic acid methyl ester

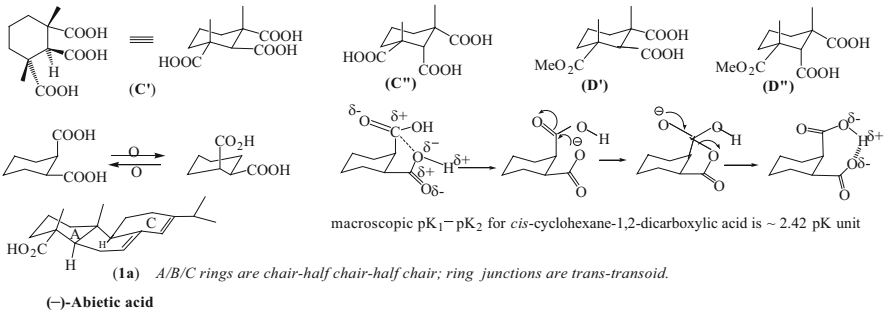


Fig. 8.18 Field effect: dissociation constants of *cis*-cyclohexane-1,2-dicarboxylic acid

8.3.5 Stereochemistry and Molecular Conformation

Compound (**c**) (cyclohexane-1,3-dimethyl-1,2,3-tricarboxylic acid, Fig. 8.12) contains three of the four asymmetric centers of (–)-abietic acid, but has been found to be optically inactive though these three chiral centers of (–)-abietic acid were not touched during its formation. Two centers are quaternary and the third one does not have any provision for racemization. Hence in all probability, the molecule should have a plane of symmetry and should thus be expressed as (C′) or (C″) (Fig. 8.18). This is one of the major problems that gave birth to *conformational analysis* [26]. Barton and Schmeidler [27] were able to determine the stereochemistry of the A/B ring junction in abietic acid and hence in a number of other resin acids. They used measured values of the dissociation constants of the unsymmetrical monomethyl ester of the tricarboxylic acid (**C′**) or (**C″**). It was concluded that the difference between the "microscopic" pK_1 and pK_2 values for the asymmetric monomethyl ester (**D′**) or (**D″**) (Fig. 8.18) was 1.11 pK units. This agrees well with the analogous value of 1.15 pK units for *trans*-cyclohexane-1,2-dicarboxylic acid and differs from that (1.80 pK units) for *cis*-cyclohexane-1,2-dicarboxylic acid. The monomethyl ester should therefore be (**D′**) and not (**D″**). The secondary COOH group in the tricarboxylic acid (**C′**) is thus shown to be *trans* to the other two

COOH groups, which had earlier been shown to be *cis* to each other. Thus A/B ring junction in abietic acid and related compounds was shown to be *trans*.

Dissociation constants of cyclohexane-1,2-dicarboxylic acids. In general, the closer in space the COOH groups are, greater is the difference of the pK values; the positive dipole of one of the COOH groups eases the departure of the proton from a closely proximate OH of another COOH by a field effect. But the negative charge of the COO^- ion in the ionized acid prevents the easy departure of the second H^+ from the other proximate COOH group, as delineated in Fig. 8.18.

The relative stereochemistry in abietic acid is shown to be *trans*-transoid by its resistance to acid isomerization. Had this been a *trans-cisoid* system, it would have picked up H^+ from axial face through transient double bond isomerization to form the stereochemically comfortable conformation. The molecular conformation (–)-abietic acid may thus be represented as (**1a**) (Fig. 8.18).

8.3.6 A Few Interesting Reactions

A few interesting reactions of abietic acid will be discussed (Figs. 8.19, 8.20, and 8.21).

(i) When dehydroabietic acid is treated with conc. H_2SO_4 at low temperature, a conformationally interesting product is obtained (Fig. 8.19).

The ring size of the lactone was much debated [29]. However, Barton gave the correct structure with a five-membered lactone ring (γ-lactone). He further suggested that the reaction should proceed through an olefinic acid and the relationship of C4-COOH group and C5-H should be changed from *cis* to *trans* relationship in order to form a sterically comfortable lactone (Fig. 8.19). His

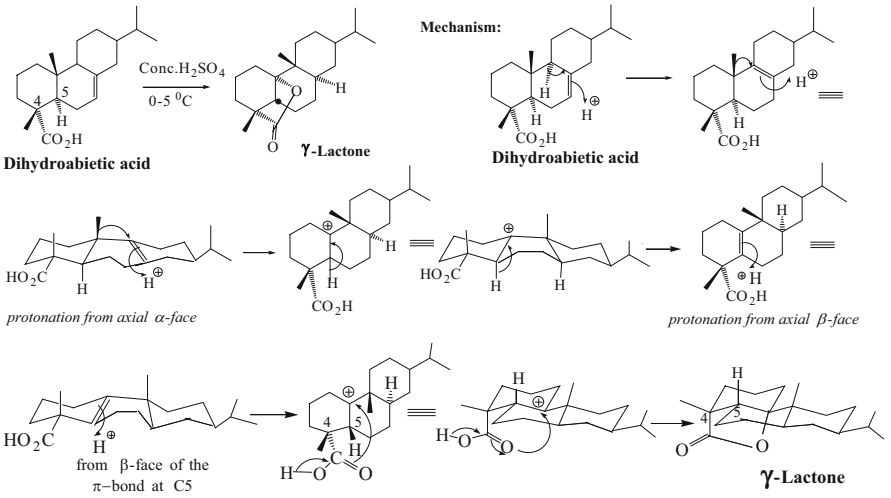


Fig. 8.19 Mechanism for the formation of γ-Lactone from dihydroabietic acid

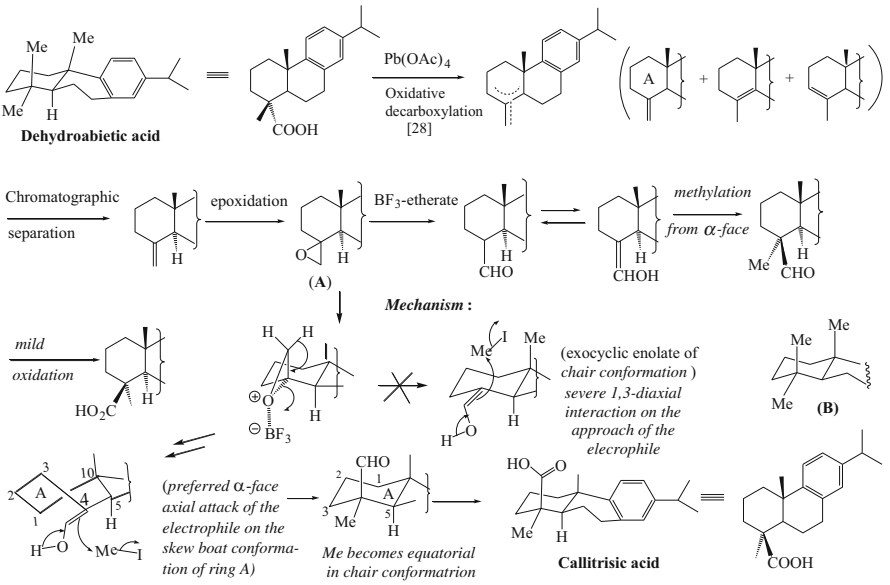


Fig. 8.20 Conversion of dehydroabietic acid to callitrisic acid

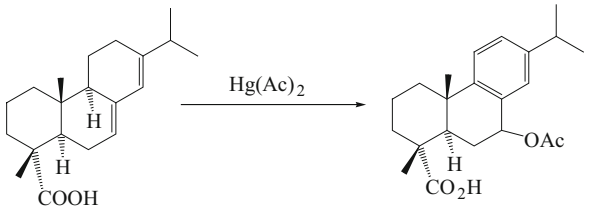


Fig. 8.21 Action of mercuric acetate on abietic acid

conjecture has been confirmed by X-ray crystallographic studies of the lactone formed from dihydroisopimaric acid under similar condition [30].

(ii) C4-epimerization of Dehydroabietic Acid. Conversion of Dehydroabietic Acid to Callitrisic Acid [31] (Fig. 8.20) .

Incidentally, it may be mentioned that in cases of C4-axial acids (like callitrisic acid) the C10-Me suffers deshielding by ~17 cps because their 1,3-diaxial relationship brings the C10-Me under the deshielding zone of the carboxyl carbonyl in their average conformation (Me comes in the plane of the carbonyl). Equatorial COOH which has no such relationship shows only a deshielding of ~4 cps (compared to **B**), thus leaving a net deshielding of ~13 cps for axial acids [32].

(iii) One interesting example is where Hg^{II} acetate incorporates allylic oxidative rearrangement and simultaneous dehydrogenation in abietic acid.

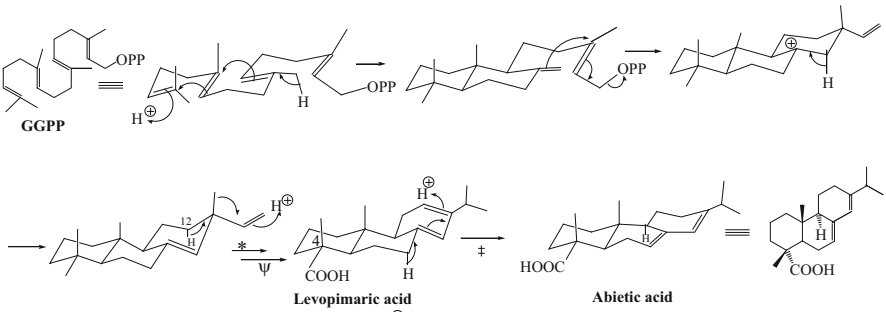


Fig. 8.22 Probable biogenesis of levopimaric acid and its isomerization to abietic acid

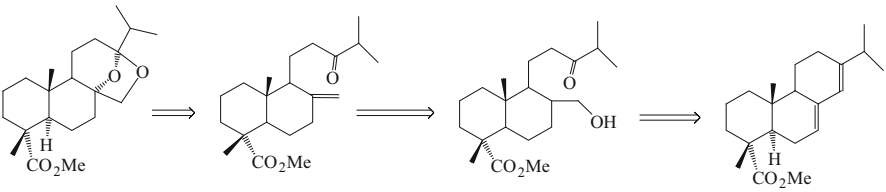


Fig. 8.23 Retrosynthesis of ambraketal

8.3.7 Biosyntheses of Abietic Acid

Levopimaric acid synthase catalyzes the stepwise formation of levopimaric acid from geranyl geranyl pyrophosphate (GGPP) folding it properly and proceeding through the steps shown in Fig. 8.22. Levopimaric acid undergoes acid-catalyzed rearrangement to produce abietic acid. The latter is considered to be a secondary resin acid and thus the isomerization of the homoannular diene to heteroannular diene may take place in vitro during isolation.

8.3.8 Uses as a Synthon

Ambergris, a metabolic product of the sperm whale, is long known as one of the most valuable animal perfumes (besides civet, musk, and castreum), and its tincture has been used as a fixative for rare perfumes. It also helps in making the fragrance of other perfumes to last long. Ambraketal (Fig. 8.24) is among the most expensive synthetic equivalents of the scarce natural ambergris. Ambraketal possesses long-lasting ambergris-type fragrance and many efforts have been made for the synthesis of ambraketal or its analogues all starting from (–)-abietic acid [25].

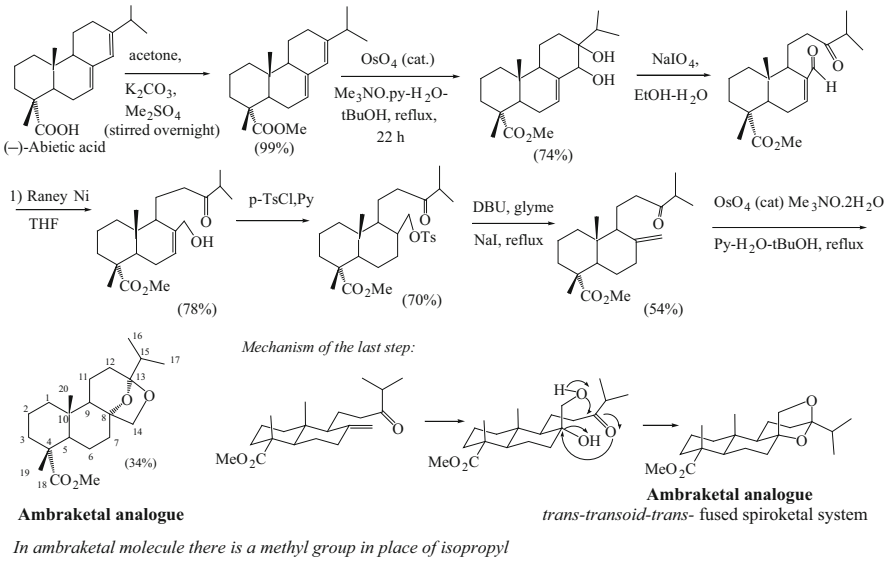


Fig. 8.24 Synthesis of an ambraketal analogue [Methyl (8*R*,13*S*)-8α,13:13,14-diepoxiabiet-19-oate] [25]

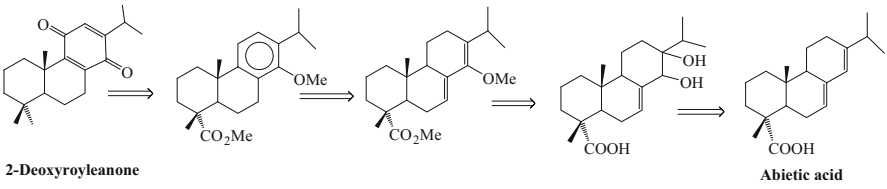


Fig. 8.25 Retrosynthesis of 12-deoxyroyleanone

A synthesis of an ambraketal analogue has been designed by CeuCosta et al. [25] starting from (–)-abietic acid. The synthetic strategy is shown in Fig. 8.23 and the actual synthesis is delineated in Fig. 8.24.

The first enantiospecific synthesis of the antileishmanial, 12-deoxyroyleanone (Fig. 8.25), has been achieved from (–)-abietic acid in 11 steps [33] in a 25 % overall yield. The strategy of this semisynthesis is shown in Fig. 8.25 and the actual conversion is shown in Fig. 8.26.

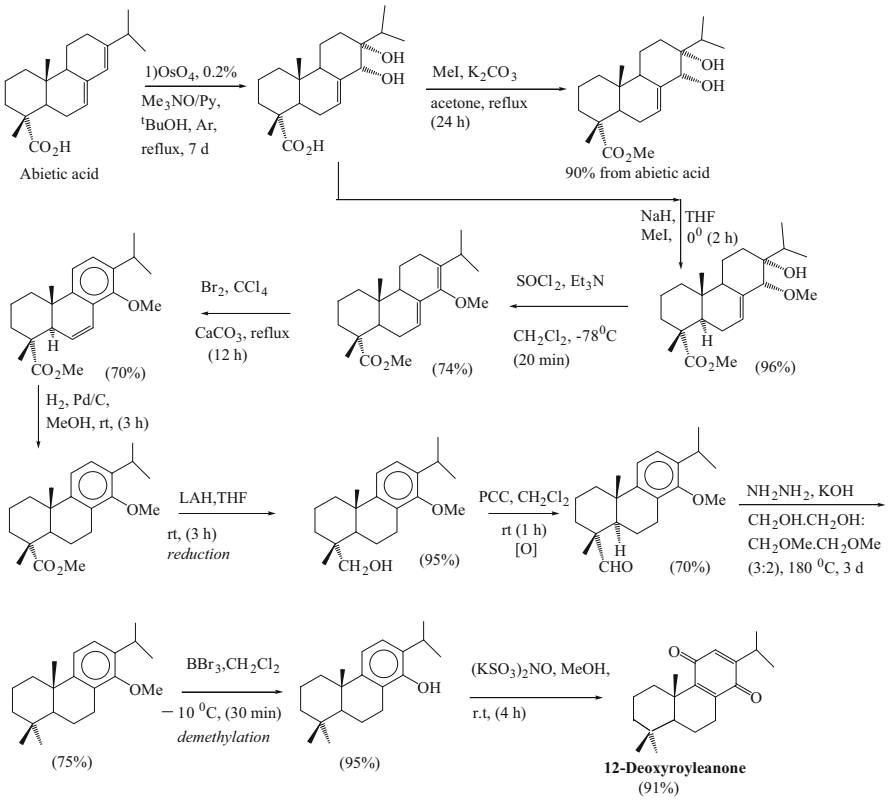


Fig. 8.26 Synthesis of 12-deoxyroyleanone starting from abietic acid

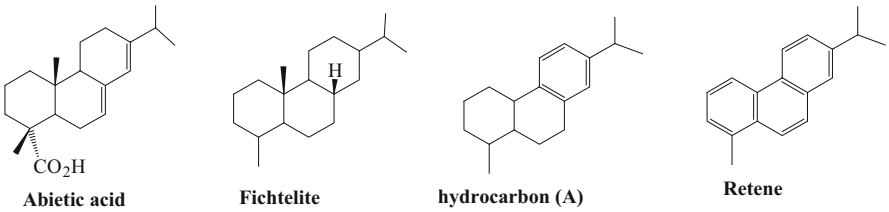


Fig. 8.27 Probable diagenetic products of abietic acid

8.3.9 Diagenetic Products of Abietic acid

Natural products which suffer chemical reactions over geological time, caused by thermal, catalysis bacterial, and other processes, are known to yield diagenetic products and the phenomenon is known as diagenesis. Abietic acid, present in the buried pine trees, results in the diagenetic products fichtelite and retene. The last two products are unknown as natural products [34].

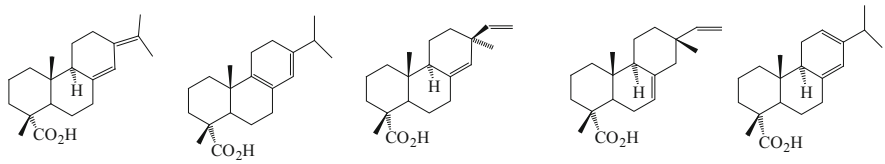


Fig. 8.28 Some resin acids related to abietic acid

It is noteworthy to mention that a hydrocarbon (A) (Fig. 8.27) has been isolated from pine forest soil. Structural similarity suggests that it could be a diagenetic intermediate in the conversion of abietic acid to retene, and hence it is suggested that compound (A) should be sought in places where fichtelite and retene are obtained (Fig. 8.27) [34].

8.3.10 Structure Diagrams of Some Related Resin Acids

Some common diterpenes resin acids structurally related to abietic acid are given in Fig. 8.28.

8.4 Taxol®: A Nitrogenous Diterpene Ester with Unique Antitumor Activity

8.4.1 Introduction. Occurrence

As a part of the screening program of the antitumor agents of plant origin, initiated in 1960 under Dr. Jonathan L. Hartwell, NCI (The United States National Cancer Institute, Bethesda, MD), extracts of bark, twigs, leaves, and fruits of *Taxus brevifolia* (Pacific Yew) (Fam. Taxaceae), one of the slowest growing trees, were examined in 1966 and identified as the most important samples [35]. The bark was first collected by a botanist Arthur S. Barclay in 1962 for chemical and biological studies [36]. Around 0.5 g of Taxol$^{(R)}$ (a registered trademark for BMS), m.p. 213–216 °C (dec.), $[\alpha]_D^{20}$ – 49 (MeOH), was isolated from 12 kg of air-dried stem and bark [35]. The name was given before the structure was known. However, it was found to contain an alcoholic OH group and hence the name is justified. All 11 species of *Taxus* contain it with varying yields; *Taxus brevifolia* ("yew") is one of the seven major species. The locations of the species are scattered and also sometimes in remote areas [37]. The production of taxol and taxane by *Taxomyces* andreanae, an endophytic fungus of Pacific yew, has been reported. The yew tree has long been known for making bows. The bark extract is recorded to be poisonous. Cativolcus, a chieftain of a Gaelic tribe, committed suicide by drinking tea

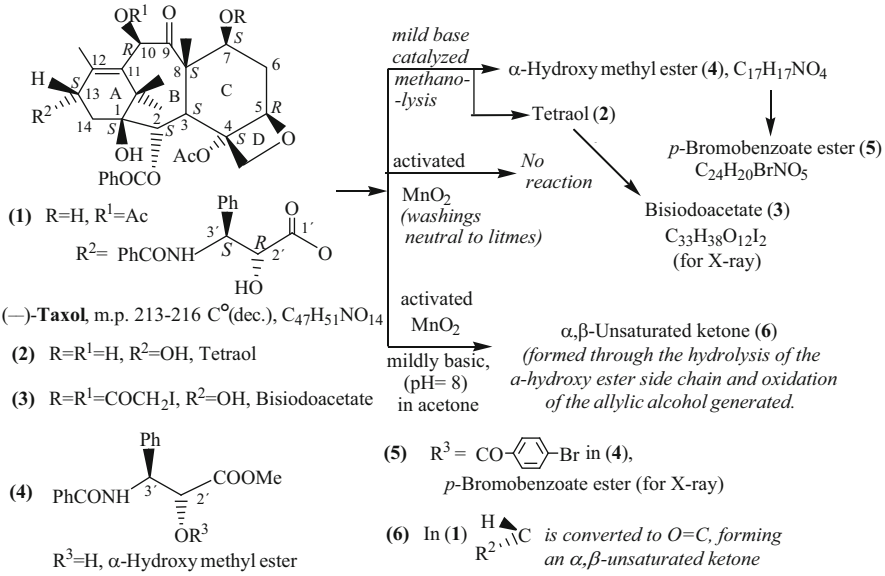


Fig. 8.29 Reaction products of taxol and their derivatives for structural elucidation

made from the bark of the yew after his defeat at the hands of Roman legions [36], as recorded by Julius Caesar in one of his books. The genus name *Taxus* perhaps had its origin from two Greek words, "toxo" (bow) and "toxikon" (poison) [36]. Taxol's remarkable antitumor activity has been discussed in Chap. 33.

8.4.2 Structure [38–40]

A high-resolution mass spectrum of taxol showed the molecular ion peak at *m*/*z* 853 and the elemental composition to be $C_{47}H_{51}NO_{14}$. It is a highly oxygenated molecule of a complex structure. Unfortunately suitable crystalline halogenated derivative could not be obtained [38] for X-ray crystallographic analysis. The structure **1** of taxol has been determined from the reactions shown in Fig. 8.29. The formation of (**6**) defined the location of the ester with nitrogen containing α-hydroxy acid at C13. Taxol was subjected to a mild base-catalyzed methanolysis at 0 °C affording a nitrogenous α-hydroxy methyl ester (**4**) and a tetraol (**2**). Their structures and absolute configuration were solved by X-ray crystallographic analysis of their *p*-bromobenzoate (**5**) and bisiodoacetate (**3**) derivatives. Its inertness to carefully washed (neutral to litmus) and activated MnO_2 indicated that the two esters were located at the allylic positions C10 and C13. The 11 stereogenic centers of taxol have 1*S*, 2*S*, 3*S*, 4*S*, 5*R*, 7*S*, 8*S*, 10*R*, 13*S*, 2′*R*, and 3′*S* configurations (Fig. 8.29), as revealed from X-ray analysis. The chemistry of taxol and related taxoids has been reviewed [39] in detail in 2003.

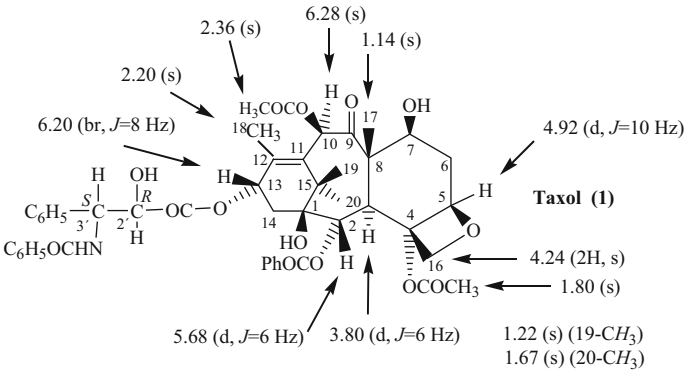
The spectral data of taxol (**1**) are given in Figure **8.30**

UV: λ_{max} (MeOH) 227 nm (ε 29,800), 273 (1700)

IR: ν_{max} (Nujol) : 3300-3500 (OH, NH), 1730 (ester), 1710 (ketone), 1650 (amide) cm^{-1}

$\mathbf{M}^+$ at *m/z* 853 (calcd. for $C_{47}H_{51}NO_{14}$, 853)

^{1}H NMR spectral data (100 MHz, $CDCl_3$, TMS):



**In the reference [38] the absolute configuration of C3′ has been drawn to have (R)configuration by oversight.*

Fig. 8.30 The spectral data of taxol

It is worth mentioning that Halsall [41] reported in 1970 the isolation (from *Taxus baccata*) of the diterpene baccatin-V, a naturally occurring oxetane similar to the tetraol (**2**), differing only in the configuration of the OH group at C7, i.e., the 7-epimer of the tetraol (**2**). A few other taxane derivatives have been reported from the same plant [42] in 1969.

8.4.3 Spectral Data [35, 38]

The spectral data of taxol (**1**) are given in Fig. 8.30.

8.4.4 Conformation of Taxol (1C)

The following characteristic features of the preferred conformation (**1C**) (Fig. 8.31) of the (−)-taxol molecule are evident from a careful analysis of its Dreiding model.

The 6-membered ring A assumes a half-chair-like conformation. The C13α–O bond of the side chain is having a pseudoaxial type (a′) orientation in ring A. The carbon atoms C15, C11, C12, and C13 are almost planar, while C1 and C14 are nonplanar.

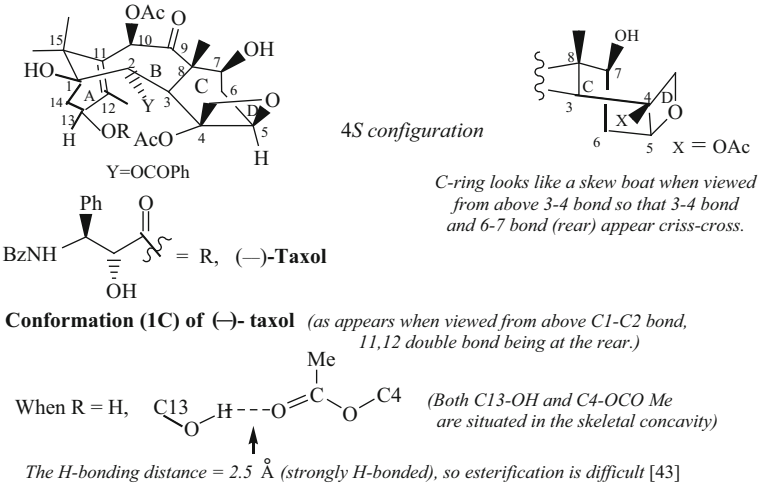


Fig. 8.31 Conformation of the taxol molecule

1. The 8-membred ring B is quite flexible. However, its preferred conformation is shown (one perspective view). In it the nonbonded interaction between 8-β-methyl and 15β-methyl is completely avoided. This interaction becomes severe in some other conformations of the flexible ring B.
2. In ring B, in the preferred conformation (**1C**) the carbon atoms C3, C8, C9, C10, and C11 come almost in one plane, while C2, C1, and C15 are above this plane.
3. The ring C looks like a skew boat (Fig. 8.31) when viewed from a particular direction.
4. The oxetane ring D is almost perpendicular to the puckered plane of ring C.

Thus the upper half of the taxol molecule forms its convex, more exposed surface, while the lower half constitutes its concave, more crowded face. Among other factors the shape of the taxol molecule or its any analogue presumably has an important role in its bioactivity (cf. Sect. 33.6.3 and [90] and [91]).

8.4.5 Synthesis of Taxol

The remarkable antitumor activity of taxol and its dense substitutional decoration along the periphery of the gross tricyclo[9.3.1.$0^{3,8}$]pentadecane skeleton involving seven oxygen atoms directly hooked to the skeleton as C-O bonds with their configurational identity and one oxygen atom as C=O throw a great challenge to the synthetic organic chemists community. Nearly 30 or more groups were involved in that hot race [36] and it became a very crowded field for accomplishing the same goal. Nearly 23 years (1971–1994) after its reported isolation and structure elucidation [38] its total synthesis first appeared in the literature [36, 44–49]. Of the first

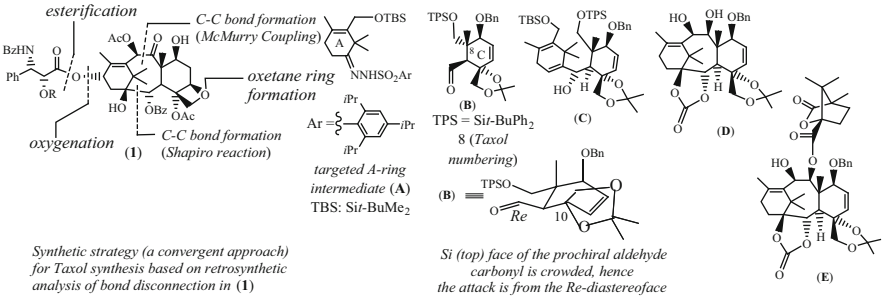


Fig. 8.32 Synthetic strategy for taxol (K. C. Nicolaou) [44]

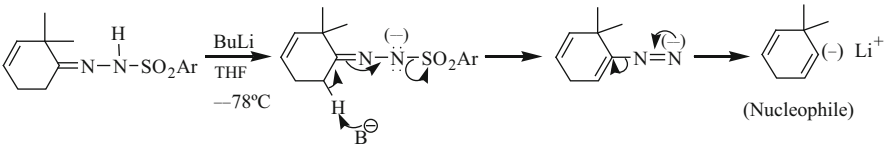
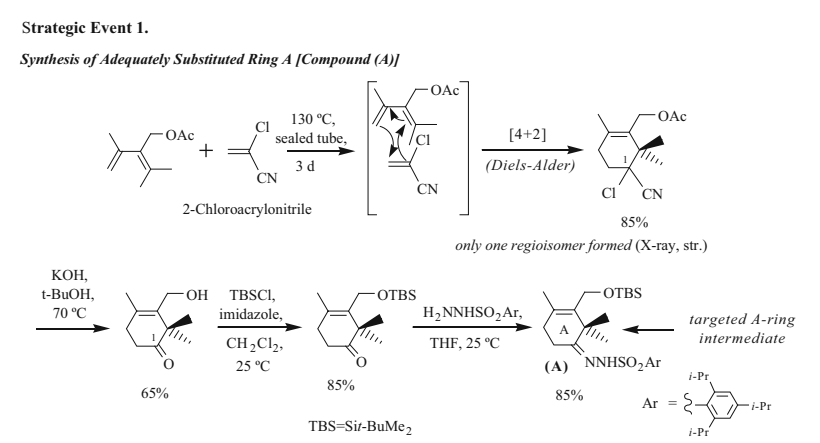


Fig. 8.33 The formation of the nucleophile used in Shapiro reaction

two syntheses of taxol the one by Nicolaou et al. [44–47] will be discussed briefly. The other synthesis by Holton et al. appeared in the literature within a span of few days only [48, 49]. Later on, many more syntheses of taxol have been reported. The ecstasy and agony during the conquest of taxol have been elegantly narrated by Nicolaou and Guy [36], which is an inspirational story to the research students.

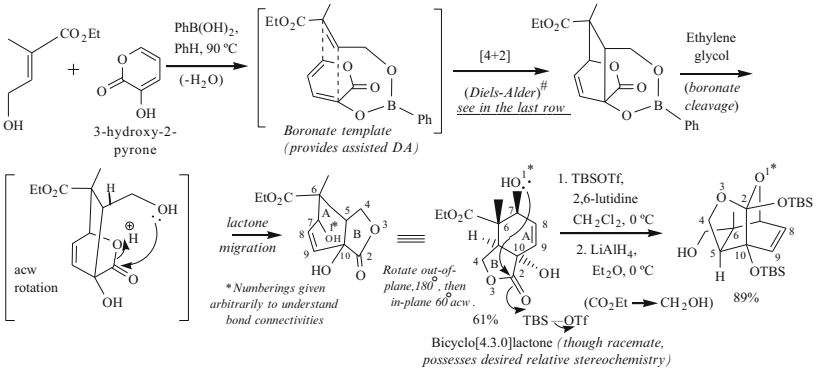
Strategy for the Synthesis of Taxol by Nicolaou: The synthetic strategy as given and followed by the authors [36, 44–47] is shown in structure (**1**) (Fig. 8.32). Intermediates are identified chemically and by X-ray analysis when needed. The *chronology of the strategic events in the synthesis of taxol* is summarized below:

(1) Construction of appropriately functionalized ring A (**A**)
(2) Synthesis of appropriately functionalized ring C (**B**), another targeted intermediate (use of Narasaka protocol of phenylboronic acid)
(3) Union of ring A and ring C precursors [(**A**) and (**B**)], Fig. 8.32) to form an intermediate (**C**) using *Shapiro reaction,* in which ring A hydrazone behaves as a latent nucleophile (Fig. 8.33). The latter could react with the aldehyde group of ring C precursor. The formation of only one stereoisomer is caused by the attack of the nucleophile from the less hindered *Re* (*β* -diastereoface of the chelated aldehyde (see Fig. 8.34, Strategic Event 3, 1st row). The 8-methyl offers hindrance to the approach of the nucleophile from *Si* (α)-diastereoface. The alcoholic OH at C2 being allylic is useful in subsequent functionalization at C1.
(4) Prior to the C9–C10 coupling a hydroxyl group is generated at C1 and blocked as carbonate with C2-OH. The carbonate will serve as the potential source of



Strategic Event 2.

Synthesis of Appropriately Substituted Ring C [Compound (B)] *(Application of Narasaka reaction using boronate as a template to tether the diene and dienophile in a specific orientation (endo) for allowing rigorously regiochemically controlled intramolecular Diels-Alder regioisomer formation)*



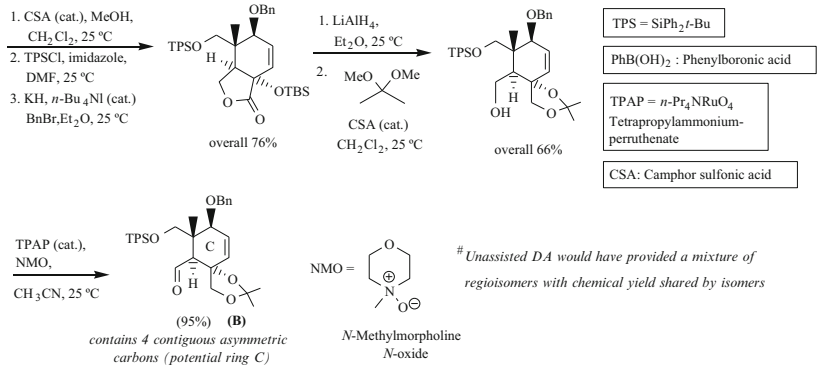
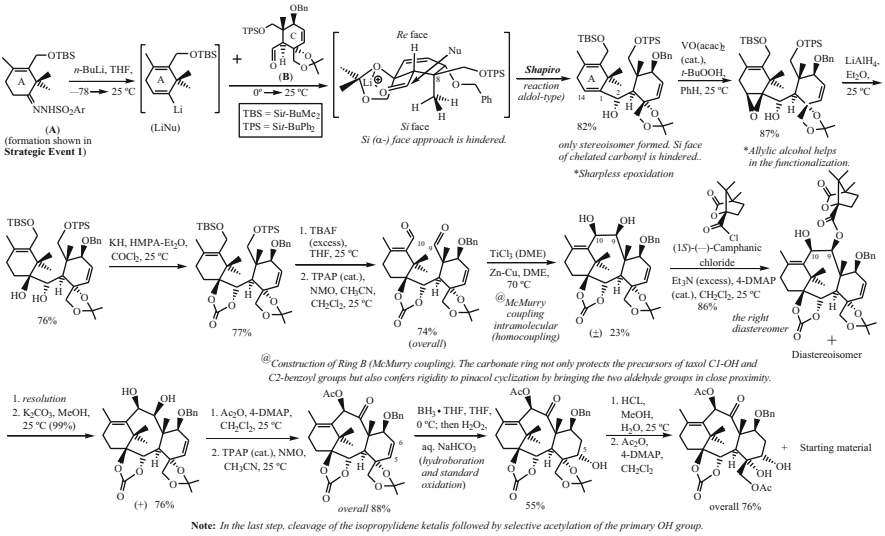


Fig. 8.34 (continued)

Strategic Events 3-7.

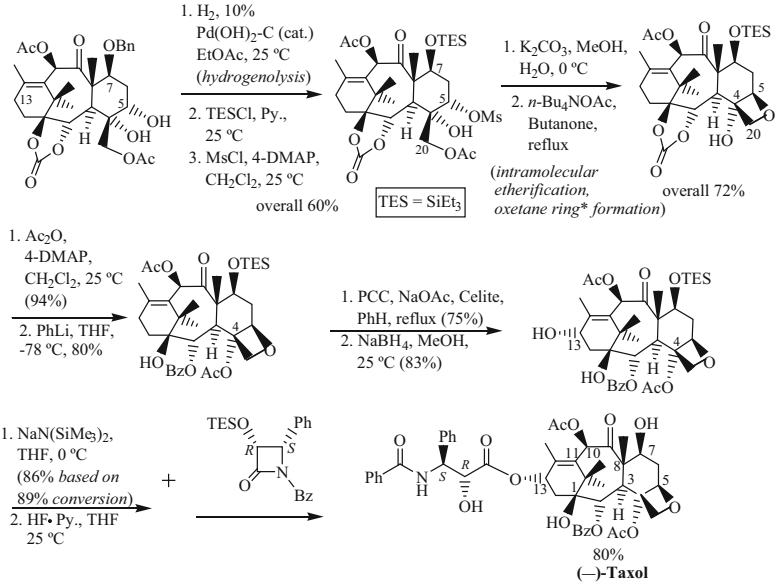
Union between Ring A and Ring C Precursors *[***(A)*** and ***(B)***] (Application of Shapiro Reaction) (reaction of C1, C2, C3, three contiguous asymmetric carbon atoms with desired relative stereochemistry of taxol, and resolution of McMurry coupling product)*



Note: *In the last step, cleavage of the isopropylidene ketalis followed by selective acetylation of the primary OH group.*

The Remaining Strategic Events

Formation of hydroxyoxetane system. Generation of hydroxyl at C13(S) and its subsequent esterification with N-benzoyl β-lactam derivative



**The oxetane ring is generated by displacement of C5-mesylate by the C20-OH. The latter is formed by the selective deacelylation of C20-acetate. During this intramolecular 4-membered cyclic ether formation the configuration at C5 is reversed (SN^2 displacement).*

Fig. 8.34 Total synthesis of taxol (Nicolaou) [44]

C1-OH and C2-benzoate of taxol. This carbonate will further bring C9 and C10 closer to effect in a better way the subsequent McMurry coupling (single electron transfer—intramolecular homocoupling).

(5) Ring B is thus generated by McMurry coupling between C9 and C10 of the carbonate derivative (±)-(**D**). At every step racemates are formed.
(6) The resolution of (±)-(**D**) by (1*S*)-(–)-camphanic acid chloride and the stereoisomerically pure (+)-enantiomer has been used for subsequent steps. An interesting observation is the formation of the ester (**E**) with (–)-camphanic acid at hindered C9-OH and not with C10-OH while with Ac_2O the acetyl derivative of C10-OH and not of C9-OH is formed. These two selective esterifications are not obvious and do not offer immediate rationalization.
(7) Formation of 9-keto-10 acetate as in natural taxol.
(8) Generation of an α-OH at C5 and the formation of an oxetane ring using mesylate protocol.
(9) The other steps follow the usual rational sequence.

The entire synthetic reaction strategy is delineated in Fig. 8.32.

The formation of an organolithium base from a tosylhydrazone of a ketone is shown in Fig. 8.33. The lithiated anion acts as a nucleophile in Shapiro reaction.

Total synthesis of taxol by Nicolaou by the convergent strategic events already defined is delineated in Fig. 8.34. These strategies opened a chemical pathway for the production of not only the natural product itself but also a variety of designed taxoids [44].

8.4.6 Search for Commercial Sources for Taxol

In most cases of the drugs of plant origin, the plants cannot serve as the source for commercial supply of the drugs to the users. The yield of taxol is low and the main source plant, *Taxus brevifolia*, is a slowly growing tree, which takes nearly 200 years to mature. For drug extraction, the bark is to be stripped and the full course of the drug per patient needs more than three plants [50]. This is indeed a threat to the species. Though taxol occurs in all the *Taxus* species, the yields are not encouraging. Further, time-consuming multistep synthesis of taxol will not be economically viable for commercial supply. The endeavor for the synthesis of such a fantastic molecule has always been a great challenge, intellectually rewarding, and chemically explorative, but could not be a means to meet the market demand. Hence other ways of preparation of this anticancer drug of great demand have been explored.

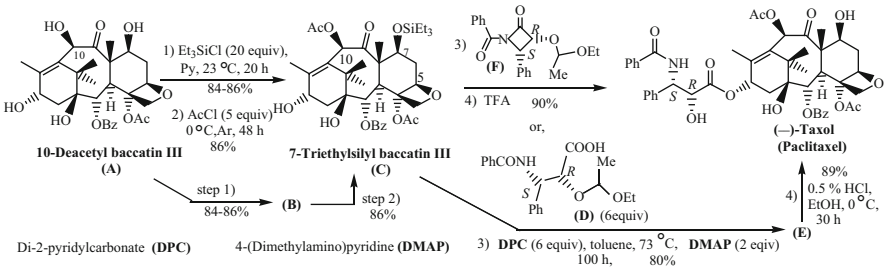


Fig. 8.35 Semisynthesis of (–)-taxol (paclitaxel)

8.4.6.1 Semisynthesis [43, 51]

The reported yields of taxol from the bark of various American, European, and Asian *Taxus* species (yew) range from 40 to 165 mg/kg. However, the yew is one of the slowest growing trees in the world. The several multistep syntheses of this complex molecule taxol have already been achieved. They are of high academic interest, but of little practical value. So an efficient partial synthesis (semisynthesis) of taxol from an easily and abundantly available taxol congener would be much useful for getting sufficient supply of this exceptionally promising cancer chemotherapeutic agent having a broad spectrum of antileukemic and tumor-inhibiting activity.

10-Deacetylbaccatin III (**A**) is a congener of taxol but is far less active than taxol. However, it is obtained in ~1 g/kg of fresh leaves [43], without harming the tree. A highly efficient 4-step conversion of 10-deacetylbaccatin III [43] into taxol is delineated in Fig. 8.35. In this methodology 10-deacetylbaccatin III (**A**) was triethylsilylated under carefully controlled condition to give 7-triethylsilyl-10-deacetylbaccatin III (**B**) in 84–86 % yield, keeping 10-OH unaffected. Thus, 7-OH is protected. Acetylation of (**A**) gives the 7-acetyl derivative and not baccatin III. Acetylation of (**B**) under controlled condition furnished 7-triethylsilyl-baccatin III (**C**). C13-OH Group is situated in the skeletal concavity of (**C**) and is able to form a stable H-bond with the C4 acetate (C13.OH. . .O = C(Me).O.C4 distance = 2.5 Å, as measured by a Dreiding model and molecular mechanics [43]) (conformation **1C**, Fig. 8.31). Hence its esterification is not possible. The esterification with optically pure (2*R*,3*S*)-N-benzoyl-O-(1-ethoxyethyl)-3-phenylisoserine (**D**) under the reaction condition shown produced (**E**) in 80 % yield [43]. The carefully chosen protecting group could be removed by treatment with 0.5 % HCl to give (−)-taxol (paclitaxel) in 38 % overall yield [43]. Further improvement was made acylating with a less sterically demanding acylating agent, the β-lactam (**F**), to give (−)-taxol in up to 90 % yield [51]. Obviously this latter methodology (steps 3 and 4) may be the preferred one for its commercial production in future. The semisynthesis is expected to serve to alleviate the shortage of taxol and thus greatly facilitate its application in cancer chemotherapy.

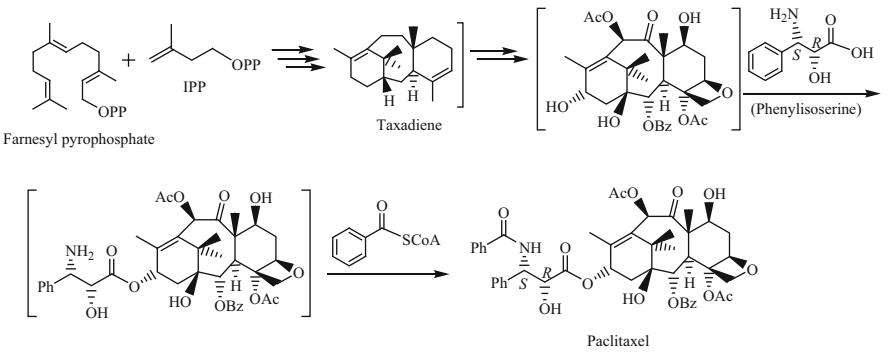


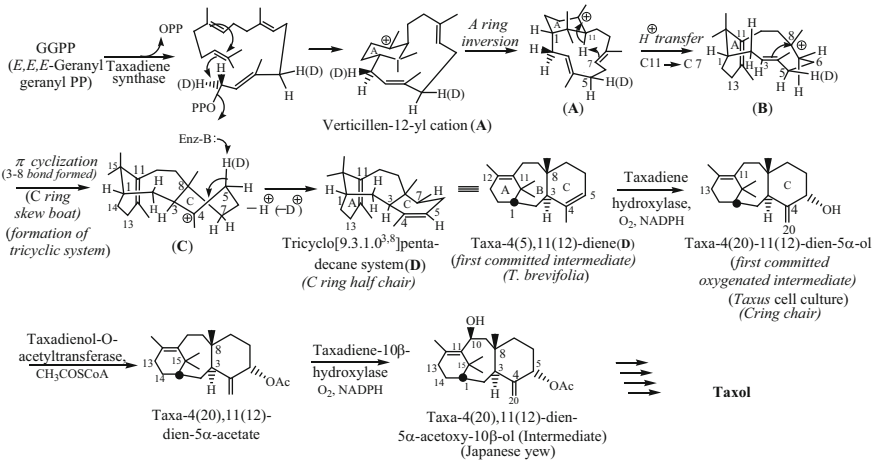
Fig. 8.36 Fermentation process for taxol formation

8.4.6.2 Application of Biocatalysis. Fermentation Process [52]

A fermentation process has been developed using *Taxus* cells for the isolation of taxol from the culture media. The entire process is catalyzed by a string of enzymes dedicated to specific steps. In this process isopentenyl diphosphate and farnesyl diphosphate are allowed to combine to form geranylgeranyl PP (GGPP), the universal molecule in the diterpene biosynthesis, catalyzed by geranylgernanyl diphosphate synthetase. The GGPP thus formed subsequently cyclizes to baccatin through the participation of a series of enzymes which cause hydroxylation, acetylation, oxidation, and the generation of the oxetane ring. Because of the specificity of enzymes these enzymic transformations follow their sequence according to the substrates they are meant for. The side chain at C13 is attached through enzymic transfer of phenylisoserine and then benzoylation (Fig. 8.36). Methyl jasmonate acts as elicitor (compounds that can enhance the production of secondary metabolites in an enzymic process) and enhances the production of paclitaxel (110 mg L^{-1}) in cell-suspension culture media.

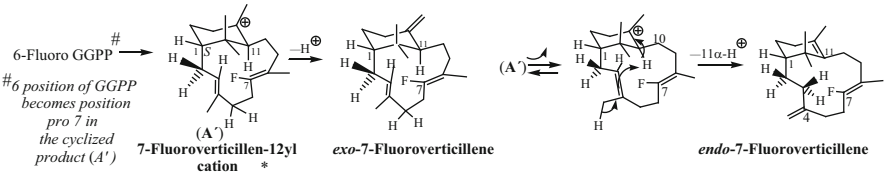
The plant cell fermentation process is a clean process, and it eliminates all steps of the semisynthesis.

Microbes thriving on plants sometimes produce the same compound as their hosts. This property of the microbes has been thought to be a clean process for the production of pharmacologically useful compounds. With this idea, search for yew-associated microbes that could produce taxol has been made. In this endeavor, 25 *Taxus brevifolia* species from different locations have been collected and nearly 200 microbes associated with these plants have been studied, of which only *Taxomyces andreanae*, an endophytic fungus showed the ability of producing taxol [37, 53]. These microbes are collected and made free from associated traces of taxol that have accompanied from the plant. They are allowed to grow in a suitable culture medium, and after 21 days' incubation, the culture is filtered, extracted with CH_2Cl_2 or $CHCl_3$/MeOH. Removal of the solvent gave taxol which is purified. When compared with yew-taxol, the fungal taxol showed identical spectral properties. The yield is low. However, efforts are going on to improve



Note: *Ring A of cation (**A**) is generated from the particular folding of GGPP, as shown, made by the specific enzyme taxadiene synthase.*

Fig. 8.37 Biosynthesis of taxol



Proton transfer from C11 to C7, like in* (A**) (**Figure 8.37**) *is arrested in* (**A′**) *because of the decreased basicity of the π-bond by the F substituent.*

Fig. 8.38 Chemical identity of the intermediates and the nonformation of the tricyclic system

the yield with the application of genetic engineering and to make the process commercially viable.

In the fermentation process, taxol is generated from the genetically engineered *Taxus* species cell culture media following the biosynthetic pathway [52].

8.4.7 Biosynthesis of Taxol

The study of the biosynthesis of taxol showed that the taxane skeleton is not of mevalonoid origin (labeling experiment) [54] and that the first committed intermediate in the biosynthetic pathway has been taxa-4(5),11(12)-diene, which has also been isolated from *T. brevifolia* [54]. Soon after the isolation of 4(20),11(12)-dien-5α-ol (Fig. 8.37) from *Taxus* cell culture media it has been proved to be the first oxygenated intermediate in this biosynthetic pathway [55]. These intermediates have been synthesized with appropriate labels and biosynthetic experiments were

carried out to show their intermediacy in the process. Further identification of other intermediates will map the entire biosynthetic path to taxol. Another intermediate taxa-4(20),11(12)-dien-5α-acetoxy-10β-ol (Fig. 8.37), isolated from Japanese yew, has been shown to be an intermediate in taxol biosynthesis [56].

During the cyclization of GGPP to taxadiene, a bicyclo[9,3.1]-pentadeca-3,7-diene precursor (verticillen-12yl carbocation) is formed (Fig. 8.37). This is followed by a proton transfer from C11 to C7, prior to further cyclization to tricycle [9.3.1.$0^{3,8}$] pentadecane skeleton (*cf* taxane). The event of hydrogen transfer from C11 to C7 [(**A**) to (**B**) in Fig. 8.37] has been elegantly shown (Fig. 8.38) [57] using 6-fluorogeranylgeranyl PP. Further cyclization of the latter has been arrested at verticillene stage as the fluorine substituent at C7 decreases its π basicity sufficiently to prevent proton transfer at this position to form tricyclo[9.3.1.$0^{3,8}$] pentadecane system like (**C**). Thus 7-fluoroverticellen-12-yl cation faces other fates and undergoes exocyclic or endocyclic elimination of proton to form a few verticillene derivatives (Fig. 8.37), of which exo-fluoroverticillene is important. The stereochemical description of these derivatives is obtained from their extensive NMR spectral studies [57]. The ring juncture B/C in (**D**) is *trans* with (3*R*)-H3-α and (8*S*)-Me8-β configurations (*cf* taxadiene).

8.4.8 Uses

Taxol is used for an important first-line treatment of ovarian and breast cancer and head and neck cancer and has potential applications in the treatment of other cancers. Some studies on the structure–activity relationships of taxol have been reported [40].

The antitumor and antileukemic activity and structure–activity relationship of taxol and its derivatives/analogues have been briefly discussed in Chap. 33 (Sect. 33.6.3).

8.5 Gibberellins

8.5.1 Introduction. Biological Activity

Gibberellins belong to an odd class of diterpenes. It was discovered in late 1820s that a rice plant (*Oryza sativa,* Gramineae) disease was caused by a fungus identified later as *Gibberella fujikuroi*. The disease known as *bakanae* in Japan

causes the elongation of the rice seedlings more rapidly than the normal seedlings when ultimately they wilt and die. This observation was published in 1828 [58, 59]. A compound thought to be responsible for such elongation was isolated in 1938 and named gibberellin by Yabuta after the name of the fungus. Later it was shown by Takahashi [60] to be a mixture of three compounds GA_1, GA_2, and GA_3. All compounds possessing this type of biological activity with gibbane skeleton are called gibberellins and are represented by the general formula GA_n (*n* represents a digit given according to the chronological order of the discovery) [61]. 112 Gibberellins have been characterized till 1997 [62, 63]. Gibberellins regulate a number of different rate-determining steps in plant metabolism, e.g., breaking the dormancy, inhibition of senescence, a primary stimulus to germination (especially when the pericarp is removed), overcoming juvenility, and determining the developmental sequence of sex organs of flowers.

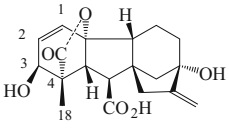
In short, gibberellins play an important role in almost all phases of plant growth. Of all the bioactive gibberellins GA_3 has been the most important one and is of wide occurrence. It is named Gibberellic acid. Gibberellins also occur in higher plants, e.g., C*itrus reticulate*, *Phaseolus vulgaris*, and *P. multiflorus*.

8.5.2 Structure. Synthesis

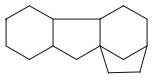
Unlike other tricyclic and tetracyclic diterpenes, gibberellins do not yield phenanthrene derivatives on dehydrogenation but yield fluorene derivatives.

The gross structure of gibberellic acid (GA_3) was elucidated as (**1**) [60]. Its complete stereostructure was subsequently determined by X-ray diffraction studies [64] of the di-*p*-bromobenzoate of the methyl ester of gibberellic acid (GA_3).

It has high density of functional groups attached to the strained gibbane skeleton (**2**), and it is labile toward many reagents. Its synthesis [61, 64] needed a lot of manipulation for the introduction of functional groups that become ultimately the integral part of its structure and the construction of the carbon framework. Corey who synthesized gibberellic acid [65, 66] commented [67].



(1) GA$_3$



Gibbane (2)

m.p. 233-235 ^{0}, $[\alpha]_D$ +52(EtOH)

"The plant bioregulator gibberellic acid resisted synthesis for more than two decades, because it abounds in all the elements contributing to complexity, including reactive and dense functionality, in an usually forbidding arrangement."

8.5.3 Biosynthesis

Biosynthesis of GA_3 has been discussed in Sect. 8.1.5. Both C_{20} Gibberellins and C_{19} (in which C20 is lost) Gibberellins are known to occur [62].

8.5.4 Uses

Gibberellic acid is used in agriculture to stimulate the swelling of fruits, e.g., grapes and tomatoes. It is also used to break the dormancy of seeds, e.g., lettuce and peach, to accelerate the germination of barley, and also for various other agricultural purposes. For detailed studies on Gibberellins, see [62].

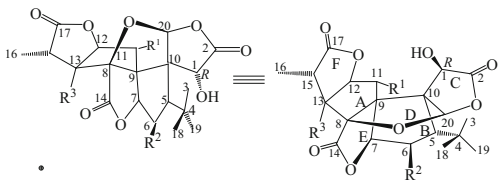
8.6 Ginkgolides

8.6.1 Introduction. Occurrence

The plant *Ginkgo biloba* has been regrown in the center of Hiroshima from her charred womb caused by the heat that resides only in the heart of Stars [68], within 1 year of the beastliest day (August 6, 1945) in Man's history, perhaps as a symbol of Hiroshima's strong will to live and our humble vow "Peace in this World" [68].

In 1923, a devastating earthquake shook Tokyo and a great fire broke out which engulfed everything on its way; however, a temple surrounded by *Gingko* trees survived. Perhaps the liberated chemicals from the trees acted as a fire retardant [69].

Ginkgo biloba (order, Ginkgoales) is a mono-species genus with longest survival history (250 million years) with unbelievable endurance ignoring the Nature's evolution of millions of years and Her environmental pressures and changes. *Ginkgo biloba* was found in the fossil, in the love-lyrics of Johann Wolfgang von Goethe (1749–1832) and in Charles Robert Darwin's (1809–1882) interest who nicknamed it as "living fossil" in 1859 since the species has not changed over millions of years [69]. The beautiful bipartite-shaped leaves of the plant add beauty to its gait. The painters use the tree and the leaves as their subjects and the beautiful shape of the leaves is copied in the hairstyle of the brides and sumo-wrestlers—all have top knots in the style of Ginkgo leaves. The trees are now planted as roadside ornamental trees in oriental cities. They are precious collections in various museum premises and parks all over the globe.



Structure	*Name*	R^1	R^2	R^3	
(**1**)	Ginkgolide A	H	H	OH	$C_{20}H_{24}O_9$
(**2**)	Ginkgolide B	OH	H	OH	$C_{20}H_{24}O_{10}$
(**3**)	Ginkgolide C	OH	OH	OH	$C_{20}H_{24}O_{11}$
(**4**)	Ginkgolide J	H	OH	OH	$C_{20}H_{24}O_{10}$
(**5**)	Ginkgolide M	OH	OH	H	$C_{20}H_{24}O_{10}$

Fig. 8.39 Stereostructures of ginkgolides

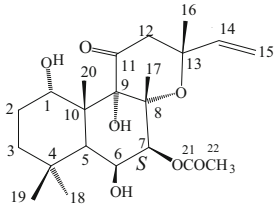
8.6.2 Chemical Constituents. Ginkgolides; Biosynthesis. Synthesis

The chemical constituents, called ginkgolides, a family of polycyclic terpenoids were first isolated by Furukawa in 1932 and the structures were elucidated by Nakanishi in a series of papers [70–73], based on chemical and NMR spectroscopic studies [structures (**1**)–(**5**)] (Fig. 8.39). Finally, the correctness of the structures was fully supported by X-ray crystallographic analysis [74]. They all belong to diterpenes with heavily oxygenated functionalities and the ratio of carbon:oxygen ~ 10:4.5 → 10:5.5, and other ginkgolides discovered differ in the positions of hydroxyls. The ginkgolides (**1**)–(**5**) have three lactones in the same positions. Later the biosynthesis of ginkgolides has been extensively studied by Arigoni [75], which is already outlined briefly in Fig. 8.10. Serious attempts have been made to synthesize ginkgolides as their complex scaffolds offer a great challenge to the synthetic organic chemists. Corey has elegantly synthesized ginkgolide B [76]. It is a therapeutic agent of potentially wide application—a potent antagonist of platelet-activating factor, and also an insect antifeedant.

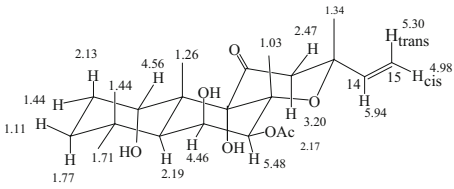
8.7 Forskolin

8.7.1 Occurrence, Stereostructure

Forskolin (**1**), $C_{22}H_{34}O_7$, m.p. 230–238 °C, $[\alpha]_D$ –26.9, a diterpene of labdane skeleton having an additional heterocyclic ring (Fig. 8.40), has been isolated from the roots of *Coleus forskohlü* (Labiatae) [77, 78]. Its structure has been shown to be 7β-acetoxy-8,13-epoxy-1α,6β,9α-trihydroxy-lab-14-ene-11-one (**1**) from its reactions, synthesis, spectral data, and X-ray crystallographic analysis. Coleonol, independently isolated from the same plant, was assigned the 7-epimeric stereostructure of (**1**), based on degradative and spectroscopic studies [78]. Later on it has been found to be identical with forskolin (**1**) having 7-(*S*)-β-acetoxy group [79, 80]. A series of compounds possessing the basic skeleton of forskolin with different oxygenation patterns and also with 7-epimeric hydroxy/acetoxy compounds have



Forskolin (1)
(≡ Coleonol)



Conformation of **Forskolin .**
^{1}H NMRchemical shift values

^{1}H NMR : δ ($CDCl_3$) (*J* in Hz), 4.56 (dd, *J* 6.0, 3.4, H1), 1.44 (*m*, H2-eq), 2.13 (m, H2-ax), 1.11 (*m*, H3-eq), 1.77 (*m*, H3-ax), 2.19 (d, J, 1.9, H5), 4.46 (dd, 1.9, 3.9, H6), 5.48 (d, J 3.9, H7), 2.47 (d, J_{gem} 17.1, H12-eq), 3.20 (d, J_{gem} 12.1, H12-ax), 5.94 (dd, *J* 17.1, 10.2, *H14), 4.98 (d,* J 10.2, H15-*cis*), 5.30 (d, *J* 17.1, H15-*trans*), 1.34, 1.03, 1.71, 1.44 and 1.26 (*s* each, H_3 16, H_3 17, H_3 18, H_3 19, H_3 20 respectively), 2.17 (H_3 22).

^{13}C NMR : δ ($CDCl_3$), 74.5 (C1), 26.6 (C2), 36.1 (C3), 34.4 (C4), 42.9 (C5), 69.9 (C6), 76.7 (C7), 81.4 (C8), 75.0 (C9), 43.1 (C10), 205.3 (C11), 48.8 (C12), 82.7 (C13), 146.4 (C14), 110.7 (C15), 31.5 (C16), 33 (C17), 23.6 (C18), 19.8 (C19), 24.3 (C20), 169.6 (C21), 21.0 (C22).

Fig. 8.40 ^{1}H & ^{13}C NMR Spectral data of forskolin (= coleonol)

been isolated (see [81]). Biogenetic relationship of the polyoxygenated diterpenes in *Coleus forskohlü* has been studied [81].

8.7.2 NMR Spectral Data [78]

^{1}H NMR spectral assignments for coleonol (forskolin) (**1**) (Fig. 8.40) have been made by two-dimensional methods. The 7-OAc group is assigned equatorial β-configuration from the coupling constants of H7 : $J_{7,6} = J_{ae} = 3.9$ Hz. ^{13}C NMR spectral assignments (Fig. 8.40) have been made with the help of DEPT and ^{1}H-^{13}C chemical shift correlation studies.

8.7.3 Bioactivity

Forskolin possesses unique antihypertensive, intraocular pressure lowering, and platelet-inhibiting properties and is also a unique cardiotonic [77, 78] (Chap. 33). A few forskoditerpenosides have been isolated possessing relaxation effects in vitro [82].

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Further Reading

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Chapter 9
Sesterterpenoids (C_{25})

9.1 Introduction. Occurrence. Structure

As per Ruzicka's biogenetic hypothesis of isoprenoids, the sesterterpenoids (C_{25}-isoprenoids) are expected to occur in nature. However, a long sought sesterterpene was not discovered until 1958 when a sesterterpene was isolated as a metabolic product from the cultural broth of the plant pathogenic fungus *Ophiobolus miyabeanus* and named *ophiobolin*. The stereostructure of ophiobolin has been established [1] in 1965 as (**1**) on the basis of ^{1}H NMR spectral data of ophiobolin and its bromomethoxy derivative and an X-ray crystallographic analysis of the latter. The skeletal pattern of ophiobolin was unknown at the time of its structural elucidation.

9.2 Spectral Data of Ophiobolin (1) (Fig. 9.1)

Since the report of the isolation and structure elucidation of ophiobolin, *a large number of sesterterpenes with ophiobolin, epiophiobolin, and other skeletal patterns have been reported from pathogenic fungi, insect secretions, and marine sponges* [2, 3]. Himalayan lichens elaborate sesterterpenoids having interesting skeletal patterns. Quite a good number of sesterterpenoids have been obtained from sea sponges, while their occurrence in plants, especially in higher plants, is rare. All these compounds originate biogenetically from geranyl farnesyl pyrophosphate (GFPP). It has been shown by labeling experiments that in plant pathogens GFPP is formed from MVA [4, 5]. Geranylfarnesol (GF), the hydrolyzed product of GFPP, has been isolated from the insect wax of *Ceroplastes albolineatus* [6], while geranylnerolidol and also geranylfarnesol have been found in fungus *Cochliobolus heterostrophus* [3].

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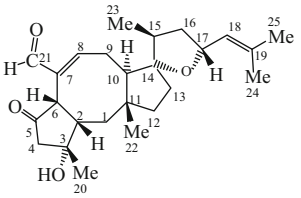


Ophiobolin (1) $C_{25}H_{36}O_4$, m.p.182^{0}C
$[a]_D^{29}$ +270

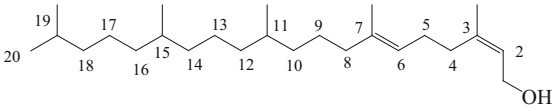
^{1}H NMR: δ 0.82 (*s*, 3H, 22Me), 1.08 (d, J = 6.5 Hz, 3H, 23Me), 1.3 (s, 3H, 20Me), 1.7 (s, 6H, 24Me, 25Me), 4.43 (q, J = 8 Hz, 1H, H17), 7.13 (t, J = 8 Hz, 1H, H8), 9.26 (*s*, 1H, H21), 5.15 (d, J = 8 Hz, H18). Additional signals at 2.46 and 2.77 (AB type q, J = 20, 2 Hz) and at 3.20 (d, J = 11, 1H) are attributed to $H_2$4 and H6 protons adjacent to >C=O group.

UV: λ_{max} (EtOH): 238 nm (ε, 13,800)

IR: υ_{max} ($CHCl_3$): 3500 (OH), 1743 (5-membered ketone), 1674 (α,β-unsaturated carbonyl) and 1633 cm^{-1}

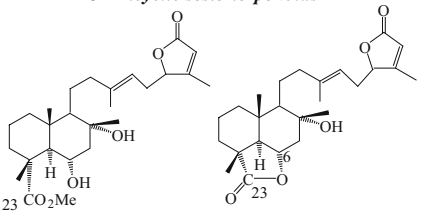
Fig. 9.1 Spectral data of ophiobolin

a ***Partly saturated acyclic C25-isoprenyl alcohol***



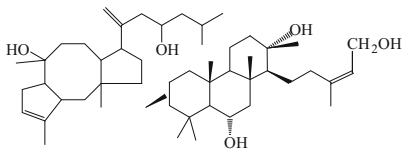
(2Z,6E)-3,7,11,15,19-Pentamethylicosa-2,6-dien-1-ol
(*Solanum tuberoscum,* Solanaceae, potato leaves/lipids) [7]

b ***Bicyclic sesterterpenoids***



Salvileucolide methyl ester **Salvileucolide 6,23-lactone**
Salvia hypoleuca (Labiatae [8])

c ***Tricyclic sesterterpenoids***

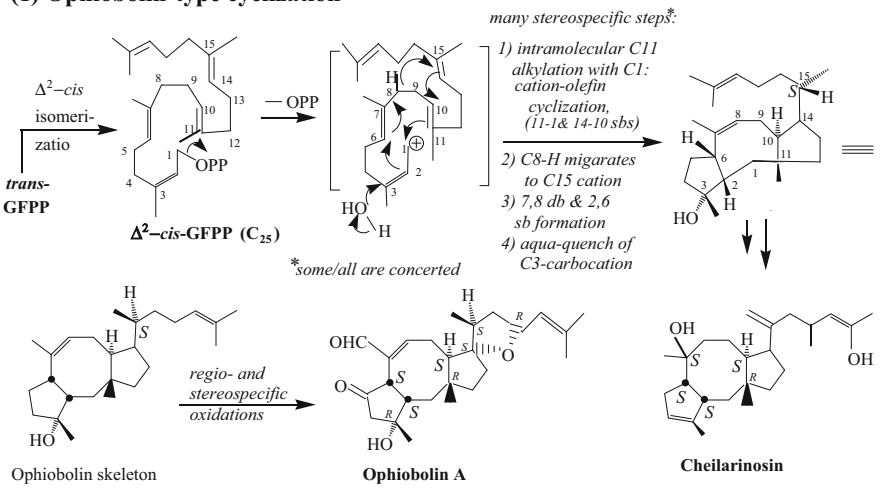


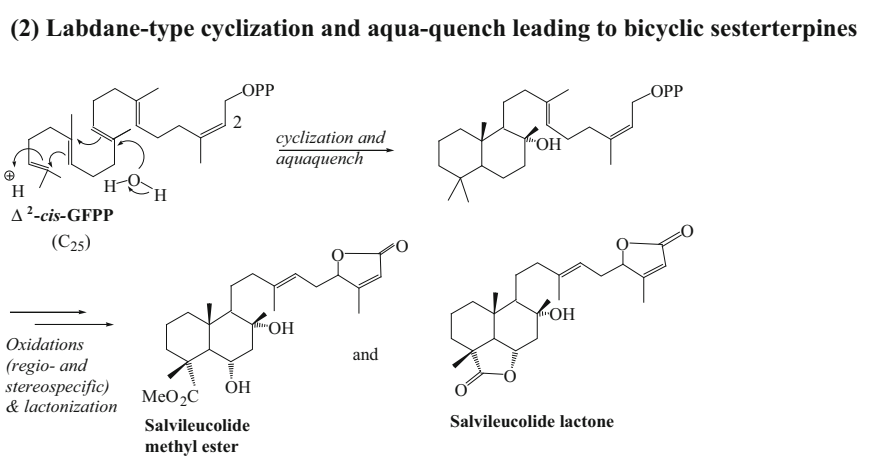
Cheilarinosin [9] **Cheilanthatriol** [10,11]
Cheitanthes farinosa (Fern) (Cheilanthaceae)

Fig. 9.2 Sesterterpenoids of plant origin

9.3 Sesterterpenoids of Plant Origin

Only few plant-based sesterterpenoids are known. Some examples with structures and sources are shown in Fig. 9.2.





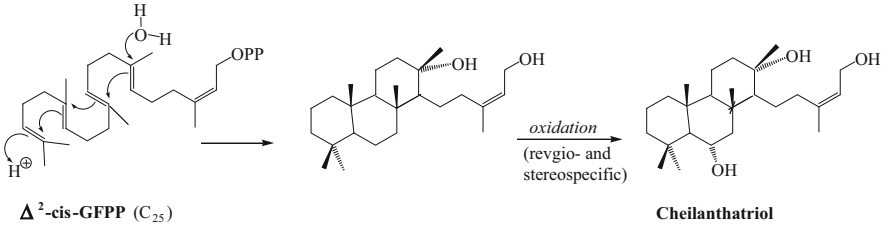


Fig. 9.3 Biogenesis of sesterterpenes having different skeletons

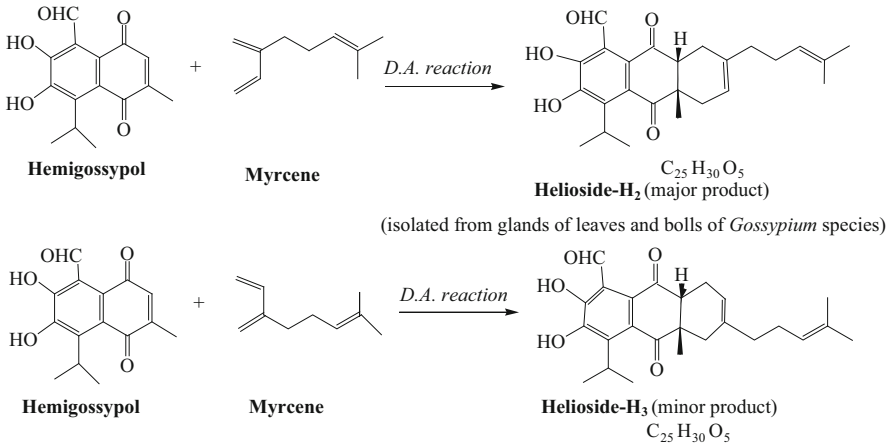


Fig. 9.4 C_{25} Natural compounds but biogenetically not related to GFPP

9.4 Biosynthesis of Some Sesterterpenoids (Fig. 9.3)

The biogenetic cyclizations of some sesterterpene skeletal patterns are delineated in Fig. 9.3.

9.5 Natural C_{25} Compounds Biogenetically Not Related to Geranylfarnesyl PP

From the molecular composition the heliosides (Fig. 9.4) appear to be sesterterpene derivatives. But biogenetically they are not derived from geranylfarnesyl pyrophosphate; rather, they are formed from the Diels–Alder reaction between hemigossypol and myrcene (Fig. 9.4). Both are co-occurring in the same plant. In fact in the laboratory the Diels–Alder reaction between the two synthons forms helioside-H_2 as the major product, while helioside-H_3 has been obtained from repeated chromatography of the mother liquor of helioside-H_2 [12, 13].

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Chapter 10
Triterpenes (C_{30})

10.1 Introduction. Biogenesis. Functions of Enzymes

Triterpenoids belong to a large and structurally diverse class of natural products which occur abundantly in plant kingdom, especially in higher plants in cases of pentacyclic and tetracyclic triterpenes, while mono-, bi-, and tricyclic triterpenes are prevalent in ferns and nonflowering lower plants (cryptogams). Some of them were found in fossils as such and also as their diagenetic products.

The biogenetic precursor of this class of compounds is squalene, an isoprene-derived C_{30} linear polyene hydrocarbon with $\mathbf{C_2}$-symmetry (Chap. 5). Under the influence of different enzymes, collectively called tripterpene synthases, nearly 200 different triterpene skeletons have been generated [1] from *squalene, oxidosqualene*, and *bisoxidosqualene*. Till 2007, more than 30 *oxidosqualene cyclases* (OSCs) have been cloned and characterized [2–4]. β-*Amyrin synthase* and *lupeol synthase* are the first two synthases to be cloned. Mutagenesis studies on these synthases showed that exchange of only one amino acid dramatically changes the enzyme's own specificity and endowed them with different product specificity (cf. Chap. 11, Ref. [32]). Some of the synthases are monofunctional (e.g., β-amyrin synthase). They elaborate single triterpene each, whereas multifunctional triterpene synthases are capable of producing more than one triterpene, e.g., *mixed amyrin synthase* isolated from peas, *Pisum sativum* (Fam. Papilionaceae). Another multifunctional synthase from *Arabidopsis thaliana* (Fam. Brassicaceae) is capable of yielding as many as nine different triterpenes.

Enzymes responsible for producing 20 different triterpene skeletal patterns have been identified so far, and the rest enzymes remain unidentified. Identification of all the involving enzymes would have uncovered the origin and diversity of the triterpene skeletal patterns. However, all the triterpenes are formed by mono- and multifunctional triterpene synthases, according to specificity.

The majority of the tetracyclic triterpenes are having 6-6-6-5 cyclopentanoperhydrophenanthrene skeleton while pentacyclic skeletal patterns possess 6-6-6-6-5 cyclopentanoperhydrochrysine and 6-6-6-6-6 perhydropicene carbon skeletal

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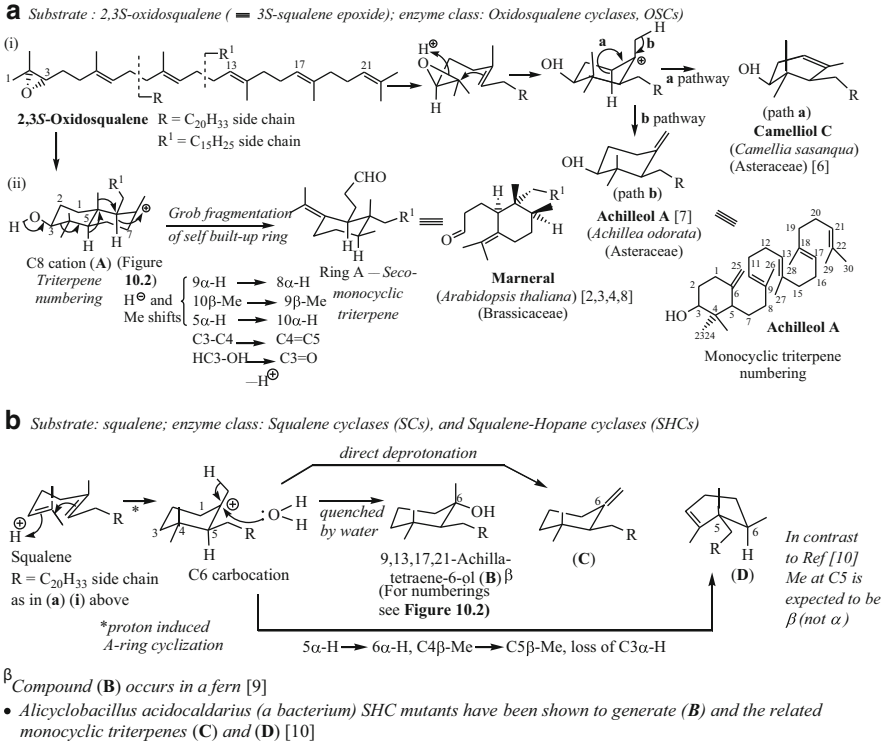
frames. In addition to the above prevalent triterpene ring systems, acyclic, monocyclic, bicyclic, tricyclic, and even hexacyclic triterpenes are known to occur and have been isolated and characterized.

The experimentally explored/unexplored but mechanistically reasonable modes of 2,3(*S*)-oxidosqualene/squalene/2,3(*S*),22(*S*),23-bis-oxidosqualene cyclization in the presence of specific enzymes to generate the mono-, di-, tri-, tetra-, and pentacyclic triterpenes are depicted in Figs. 10.1–10.16 that follow.

Unlike mono-, sesqui-, and diterpene cyclases, triterpene cyclases never use pyrophosphate (PP) substrates. The mother molecule squalene does not contain pyrophosphate group. Further, ionization through the loss of PP group in most of the former cases (Chaps. 6, 7 and 8) causes the initiation of the cyclization process, while in triterpene biosynthesis the cyclization is initiated by the electrophilic addition of a proton at the terminal double bond (squalene) or at the expoxy oxygen of oxidosqualene/bisoxidosqualene [1, 5]. Other steps are more or less similar to those for mono-, sesqui-, and diterpene bioformation. Unlike sesquiterpenes (Chap. 7) where 1,3-hydride shift is a common phenomenon, in triterpene biosynthesis, especially in tetracyclic and pentacyclic systems, antiparallel 1,2-hydride as



β *Compound* (**B**) *occurs in a fern* [9]

- *Alicyclobacillus acidocaldarius (a bacterium) SHC mutants have been shown to generate* (***B***) *and the related monocyclic triterpenes* (**C**) *and* (**D**) [10]

Fig. 10.1 Biosynthesis of some monocyclic triterpenes originating from 2,3S-oxidosqualene and squalene

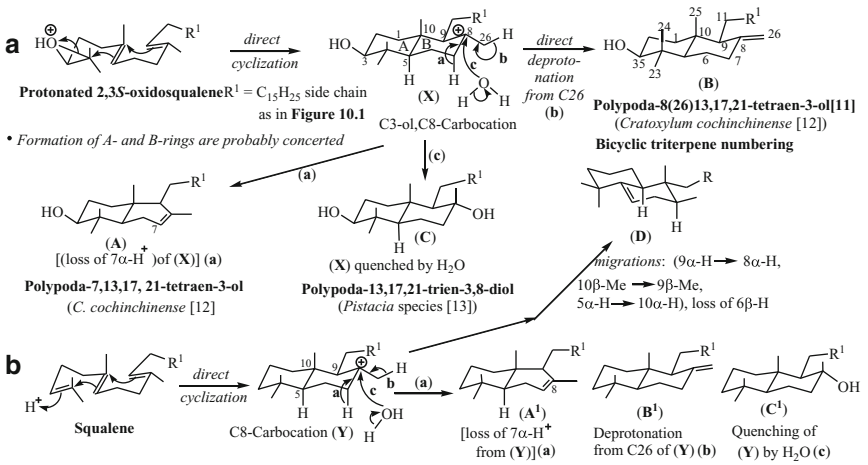
well as 1,2-alkyl (methyl) migrations are prevalent. Oxidosqualene (2,3*S*-oxo) is the obligatory precursor for all 3β-hydroxyl (in ring A) of cyclic triterpenes and also for some 3-oxo compounds (e.g., friedelin). Prior to protonation or initiation of cyclization, the triterpene synthases, as per their specificity, fold the linear substrate into a compatible conformation (which does not change much during cyclization) to bring the reactive sites within bonding distances. Proton addition at the terminal double bond or opening of the protonated epoxide triggers the cyclization when cation–olefin cyclization preferably in Markovnikov fashion (each time a low energy carbocation is generated) starts following the stereoelectronic requirement of the processes. The cation reaches its home carbon depending on the specificity of the cyclase in operation, and a specific proton is eliminated to quench the carbocation as olefin; sometimes the carbocation is quenched by some nucleophile, mainly water (aqua quench). All the above events will be mechanistically (organic chemistry based) explained through the formation of some selected triterpenes of different skeletal patterns (Figs. 10.1–10.16). Whether cyclization to the different carbocations and the host of 1,2 migrations is concerted or nonconcerted still remains to be settled; however, the stereospecificity in these processes is maintained by the specific enzymatic folding of the substrate molecules in definite conformations for cyclizations giving rise to innumerable skeletal patterns of triterpenes through the intermediacy of various carbocations. It is to be noted that squalene numbering remains unchanged as long as the molecule is acyclic; the numbering changes when cyclization takes place, since C5 of squalene after cyclization of ring A becomes C1 and the starting gem dimethyl groups of squalene are numbered only at the end. Numbering also depends upon the number of consecutive fused rings—as will be applicable for mono-, bi-, tri-, tetra-, and pentacyclic triterpenes (Sects. 10.1.1–10.1.5).

10.1.1 Monocyclic Triterpenes

10.1.2 Bicyclic Triterpenes

Bicyclic triterpenes are mostly *trans*-decalin derivatives. Electrophilic proton addition at the epoxy or terminal double bond of epoxysqualene [*cf.,* (a)] or squalene [*cf.*, (b)] and ring A and B formations are concerted followed by quenching of carbocations when direct cyclization leads to products formation. Sometimes migrations of hydrides and methyls take place prior to final carbocation quenching leading to the products (Fig. 10.2). All the bicyclic triterpenes of Fig. 10.2 are *trans*-decalin derivatives, the conformations of which are shown in the figure.

c Lansic acid [16, 17]. The major constituent of the latex of the fruit peels of *Lansium domesticum* Jack v. duku (Meliaceae) serves as an example of a bicyclic triterpene which unlike the common bicyclic triterpene does not contain a decalin ring system. Its structure revealed the presence of two substituted six-membered



- For R and R^1 see the first structure of **Figure 10.1**
- *The bicyclic triterpenes* (**A**^{1}) *and* (**B**^{1}) *occur in the fern genus* ***Polystichum*** [14] *and have also been obtained from* ***A. acidocaldarius*** *SHC mutants.*
- *The triterpene alcohol* (**C**^{1}) *is from the fern Polypodiodes and the triterpene* (**D**) *has been generated by A. acidocaldarius SHC mutants but is not found in nature* [15]

Fig. 10.2 Biosynthesis of some bicyclic triterpenes originating from 2,3*S*-oxidosqualene and squalene

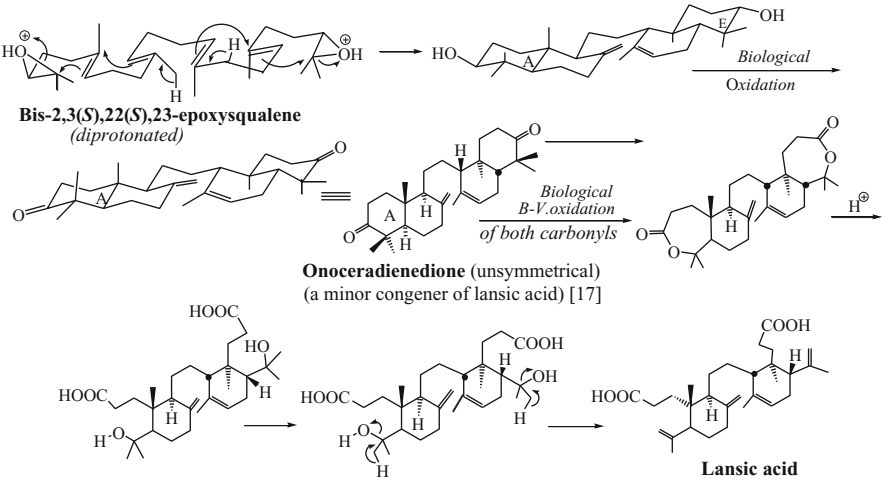


Fig. 10.3 Biogenetic formation of lansic acid

rings linked with a C_2-linker. It has been chemically correlated with α-onocerin. Biogenetically it may be derived from unsymmetrical onoceradienedione (a minor congener of lansic acid from *Lansium domesticum* [17]), as outlined in Fig. 10.3.

10.1.3 Tricyclic Triterpenes

(a) **Malabaricol** The first tricyclic triterpene, malabaricol, has been isolated by Sukh Dev [18] from *Ailanthus malabarica* (Simaroubaceae). Malabaricol is proposed to be biogenetically formed from 2,3*S*-oxidosqualene as shown in Fig. 10.4. The stereochemistry of (+)-malabaricol has been settled by correlating it with (+)-ambreinolide of known structure and stereochemistry [19]. Malabaricanediol, a precursor of malabaricol, is structurally consistent with cyclization from 2,3-epoxy-18,19-dihydrosqualene-18,19-diol.

Later, Bohlmann et al. isolated malabarica-14(27),17,21-trien-3-ol (**B**) [20] and an aqua-quenched product (C) has been generated in the laboratory. Incidentally, it may be mentioned that isomalabaricane has been isolated from a sea sponge [21].

Sharpless [22] carried out the conversion of an epoxydiol of squalene to dl-malabaricane diol (7 % yield) using picric acid (Fig. 10.5). This conversion serves as the first examples of a nonenzymatic catalyzed conversion of squalene into a natural product. The conventional acids used ($SnCl_4$, $SnCl_3$, BF_3-etherate) for polyene cyclization affect not only epoxy group but also other

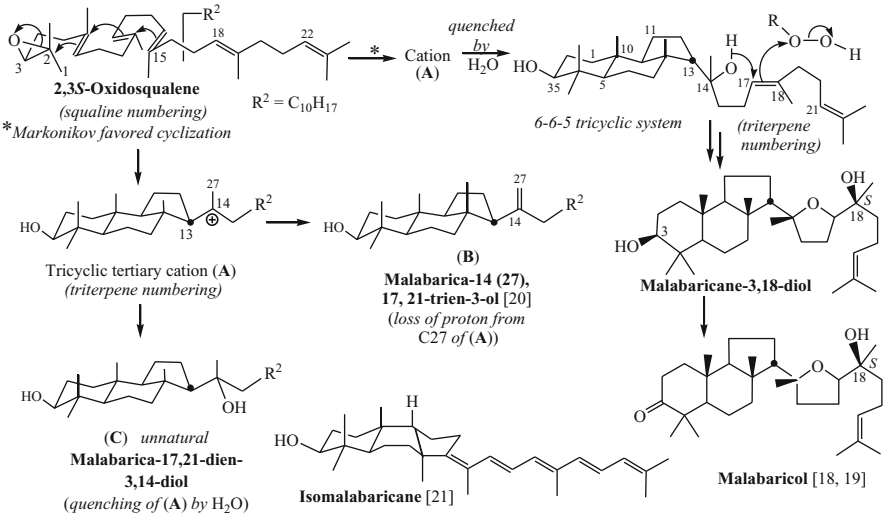


Fig. 10.4 Biosynthesis of malabaricol and related tricyclic triterpenes

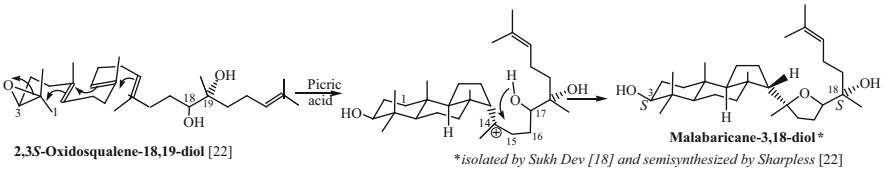


Fig. 10.5 Semisynthesis of malabaricane-3,18-diol

vulnerable centers yielding different cyclic products. The selection of picric acid, used for such cyclization, is the first case and is based on its acidity (nucleophilicity) as well as bulk which makes it convenient for the purpose. In nonenzymatic cyclization oxidosqualene gave thermodynamically favored tertiary carbocation with 6.6.5-fused tricyclic products (Markovnikov addition) as found in natural malabaricol [23]. Such a product has also been obtained from 18,19-dihydro-2,3-oxidosqualene [24].

Biogenetic pathways of some tricyclic triterpenes derived from squalene are delineated in Fig. 10.6.

(b) **Lansioside A** (Fig. 10.7) [27] isolated from the fruit peels of *Lansium domesticum* Jack v. duku (Meliaceae) is an aminosugar glycoside. The

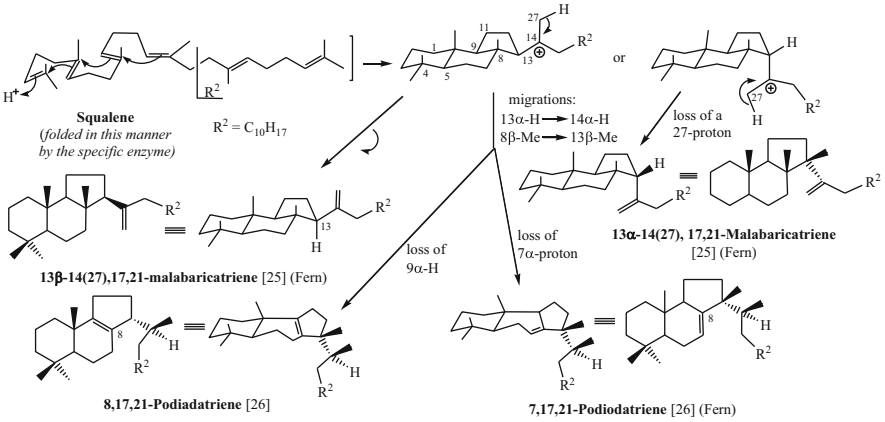


Fig. 10.6 Biogenesis of some tricyclic triterpenes derived from squalene

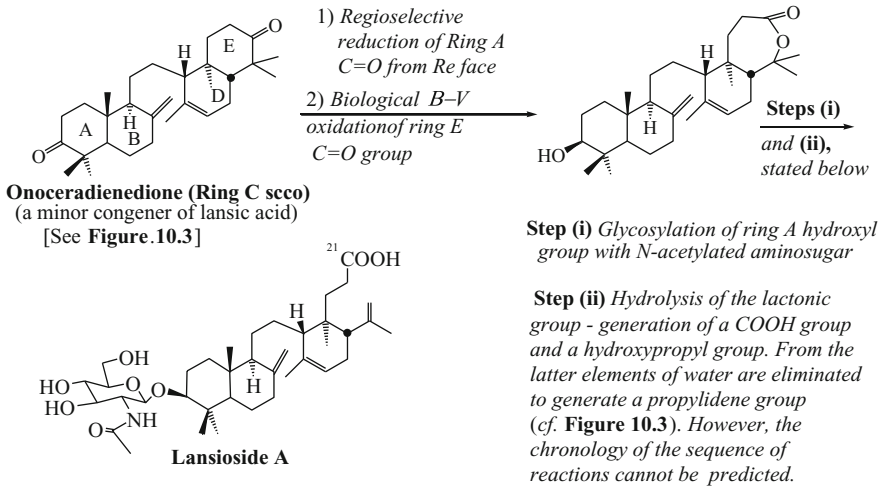


Fig. 10.7 Biogenesis of lanosioside A

aglycone part of it is a tricyclic triterpene having secoonocerane skeleton. Biogenetically it may be derived from α-onoceradione (Fig. 10.7)

Subsequently, two more tricyclic triterpenes, **lanosioside B** and **C**, have been isolated and their structures were established.

10.1.4 Tetracyclic Triterpenes. Substrates: (a) Oxidosqualene, (b) Squalene

(a) OSCs (*oxidosqualene cyclases*) can generate a large number of tetracyclic triterpenes from 2,3*S*-oxidosqualene (**1**) via two major intermediate carbocations (Fig. 10.8) as follows. (i) The 17β-p*rotosteryl carbocation* (**C**) is derived from (**1**) folded with rings A and C in *pro*-chair conformation and ring B in *pro*-boat conformation (**A**), prior to cyclization and 1,2 migrations, and biosynthesizes 6-6-6-5 tetracyclic triterpenes as shown in Fig. 10.9. The most well-known compounds that are formed from the carbocation (**C**) are lanosterol and cycloartenol. Lanosterol is responsible for the biosynthesis of cholesterol in mammals and ergosterol in fungi, while cycloartenol is responsible for the biosynthesis of phytosterols including cholesterol (Chap. 11). (ii) The 17β-*dammarenyl carbocation* (**D**) is derived from all *pro*-chair conformation (**B**) of 2,3*S*-oxidosqualene and yields a large variety of 6,6,6,5 tetracyclic triterpene alcohols from the 17β-dammarenyl cation, the detailed pathways for the bioformation of which have been outlined in Fig. 10.10. Further, a number of pentacyclic triterpenes are generated from the dammarenyl carbocation as stated in Sect. 10.1.5.

(b) ***Cyclization of Squalene:*** The enzymatic cyclization of squalene differs in several respects from that of oxidosqualene. The formation of all chair (A–B–C rings) (**F**) carbocation is the characteristic of squalene, while

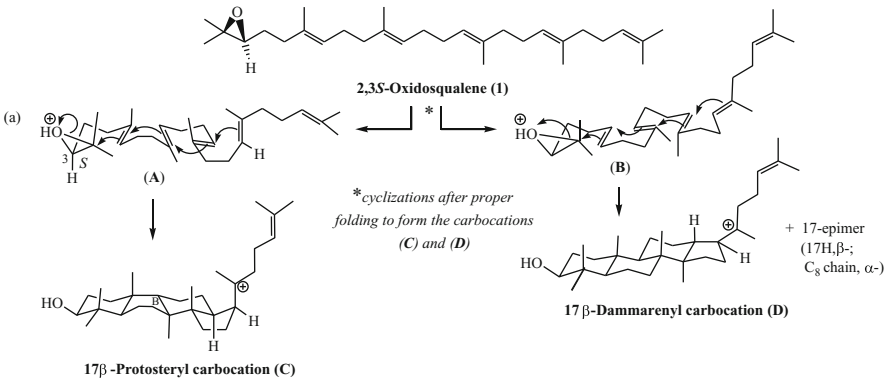


Fig. 10.8 Bioformation of tetracyclic cations (**C**) and (**D**) from 2.3*S*-oxidosqualene

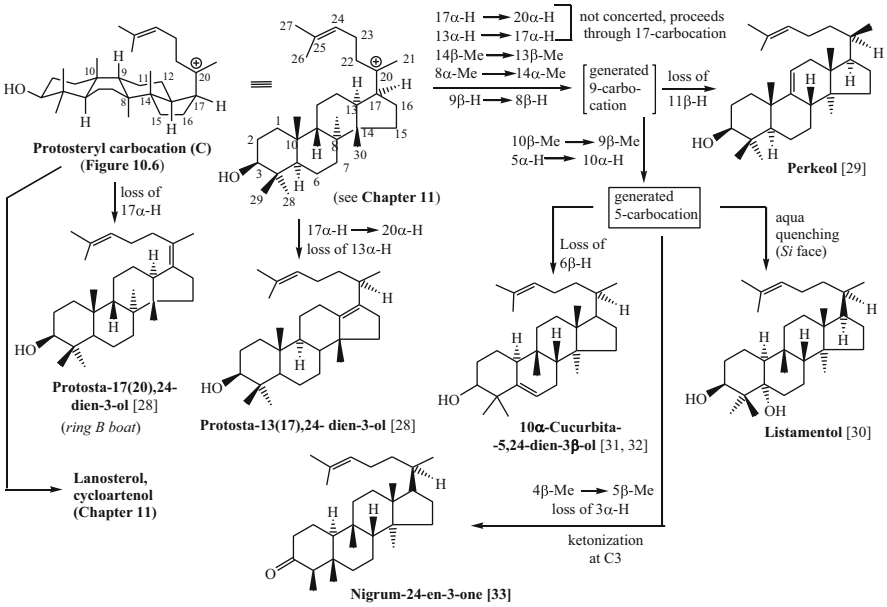


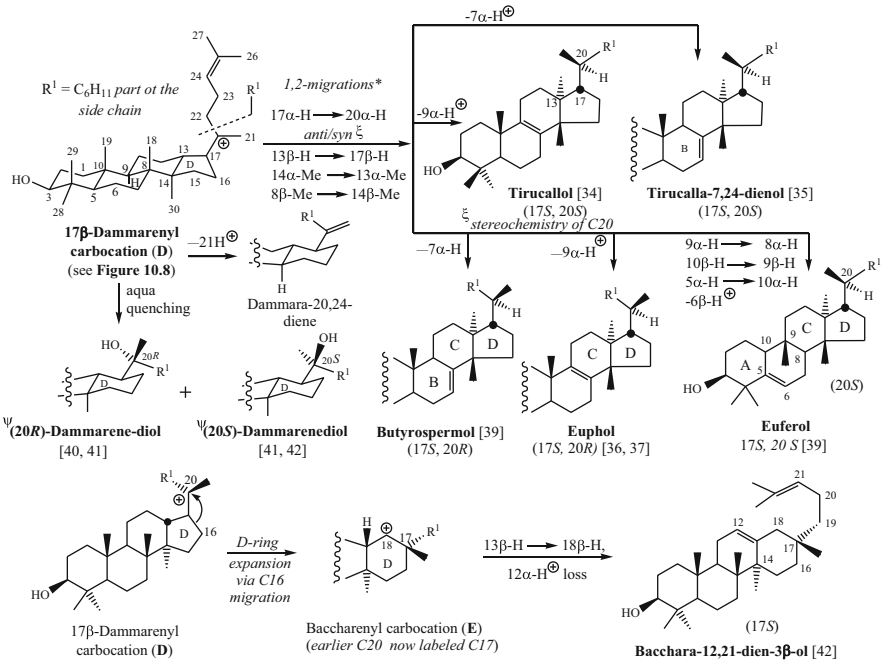
Fig. 10.9 Bioformation of 6-6-6-5 tetracyclic triterpenes derived from protosteryl carbocation (**C**)

oxidosqualene can form chair–boat–chair (A–B–C) cation (protosteryl carbocation) as well as chair–chair–chair (C–C–C) cation (dammarenyl carbocation) (Fig. 10.8) after cyclization [1, 2–4].

In case of tetracyclic triterpenes from squalene the latter will form the C–C–C all chair tetracyclic carbocation (**F**), i.e., the deoxy analog of dammarenyl cation. The carbocation (**F**) gives rise to some 6-6-6-6 or 6-6-6-5 tetracyclic triterpenes (Fig. 10.11) A particular way of folding of squalene and cyclization giving rise to 8(26),14(17)-onoceradiene, a C2-symmetric tetracyclic triterpene is also shown in Fig. 10.11.

10.1.5 Pentacyclic Triterpenes

Pentacyclic triterpenes are biosynthesized from the dammarenyl carbocation (**D**) (Fig. 10.8) or protosteryl carbocation (**C**). Formation of various isomeric carbocations due to 1,2-hydride and/or methyl migrations and ring D expansion and subsequent quenching of the carbocations through the loss of adequate protons or quenching by hydroxyl equivalent (e.g., H_2O) lead to the formation of a plethora of pentacyclic triterpenoids of diverse skeletal patterns. A few common compounds have been taken as examples and their biosynthetic pathways derived from lupanyl



**Migrations are always suprafacial. In a cyclohexane system only the axial group migrates and the migration terminus undergoes inversion of configuration, if an axial group from that center also migrates.*

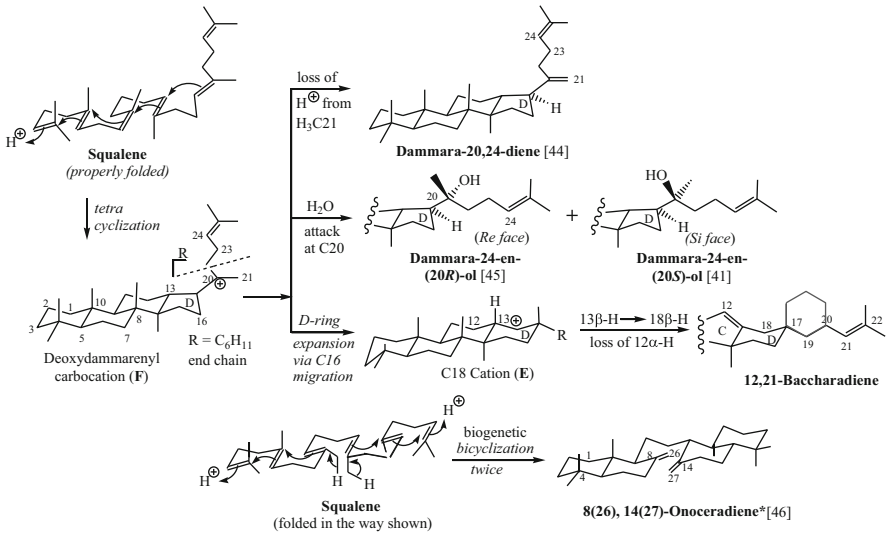
*ψC20(R) and C20(S) products are formed by quenching the carbocation (**D**) (planar at C20) with the side-chain R' **anti** to the C17-C13 bond) with an hydroxide equivalent from the lower (α-) face and upper (β- face) respectively. They may also be formed by attack of the planar carbocation with R' **syn** to C17-C13 bond from the upper face and the lower face respectively.*

*ξ 1,2-Migration of 17α-H to the α-face of the C20 carbocation in the rotamer with R' **anti** to the C17-C13 bond; followed by the 1,2-migrations and deprotonation shown produces tirucallol, or euferol with (20S)-configuration, whereas when the rotamer with R' **syn** to the C17-C13 bond is involved the final product is butyrospermol or euphol with (20R)-configuration.*

Fig. 10.10 Some tetracyclic 6-6-6-5 and 6-6-6-6 triterpenes derived from the dammarenyl cation (**D**) and bachharenyl cation (**E**)

cation (**G**) (Fig. 10.12), 18α-oleanyl cation (**H**) (Fig. 10.13), and hopyl cation (**J**) (Fig. 10.14) are delineated .

The pathway shown for the biosynthesis of eupacannol (Fig. 10.13) from bauerenol is a speculative mechanistic possibility, explaining the axial β-hydroxylation at C7. All the anti-migrations following the migration of 8β-methyl to 14-β-methyl may be continued without the involvement of bauerenol, hydroxylation taking place at any stage, even before cyclization of squalene epoxide. *Alternatively, eupacannol might be biosynthesized from squalene* (rather than from squalene epoxide), through the intermediacy of 3-deoxy-α-amyrinyl-8-cation (= deoxy-J cation). The latter undergoes migration of 14α-Me → 13α-Me, 8β-Me → 14β-Me and loss of 7α-proton to form 3-deoxybaurenol, followed by enzymatic β-axial hydroxylation at C7, and a series of methyl and hydride transfers in the stereospecific trans manner (cf. Fig. 10.13), and loss of C3 proton, and finally



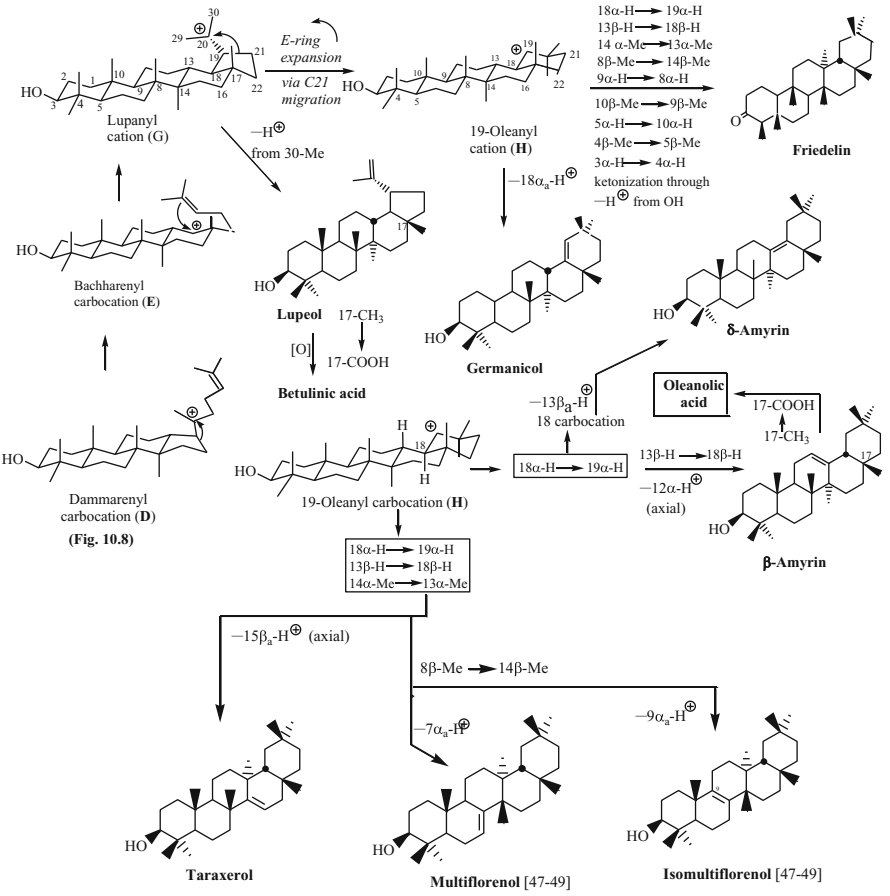
*Since squalene is symmetrical, acid catalyzed cyclization may start from either end in succession or from both ends simultaneously

Fig. 10.11 Bioformation of some 6-6-6-5 and 6-6-6-6 tetracyclic and a C_2-symmetric 6-6-$(CH_2)_2$-6-6 tetracyclic triterpenes from squalene

enzymatic reduction of the generated C3 = C4 double bond to push C4-Me to β-equatorial position (For molecular conformation see Fig. 10.33). The allylic hydroxylation at C9 in squalene itself, prior to its cyclization, might also take place, catalyzed by specific enzymes [51]. The substrate specificity of most enzymes still remains to be defined.

10.1.6 Cyclization of Bis-Oxidosqualene

The oxidation state of several triterpenoids indicates that they arise from a bis-oxidosqualene substrate. Two general modes of bis-oxidosqualene cyclizations that have been established experimentally are shown in Fig. 10.15. Squalene is epoxidized by squalene epoxidase to 2,3(*S*)-oxidosqualene, the cyclization modes of which have been outlined in Fig. 10.8. Under some conditions, when lanosterol synthase is present in restricted amount, the distal terminus of 2,3(*S*)-oxidosqualene can enter into reepoxidation by squalene epoxidase to form 2,3(*S*)-22(*S*),23-bisoxidosqualene (Fig. 10.15). The latter under the influence of different specific enzymes undergoes the conversions to a series of varied types of tetracyclic and pentacyclic triterpenes. The bioformations of a few such triterpenes, for example, reissantenol [57], 24,25(*S*)-epoxycycloartan-3(*S*)-ol [58, 59], α-onocerin [60], and serratenediol [61], derived from 2,3(*S*)-22(*S*),23-bisoxidosqulene, are delineated in Fig. 10.15.



Note: *References of the 6-6-6-6-6 pentacyclic triterpenes of common occurrence are not cited*

Fig. 10.12 Biosynthesis of lupeol (a 6-6-6-6-5 pentacyclic triterpene) and of some common 6-6-6-6-6 pentacyclic triterpenes derived from dammarenyl cation (**D**) *via* lupanyl cation (**G**)

A tetracyclic triterpene 3β-cabraleadiol [62] containing a substituted tetrahydrofuran moiety linked at 17β position would originate from 2,3-(*S*)-22-(*S*),23-bis-oxidosqualene following the probable biogenetic route depicted in Fig. 10.16 .

10.1.7 Sesquiterpene–Nortriterpene Adduct (C_{44})

Cheiloclines A-I, nine octacylic C_{44}-terpenoids reported from the root bark of *Cheiloclinium hippocratoides* (Fam. Celastraceae) [63], represent first examples

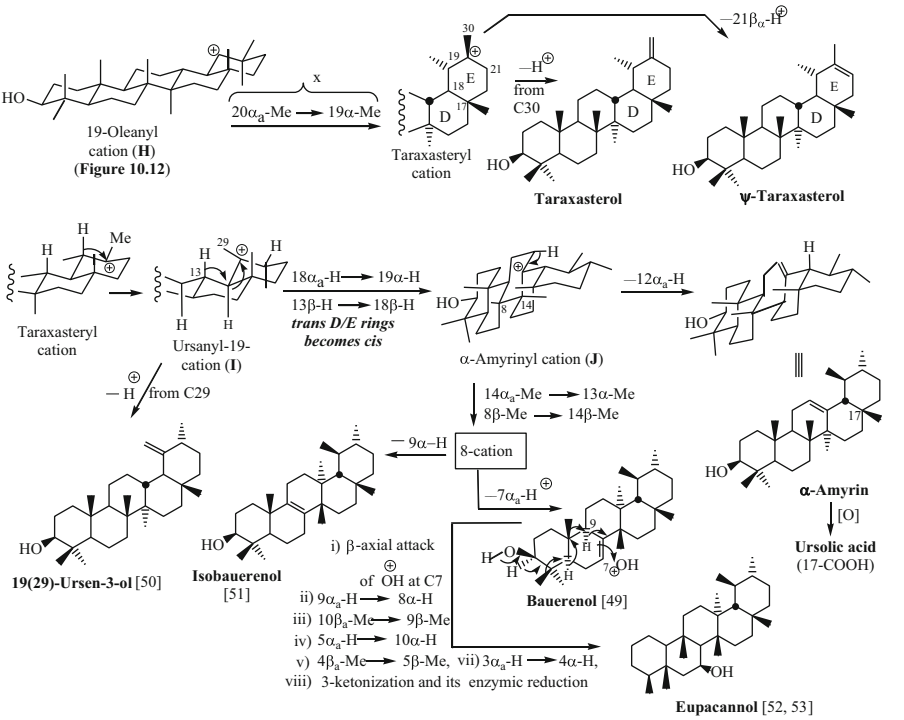
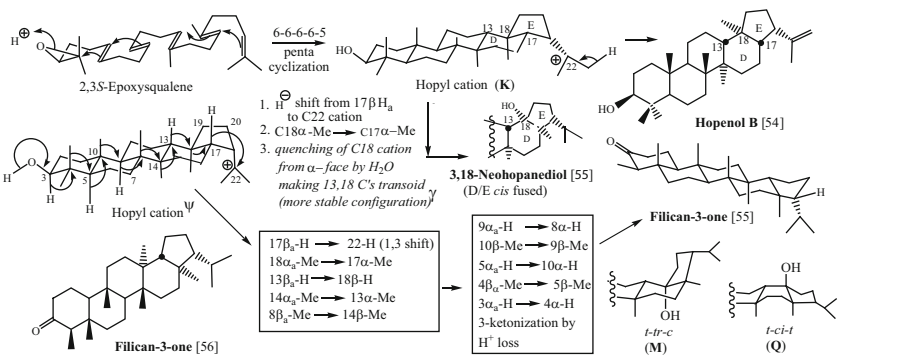


Fig. 10.13 Some 6-6-6-6-6 pentacyclic triterpenes derived from 19-oleanyl cation (**H**)



γ *The generated* ***trans-transoid-cis*** *configuration* **(M)** *of C/D/E rings is much more stable than their* ***trans-cisoid-trans configuration*** **(Q),** *(in which ring D attains. true boat conformation), resulting from quenching by H_2O from the β-face (see also Chapter* **2**).

ψ *The hopyl cation undergoes a 1-3H⊖ shift followed by a series of suprafacial1,2 anti migration and finally 3-ketonization occurs. to produce filican-3-one.*

Fig. 10.14 Biosynthesis of 6-6-6-6-5 pentacyclic triterpenes derived from 2,3*S*-epoxy squalene *via* hopyl cation (**K**)

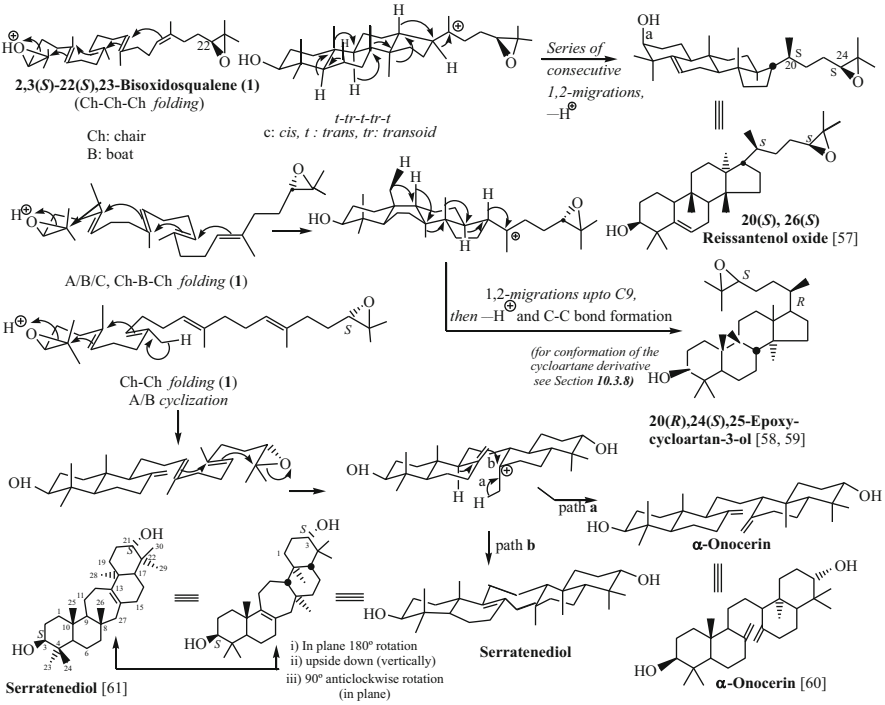


Fig. 10.15 Bioformation of tetracyclic and pentacyclic triterpenes derived from 2,3(*S*)-22(*S*),23-bisoxidosqualene

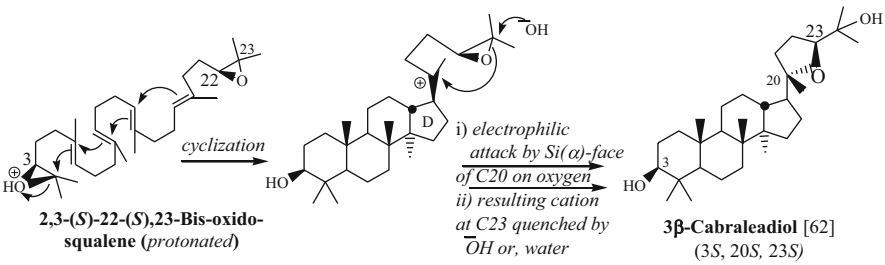


Fig. 10.16 Biosynthesis of 3β-cabraleadiol

of hetero-Diels–Alder adducts between a nortriterpenequinone and a sesquiterpene. Their structures were elucidated on the basis of COSY, ROESY, HSQC, and HMBC NMR experiments. They are composed of one aromatic triterpene unit derived from pristimerin (D:A-friedo-nor-oleanane skeleton) and one sesquiterpene unit derived from guaia-1(5),3(4), 11(12)-triene (Fig. 10.17). One such terpenoid, cheilocline A, is shown in this figure.

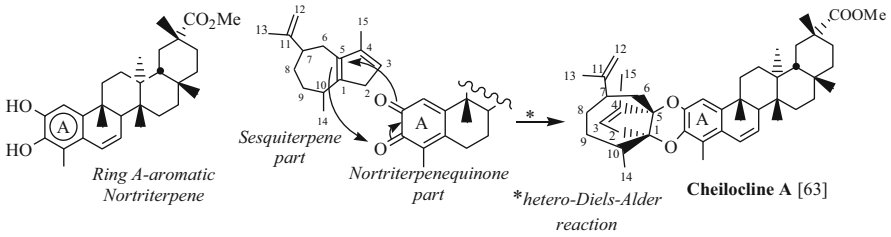


Fig. 10.17 Possible biogenetic formation of cheilocline A

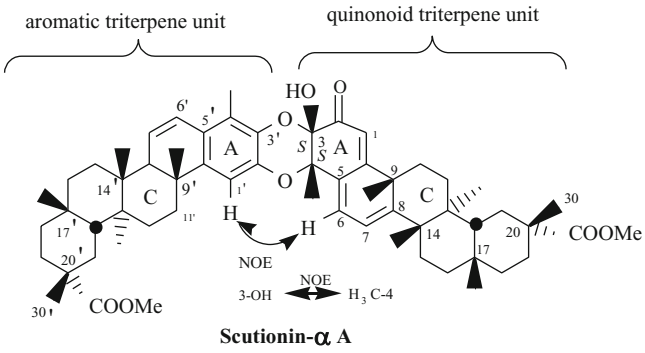


Fig. 10.18 Structure of a triterpene dimer scutionin-α A

10.1.8 Triterpene Dimers (C_{60}) and Triterpene Trimers

Triterpene dimers and triterpene trimers are also known to occur in nature. Seven dimeric compounds have been isolated [64] from the root bark of the subtropical shrub *Maytenus scutioides* (Celastraceae), distributed in North of Argentina and South of Paraguay and Bolivia. They were found to be composed of one quinoid-type triterpene, derived from co-occurring pristimerin or 7,8-dihydropristimerin and one aromatic triterpene, derived from pristimerin or 6-hydroxypristimerin linked together by two ether linkages between the two A rings. One of them scutionin-α A is shown in Fig. 10.18. The other triterpene dimers are 7,8-dihydroscutionin-αB, 7,8-dihydroscutionine-βB, scutidin-αA, the structures and absolute configurations of which have been elucidated [64].

González and his group [65] and Itokawa and his collaborators [66] have isolated more triterpene dimers and González et al. [67] have also reported triterpene trimers from Celastraceae plants.

10.2 Squalene, the Universal Precursor of Triterpenoids and Steroids

10.2.1 Occurrence. Biogenesis

Squalene, $C_{30}H_{50}$, has been named after its first source of isolation—the shark (*Squalus* species) liver oil. Later, it has been found to be ubiquitously distributed. It has been biosynthesized from two molecules of all *trans*-FPP. The puzzling mechanism of this coupling involves the alkylation of C2–C3 bond of one molecule of FPP by the second molecule of FPP and the formation of a cyclopropane intermediate (*presqualene*) followed by its opening, and the carbocation thus formed receives a hydride from NADPH (labeling experiment) serving as the H_R of the central methylene group of squalene. Its biogenesis has been exemplified in a sequence of steps in Chap. 5 (Fig. 5.20). Its structure has been settled in the conventional way by degradation and synthesis.

10.2.2 Synthesis of Squalene [68]

Cornforth applied their method of highly stereoselective olefin synthesis [69] to the synthesis of squalene in which no separation of geometrical isomers was necessary before the final step.

The C_{30} carbon skeleton is constructed according to the carbon skeletal linkage of the following three units.

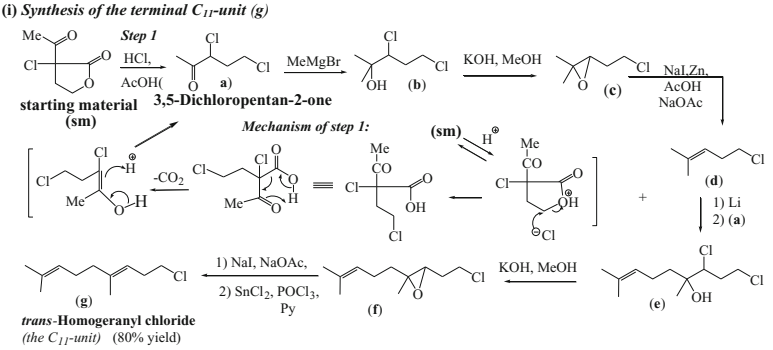
$C_{11} + C_8 + C_{11} = C_{30}$ (Fig. 10.19).

Many other syntheses of squalene have been reported later.

10.3 β-Amyrin

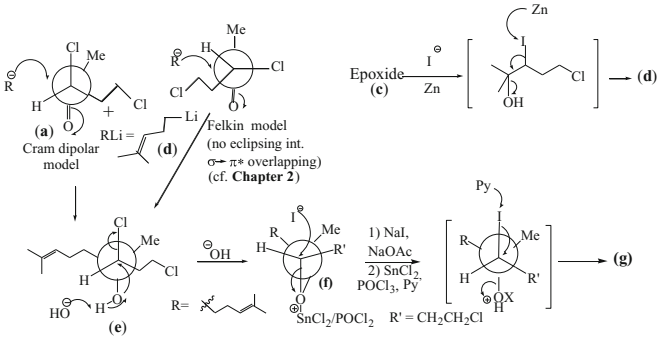
10.3.1 Occurrence. Structural Elucidation

β-Amyrin, the parent compound of the oleanane family, is the major component of Manila elemi resin and is quite prevalent in higher plants. It sometimes occurs as the major constituent as a single triterpene or in a mixture, either in the free state or as its ester like acetate, palmitate, etc. It was isolated from the latex of rubber trees and from *Erythroxylum coca* (Erythroxylaceae) [70]. Its structure has been decided on the basis of its reactions and degradation studies, some of which are delineated in Fig. 10.20. The reaction products have been identified and the elimination of other possibilities of the location of the double bond and the generated CO group and carboxylic groups during oxidative studies [71, 72] are not discussed. The



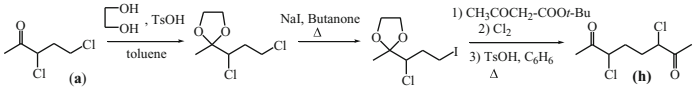
Mechanism of some steps:

The addition of the lithium alkyl [from (d)] to (a) takes place on the rotamer in which two vicinal dipolar groups $>C^{\delta+}=O^{\delta-}$ and $>C^{\delta+}\text{-}Cl^{\delta-}$ are as far as possible and antiparallel and the addition follows Cram's rule. Now, this addition can be better explained by Felkin model, as shown below.



This sequence of reactions constitutes a general stereoselective synthesis of trisubstituted olefins.

(ii) *Synthesis of the central C_8-unit*



(iii) *Building of the C_{30}-chain*

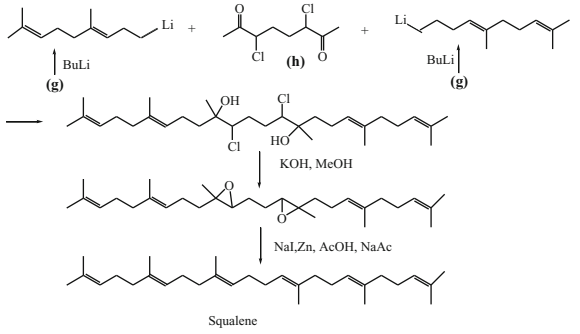


Fig. 10.19 First synthesis of squalene by Cornforth [68] (1959)

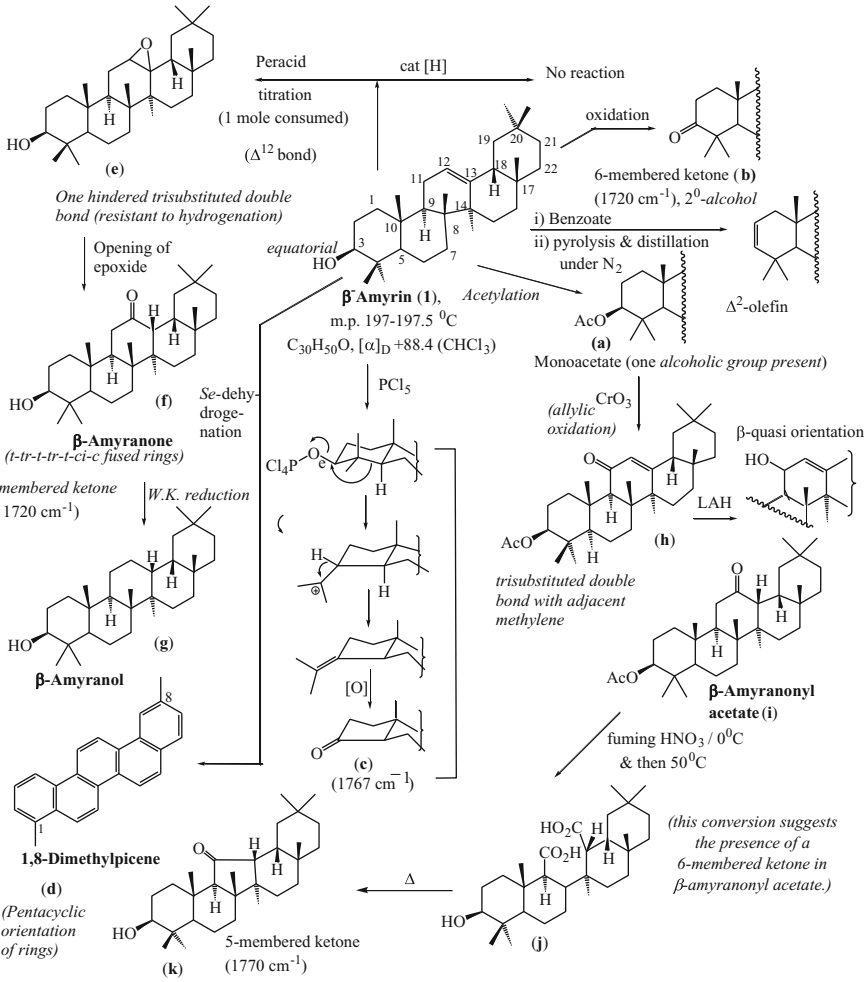


Fig. 10.20 Degradations and reactions of β-amyrin

formation and structures of the products which led to the correct structure of β-amyrin [71, 72] are exemplified in the figure.

Formation of (**a**) and (**b**) indicates the presence of a secondary OH group. Formation of (**c**) and (**d**) indicates that the secondary OH group is equatorial and is in the ring A of a perhydropicene skeleton.

It is resistant to hydrogenation; it forms an epoxide (**e**), which opens up to a ketone (**f**) and the latter could be reduced to (**g**). Further, (**a**) could be oxidized to an enone (**h**).

All these compounds suggest the presence of one hindered trisubstituted double bond with an adjacent methylene group. The compound (**i**) [≡ (**f**)-acetate] could be

oxidized to a dicarboxylic acid (**j**) which on heating gives a five-membered ketone (**k**). These indicate the presence of a six-membered ketone in (**i**).

10.3.2 Stereochemistry

Stereochemistry at C17 and C18. β-Amyrin has perhydropicene skeleton with a trisubstituted double bond in ring C. The relation of β-amyrin with oleanolic acid is shown in Fig. 10.21, which elucidates the stereochemistry at C17 and C18 [73].

Barton [74] has elegantly shown that C17 COOH in oleanolic acid is β-axial in ring D, and D/E rings are *cis*-fused as is evident from the following facts: the C17-CO and C13-O bonds in the lactone (**A**) are in 1,3-diaxial relationship in ring D (Fig. 10.21). However, opening of the lactone ring to form the thermodynamically more stable (**B**) having the 13,18 double bond is not possible due to the presence of C18–H and C13–O bonds (*e* and *a*, respectively, in ring D) in *cis* orientation. Thus, C18-H and C17-COOH in oleanolic acid must be in *e* and *a* orientation in ring D. This experiment showed that the D/E ring junction in β-amyrin and its derivatives is *cis*-fused. Had this ring junction been *trans* [(having the conformation (**C**)] (Fig. 10.22), opening of the lactone ring to form the $\Delta^{13(18)}$ compound (**B**) would have been stereochemically feasible (Fig. 10.22).

The stereochemistry at C18 and C9 has been deduced by base-catalyzed isomerization studies of methyl 11-oxo-oleanolate 3-acetate (**D**) (Fig. 10.22) [obtained by CrO_3 oxidation of acetyl oleanolic acid]. Epimerization at either or both the centers C9 and C18 of (**D**) (being activated by keto or conjugated keto group at C11) is possible. That the configuration at C9 remains unchanged has been decided by converting the epimeric product (**E**) to the 11,13(18)-diene (**F**) of

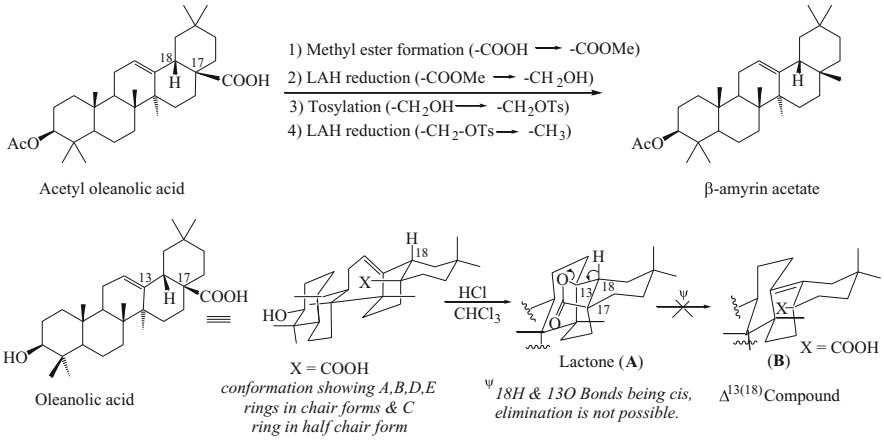


Fig. 10.21 Conversion of acetyl oleanolic acid to β-amyrin acetate and formation of the lactone from oleanolic acid

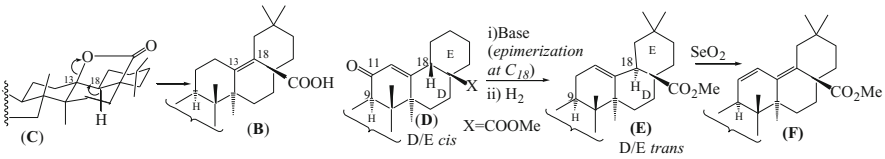
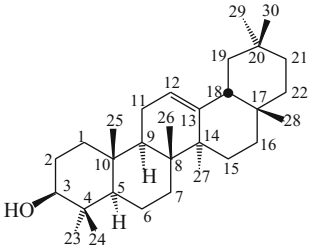


Fig. 10.22 Elucidation of stereochemistry at C18 and C9

known structure, in which stereochemistry at C18 is destroyed. Its C9 configuration remains unchanged indicating that B/C rings are *trans*-fused, which cannot be changed to the less stable *cis* fusion.

The configurations of A/B and B/C ring junctures have been settled by correlating oleanolic acid with other compounds of oleanane series, and by consideration of the stability of various perhydrophenanthrene diastereomers, of which the *trans-transoid-trans* is the most stable one (*vide* Chap. 2). In oleanane series the A–B–C system possesses the *trans-transoid-trans* stereochemistry. The synthesis of (**A**), the left-hand part precursor for the synthesis of β-amyrin from ambreinolide of known structure and stereochemistry (Fig. 10.26), establishes the stereochemistry of the A/B rings as *trans*. The absolute stereochemistries of β-amyrin and other triterpenes of oleanane series follow from their biosyntheses as described in Figs. 10.8 and 10.12.

10.3.3 Spectral Data of β-Amyrin



IR (KBr): 3350 (OH), 1392, 1368, 1140, 1048 cm^{-1}.

^{1}**H NMR** ($CDCl_3$; 400 MHz, δ) [70] 5.18 (t, J = 3.2 Hz, 1H), 3.27-3.18 (*m*, 1H), 1.13 (*s*, 3H), 1.00 (*s*, 3H), 0.96 (*s*, 3H), 0.93 (*s*, 3H), 0.86 (*s*, 3H), 0.83 (*s*, 3H), 0.79 (*s*, 3H), and very complex methylene envelope.

^{13}**C NMR**: ($CDCl_3$; 400 MHz, δ) C1 (38.5), C2 (27.0), C3 (78.9), C4 (38.7), C5 (55.1), C6 (18.3), C7 (32.7), C8 (39.7), C9 (47.6), C10 (37.0), C11 (23.5), C12 (121.9), C13 (145), C14 (41.7), C15 (26.2), C16 (27.3), C17 (32.5), C18 (47.2), C19 (46.8), C20 (31.1), C21 (34.8), C22 (37.2), C23 (28.3), C24 (15.4), C25 (15.5), C26 (16.8), C27 (26.0), C28 (28.8), C29 (33.2), C30 (23.6).

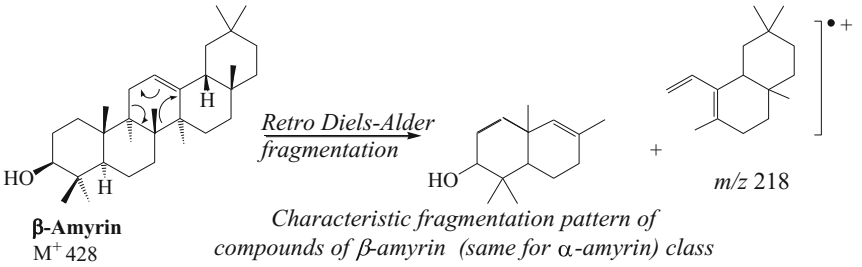


Fig. 10.23 Main mass fragmentation of β-amyrin

Mass Spectrometry

The characteristic mass fragmentation pattern [74] of β-amyrin is shown in Fig. 10.23.

10.3.4 Synthesis of β-Amyrin

Total synthesis of pentacyclic triterpenes with ring arrangement like β-amyrin by stepwise ring extension (linear synthesis) could offer great difficulty specially for having two adjacent quaternary carbon atoms at C8 and C14. Halsall and Thomas [75] suggested that this difficulty could be overcome, and a pentacyclic triterpene could be synthesized by constructing a suitable tetracyclic system with A,B and D,E rings, followed by ring closure to generate ring C. This suggestion received support from the acid-catalyzed cyclization of α-onocerin to γ-onocerin by Barton and Overton (Fig. 10.24).

Based on the above strategy Corey synthesized [76] a β-amyrin derivative, olean-11,13(18)-diene via cation–olefin cyclization of a tetracyclic intermediate containing A,B and D,E rings. During the preparation of the pentacyclic intermediate a precursor containing the A and B rings is joined to a monocyclic intermediate with a preformed E ring, and ring D is generated by an internal aldol condensation. Finally ring C is constructed by internal cation–olefin cyclization (Fig. 10.25). The two precursors (**A**) and (**B**) of the oleanane skeleton are shown in Fig. 10.25.

The pentacyclic skeleton was later on converted to β-amyrin by Barton [78]. Thus composite efforts of two groups of workers led to the total synthesis β-amyrin. The objective of Corey's work was to test the possibility of construction of a pentacyclic nucleus (perhydropicene) by cyclizing a tetracyclic intermediate with built-in rings A, B, D, and E via cation–olefin cyclization when ring C is formed (Fig. 10.26).

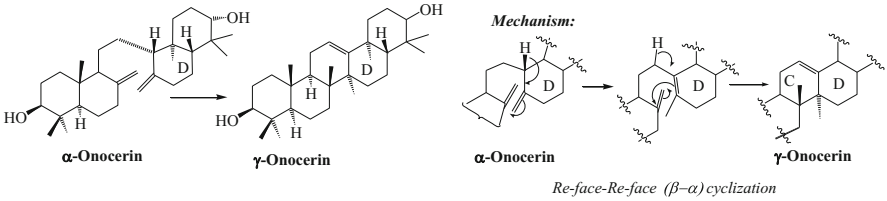


Fig. 10.24 Acid-catalyzed cyclization of α-onocerin to γ-onocerin to form the ring-C

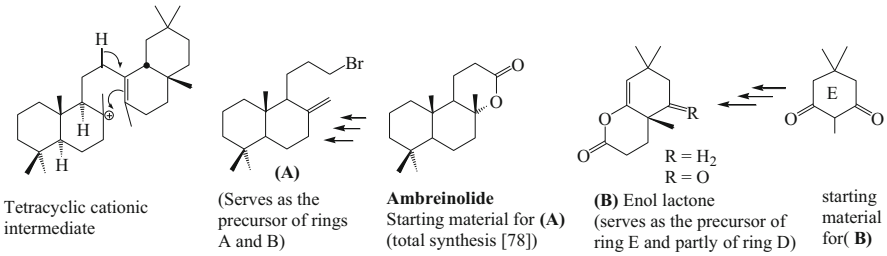


Fig. 10.25 Strategy for the synthesis of β-amyrin

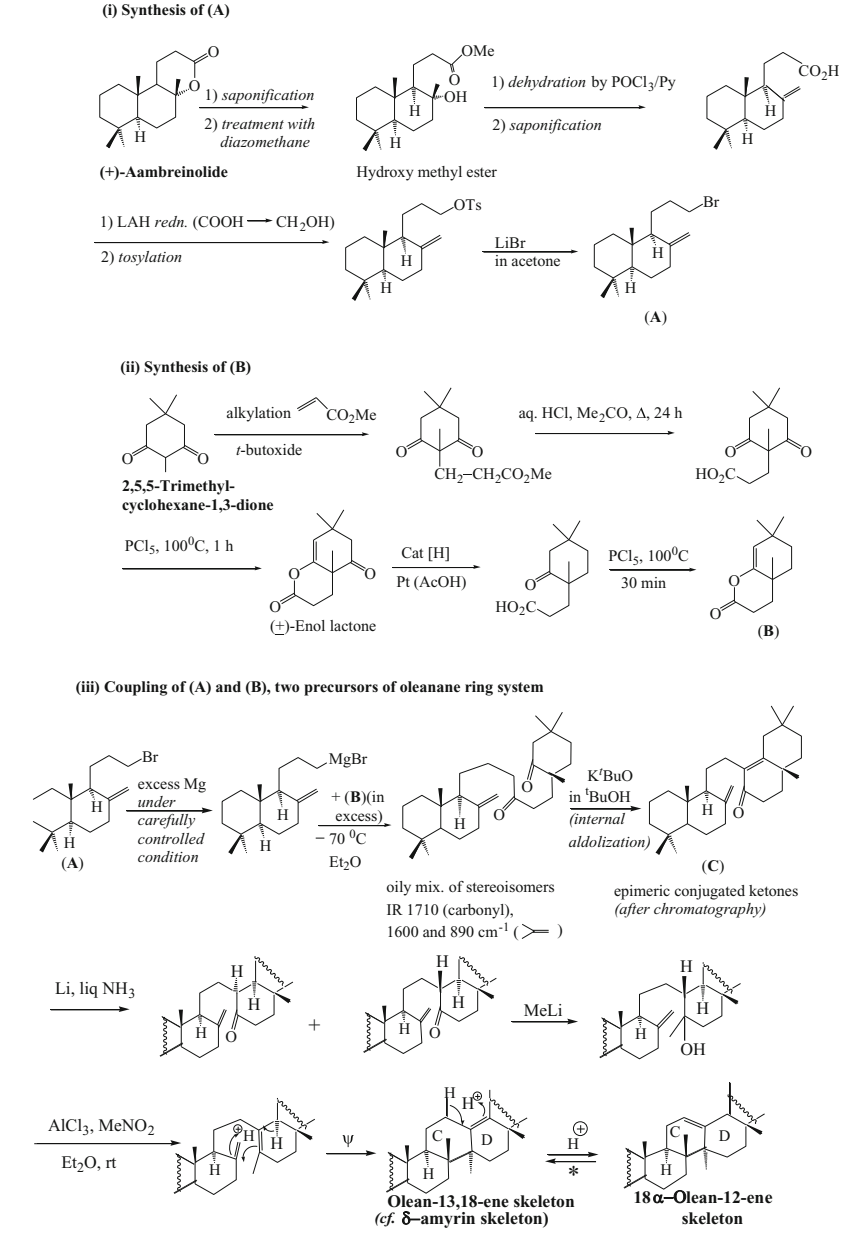
10.3.5 Formal Syntheses of β-Amyrin by Polyene Cyclization

In 1972 van Tamelen has shown that stannic chloride-nitromethane-induced cyclization of an epoxide (**A**) leads to δ-amyrin in 4 % yield [79]. The latter has been converted to 18α-olean-12-ene [B] by Brownlie and coworkers [80]. Compound [B] has been converted to β-amyrin by Barton et al. [78] (Fig. 10.27). Thus van Tamelen's synthesis of δ-amyrin constitutes the formal synthesis of β-amyrin.

Tori and coworkers reported [81] the acid-catalyzed backbone rearrangement of 3β,4β-epoxyfriedelane (**C**) to β-amyrin (Fig. 10.28), accompanied by other products. Friedelin has been synthesized by Ireland [82] and Kametani [83]. 3β,4-β-Epoxyfriedelane (**C**) has been derived from friedelin. Hence Tori's conversion constitutes another formal synthesis of β-amyrin. β-Amyrin has also been obtained as a backbone rearrangement product of taraxerol and multiflorenol (Fig. 10.28) [84].

10.3.6 Johnson's Total Synthesis of β-Amyrin by Polyene Cyclization

In 1993 W.S. Johnson et al. reported [70] a total synthesis of dl-β-amyrin (Fig. 10.29) in ~0.2 % overall yield by biomimetic polyene cyclization using fluorine atom as a cation-stabilizing (C-S) auxiliary. An appropriate polyene (**D**)



Notes: ψ *Direction of cation-olefin cyclization is important. In principle, the ring C can be formed by addition of AB ring cation to DE ring unsaturation.*

* *This acid catalyed equilibrium leans more towards tetra-substituted 13-18 double band.*

Fig. 10.26 (continued)

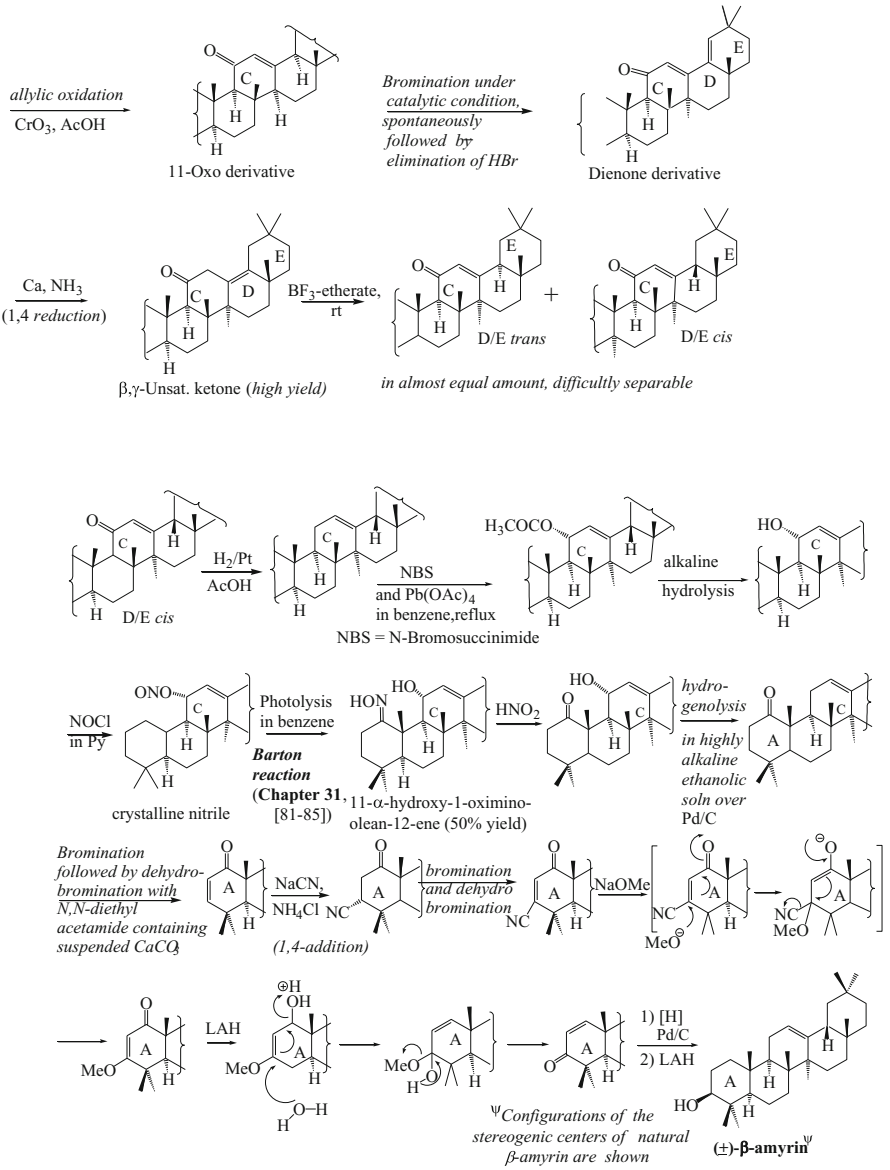


Fig. 10.26 Synthesis of (±)-β-amyrin

with tetrasubstituted *trans* fluoroalkene bond (at pro-C13) and having 3-*trans*, 7-*trans*,11-*cis*-alkene (alkene nomenclature) stereochemistry and a propargylsilane terminating group attached to a pentenoid system has been synthesized [70]. The latter was cyclized in CF_3COOH taken in CH_2Cl_2 at -70 °C to give a pentacyclic system (**E**) containing six out of the eight stereogenic centers of β-amyrin with

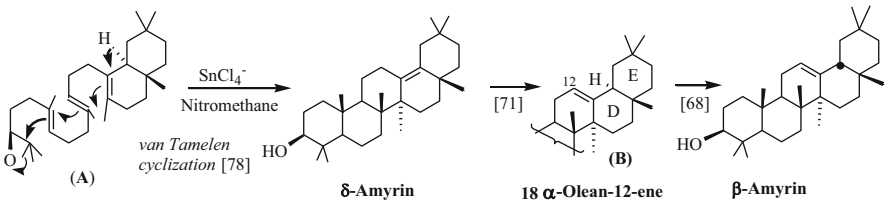


Fig. 10.27 Formal synthesis of β-amyrin via δ-amyrin by polyene cyclization

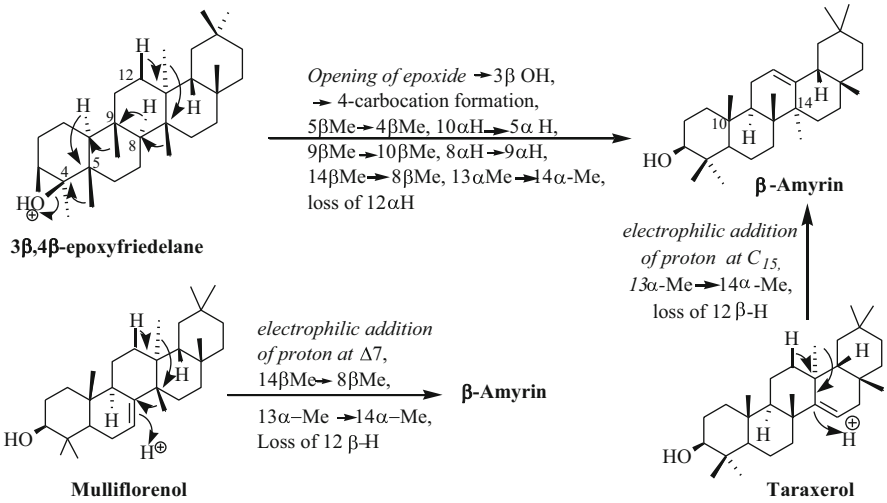


Fig. 10.28 Acid-catalyzed conversions of 3β,4β-epoxyfriedelane, taraxerol, and multiflorenol to β-amyrin

correct relative stereochemistry. Compound (**E**) was subjected to a number of steps delineated in Fig. 10.29 to finally give *dl*-β-amyrin [70].

10.3.7 3-Deoxy-β-Amyrin by Backbone Rearrangement of 3β-Fridelanol

Friedelin, a commonly occurring triterpene ketone on reduction, produces 3β-friedelanol. The latter on acid treatment produces 3-deoxy-β-amyrin. The spectacular backbone rearrangement is triggered by elimination of –OH as H_2O and is followed by a series of concerted stereospecific migration of axial H or CH_3 with electron pair on the same face as the migrating species and *anti* to the species migrating from that terminus, as delineated in Fig. 10.30.

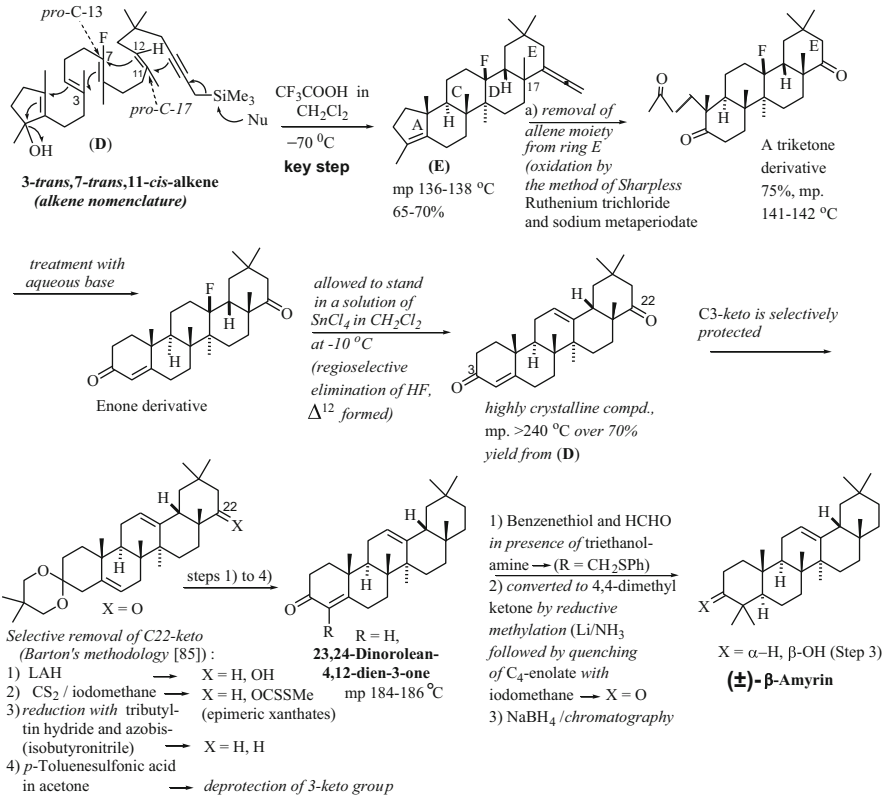


Fig. 10.29 Johnson's synthesis of (±)-β-amyrin using polyene cyclization as the key step [70] (1993)

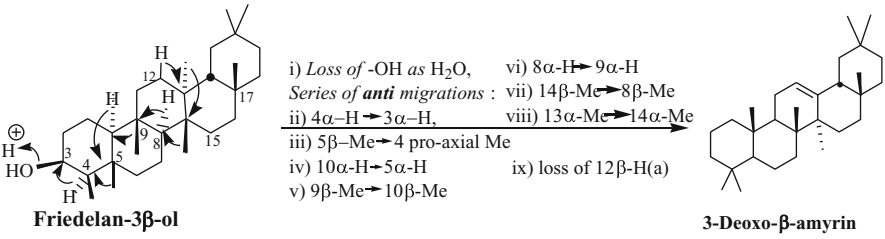


Fig. 10.30 A spectacular backbone rearrangement of friedelan-3β-ol to 3-deoxy-β-amyrin

10.3.8 Biosynthesis

For biosynthesis of β-amyrin see Fig. 10.12.

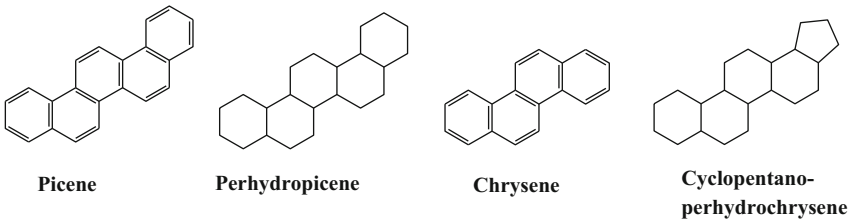


Fig. 10.31 Picene, chrysene, and their perhydroderivatives

10.4 Analysis of Molecular Conformations of Some Common Pentacyclic Triterpenes

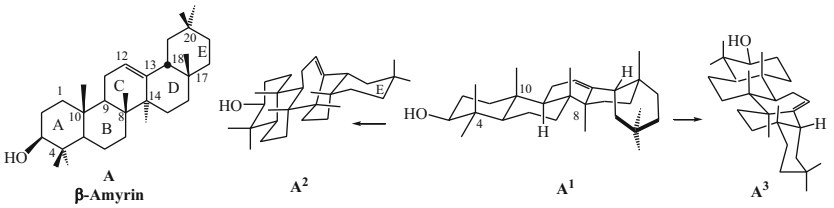
The beauty of the polycyclic terpenoid biosynthesis lies in the conversion of a single substrate into different skeletal patterns under the influence of different specific cyclases. In cases of triterpenoids the individual substrates are squalene, 2,3(*S*)-oxidosqualene, and 2,3(*S*),22(*S*),23-bisoxidosqualene. Each of them cyclizes to yield a large number of monocyclic and/or polycyclic triterpenoids of different skeletal patterns (Sects. 10.1–10.3) under the influence of specific enzymes. The basic carbocyclic rings are formed from a substrate undergoing a particular folding by a single enzyme-controlled asymmetric reaction. *The final configurations of the stereogenic centers are dictated by the extent of the antiperiplanar 1,2-hydride/alkyl shifts (migrations) and quenching of the terminal carbocation thus formed on the way towards the final product formation.*

The common fused ring systems with the pentacyclic triterpene framework belong to 6-6-6-6-6-fused cyclohexanes in the array of perhydropicene and to 6-6-6-6-5-fused cyclohexanes with a cyclopentane—in the way of cyclopentano-perhydrochrysene (Fig. 10.31).

Molecular conformations of some common pentacyclic triterpenes will be illustrated by reference of the above carbocyclic systems as revealed by careful examination of their Dreiding molecular models. The rings attain chair, half-chair, boat, or twist-boat conformations depending on the nature of ring fusion, presence or absence of endocyclic sp^2 carbons (as C=C), nonbonded interactions through space, steric crowding, etc. The preferred molecular conformation is the one which is energetically most comfortable.

10.4.1 Conformation of β-Amyrin

The molecular conformation of β-amyrin (**A**) having A/B, B/C, and C/D *trans*-fused and D/E *cis*-fused (steroid conformation) may be expressed as $\mathbf{A^1}$ or $\mathbf{A^2}$ or $\mathbf{A^3}$. In $\mathbf{A^1}$ β-bonds are going up and α-bonds are going down (Fig. 10.32). These conformations are equivalent. The conformation $\mathbf{A^1}$ is first obtained by constructing



A¹: The molecular model of **A** is held horizontally at the eye-level of the viewer in such a way that looking at the upper face of the molecule in a slanting manner (tangentially) the ring A, B, C, and D appear as chair, chair, half-chair, and chair (little distorted because of the sp^2 carbon at 13. In this perspective E ring will be viewed from the lower face.

A²: **A¹** is held at the eye-level, looking from the side of the ring E rotated 90^0 anticlockwise about the puckered plane of the molecule and viewed tangentially on the upper face in a slightly slanting position of the model to get the conformation as in **A²;** the ring E also appears as a chair form.

A³: If A^1 is rotated about 60^0 clockwise in the puckered plane of the molecule (which looking from the side of the ring E) and viewed in a slanting manner (tangentially) on the upper face, the molecular conformation like A^3 will be seen.

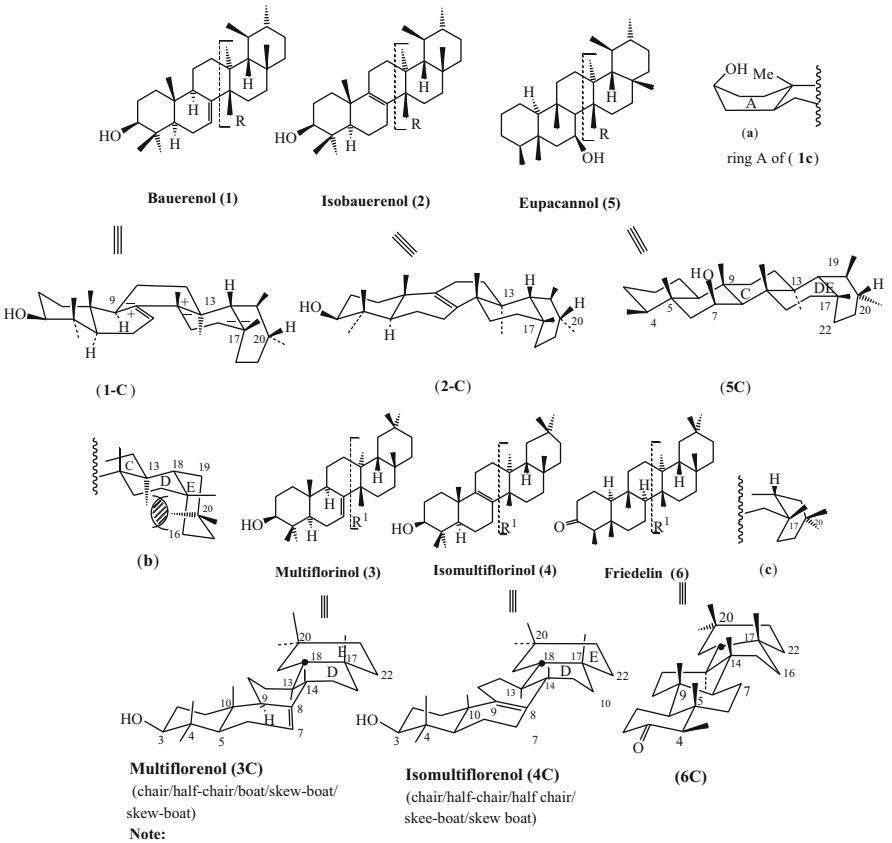
Fig. 10.32 Conformation of β-amyrin, different representations, and the manner of getting the perspective formulas

the molecule by Dreiding model and keeping the correct absolute stereochemistry of each chiral centre as in **A**. Rings A, B, D and E are in chair form, while ring C is in half chair form. Now projecting the model in different ways on the projection plane one can get the different representations of the same molecular conformation. This has been indicated in the Fig. 10.32.

β-Amyrin. The Conformations of Rings in β-Amyrin The molecule possesses chair–chair–half-chair–chair–chair conformations of rings A, B, C, D, and E, respectively, and thus involves 1,3-*syn* diaxial nonbonded interactions. To alleviate such interactions to some extent ring A may have slightly distorted chair conformation to minimize the energy content contrary to a report [86]. The corresponding boat conformation (lacking 4β-Me and 10β-Me *syn-a*xial interaction), if attained, will involve increase of energy due to flagpole–bowsprit (fp–bs) interaction between 3β-OH and 10β-Me (Fig. 10.33, Ic) in addition to the increase of energy by >5 kcal/mol due to the boat/skew-boat conformation.

10.4.2 Molecular Conformation of α-Amyrin

α-Amyrin possesses the same stereoconfiguration of β-amyrin and has 19β- and 20α-methyl groups (both equatorial in ring E chair) instead of 20-gem-dimethyl groups in molecular conformation of α-amyrin (vide Fig. 10.13) and is thus same as that of β-amyrin.



Special Note *The conformations of all the rings may appear in one view, as drawn, only in case of flat molecules, and not in cases of molecules having convex and/or concave surfaces (to avoid clumsiness). In the latter cases each ring will appear as chair/half-chair/boat/skew-boat only when looked at it in a particular direction. Usually one looks at the ring from the upper face in a slanting manner so that the front bonds appear as lower bonds. If one looks at the ring from the lower side, the front bonds will be projected as upper bonds.*

Fig. 10.33 Structures and molecular conformations of some pentacyclic triterpenes

10.4.3 Conformations of Bauerenol, Isobauerenol, Multiflorenol, and Isomultiflorenol

10.4.3.1 Conformation of Ring A

In 1968 the present authors have shown [51] from careful analysis of Dreiding models of bauerenol and isobauerenol (Fig. 10.33) that although ring A is free to assume boat form the associated extra strain will be enhanced by a strong fp–bs interaction between 10β-Me and 3β-OH(**a**), as already stated in cases of α-amyrin

and β-amyrin. Contrary to the literature report [86] on bauerenol *a slightly distorted chair conformation is, therefore, preferred in the ground state for all polycyclic triterpenes having 10β-Me and 4-gem-dimethyl groups, as in bauerenol* (**1**), *isobauerenol* (**2**), *multiflorenol* (**3**), *and isomultiflorenol* (**4**) (Fig. 10.33).

10.4.3.2 Conformations of Rings B and C

As expected, bauerenol (**1C**) will have rings B and C in half-chair and true boat conformation, respectively, to maintain the correct stereochemistry at C9, C13 andC14. The opposite torsion angle signs at 9-8 and 14-13 bonds (1st and 3rd bonds in ring C) also dictate that ring C must possess boat conformation (*cf.* Sect. 2.14.2).

10.4.3.3 Conformations of Rings D and E

The nonbonded interactions existing in different possible conformations of the *cis*-fused nonsteroid forms of D/E rings of bauerenol and multiflorenol, as revealed from careful analysis of their Dreiding models, have been recorded in Table 10.1, in their possible decreasing order of enthalpy (or increasing order of stability). Careful analysis of the additional nonbonded interactions present in the four possible conformations in case of bauerenol (**1**) [(i) to (iv)] and multiflorenol (**3**) [(v) to (viii)] leads to the increasing order of relative stability of the different conformers in each case, as shown in the Table, and also to the facts that conformer (iv) with *cis*-fused D/E chair/skew-boat form and the conformer (viii) with *cis*-fused D/E skew-boat/skew-boat form are the most stable and hence the predominant conformers of bauerenol and multiflorenol, respectively. The enthalpy difference measured qualitatively also indicates that the conformations (iv) and (viii) of the D/E rings of bauerenol and multiflorenol are expected to be their only predominant conformations.

Again the torsion angle signs (−/−) at the D/E ring junction are consistent with the *cis*-fused D/E rings of non-steroid form (**1C**) (*cf.* Sect. 2.15.1.3). In isobauerenol (**2**) [51] both the rings B and C are in half-chair conformation. Like Bauerenol D/E rings of isobauerenol are *cis*-fused and of nonsteroid form. In both bauerenol and isobauerenol ring D prefers chair form, but ring E must exist in a boat (or skew-boat) conformation with 17β-Me and 20β-H at the fp–bs positions, since a severe steric interference between 13α-Me and 20α-Me in addition to 1,3-*syn* diaxial interaction between 17β-Me and 19β-Me makes it impossible for rings D and E to attain chair conformation as shown in (**b**) [51] (Fig. 10.33).

Chemical Support Facile SeO_2/AcOH oxidation of bauerenyl acetate to form the corresponding 7,9(11)-hetereoannular diene and inertness of isobauerenyl acetate to SeO_2/AcOH even under drastic condition have been explained [51] on the basis of their proposed conformations. These facts reflect the relative instability of bauerenyl acetate due to its nonbonded interactions, already mentioned, and

Table 10.1 Nonbonded interactions existing in different conformations of D/E rings of bauerenol (1) and multiflorenol (3)

	Conformations of rings D/E	Nonbonded interspace interactions + additional interactions over chair-chair conformations
Bauerenol (in order of decreasing enthalpy/increasing stability)		
(i)	Chair/chair	13α-Me↔20α-Me (v. strong), cannot be attained, 17β-Me↔19β-Me (*syn* axial), 13α-Me↔17-20 bond, 14β-Me (axial) in ring D, 19β-Me*(a)* in ring E
(ii)	Skew boat/skew-boat	13α-Me↔16α-H (*fp↔bs*), 14β-Me↔17β-Me (fp↔bs alleviated), additional enthalpy for two skew-boats.
(iii)	Boat/chair	14β-Me↔17β-Me (*fp↔bs)* (v. strong) + additional enthalpy for true boat
(iv)	Chair/skew-boat	17β-Me↔20β-H(*fp↔bs*) (alleviated in skew-boat), 13α-Me(*a*) (ring D) ↔ 17-22bond (*syn*-diaxial type) + (additional enthalpy for skew-boat);
Multiflorenol (in order of decreasing enthalpy/increasing stability)		
(v)	Boat/chair	14β-Me ↔ 17β-Me (*fp↔bs*), + additional enthalpy for true boat
(vi)	Chair/chair	13α-Me (a)(ring D) ↔ 20α-Me (*a*)(ring E)(v.strong) *does not allow this conformation*, 13α-Me ↔ 17-22 (bond), 17β-Me↔20α-Me (axial in ring E)
(vii)	Chair/skew-boat	17β-Me ↔ 20β-Me (fp ↔ bs, alleviated in skew-boat), 13α-Me ↔ 21α-H (*fp ↔ bs*, alleviated in alternative skew boat, 14β-Me (*a* in ring D, additional enthalpy for skew boat.
(viii)	Skew-boat/skew-boat (worked through 15-16 bond in eye level)	13α-Me ↔ 16α-H (*fp ↔ bs*), + additional enthalpy for two skew-boats

Note The enthalpy due to the remaining part (A/B/C-fused rings) is same for all possible conformations of D/E rings of bauerenol and multiflorenol

involvement of low activation energy of this reaction (involving release of strain). On the contrary, isobauerenyl acetate, which is free from any severe nonbonded interaction has to involve high-energy TS of boat conformation of ring C and severe nonbonded interaction for undergoing this reaction, remains unchanged. These facts have been explained mechanistically [51].

Multiflorenol (3) and Isomultiflorenol (4) It has been suggested [51] that multiflorenol (**3**) and isomultiflorenol (**4**)—both having geminal methyls at C20—will have conformations of A, B, C ring identical with those of bauerenol and isobauerenol, respectively. Both will have rings D and E in the boat or skew-boat form, as shown in (**3C**) and (**4C**) (Fig. 10.34), to avoid strong fp–bs interaction between 17-Me and 20β-Me groups in case; rings D and E assume chair and boat conformation respectively as shown in (**C**).

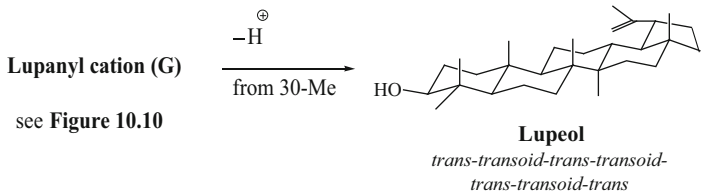


Fig. 10.34 Molecular conformation of lupeol

10.4.4 Molecular Conformations of Eupacannol, Friedelin, and Derivatives

From the structures of bauerenol (**1**), isobauerenol (**2**), and eupacannol (**5**), it is evident that their **R** part is common (Fig. 10.34); hence the conformations of D and E rings will be similar in these cases [*cf.* (**1C**), (**2C**) and (**5C**)]. Thus, in eupacannol [52, 53] also *cis*-fused D/E rings are in chair/skew-boat form (**5C**). R′ part is common in multiflorenol (**3**), isomultiflorenol (**4**), and friedelin (**6**); hence the *cis*-fused D and E rings will be similarly adopting skew-boat/skew-boat conformations.

From the conformational analyses (with the Dreiding model) of multiflorenol, bauerenol and their isomers it is well understood that during the biosynthesis of pentacyclic triterpenes (perhydropicene type) in plant cells, when the 1,2 *anti*-migrations advance to such an extent that the terminal carbonium ion (prior to quenching) gets a home beyond C13 in ring D or C or B or A, all triterpenoids (olefin or alcohol) derived from that carbonium ion will have D/E *cis*-fused rings. If the carbonium ion gets a home in ring B or A the *cis*-fused D/E rings will be in *skew-boat*/*skew-boat* conformation with *geminal methyls at C20*, while with *C19, C20 vicinal methyls* in *chair-skew boat* conformation.

Our conjecture made in 1968 [51] has been shown to be correct by detection in 1983 of D/E boat conformations in friedelin by methyl-to-methyl nuclear Overhauser enhancement studies [87], and also by ^{1}H NMR data of some friedelin derivatives isolated from the roots of *Martonia diffusa* (Celastraceae) [88]—with special reference to the in influence of 20α-Me and its natural oxidation products and also of the oxidation of 13α-Me to CH_2OH, CHO and COOH. The bulk requirement of CH_2OH (sp^3), CHO (sp^2), and COOH (sp^2) at C20 and that of Me (sp^3) at C13-α position play an important role in acquiring the conformations of rings D and E as follows: 13α-Me and 20α-Me or 13α-Me and 20α-CH_2OH (*cis* D/E boat–boat) and 13α-Me and 20α-CHO or 13α-Me and 20α-COOH (*cis* D/E chair–chair). These results confirmed our conjecture proposed in 1968 [51]. These are well explained in terms of their interspace nonbonded interactions. Further support came from the X-ray analysis of a co-crystal of friedelin-3β-ol and friedelin (0.75/0.25), in which three cyclohexane rings adopt chair conformation, one adopts a twist-boat conformation, and the fifth is in boat conformation [89], consistent with our assignment from Dreiding model analysis.

10.4.5 Molecular Conformation of Lupeol

Lupeol, a very common pentacyclic triterpene of higher plants, is having a cyclopentanoperhydrochrysene skeleton. The molecular conformation of Lupeol is straightforward. It attains chair–chair–chair–chair five-membered cyclopentane conformation; here the lupanyl carbocation (**G**) has been quenched as an olefin forming an isopropylidene group (Fig. 10.34).

Interestingly, it has been shown that the absolute configurations of all the eight chiral centers of lupeol isolated from a fossil have been found to be identical with those of lupeol isolated from higher plants. The specimens of lupeol isolated from these two sources of a gap of millions of years are identical in all respects (and not diastereomeric—being epimeric at any one or more chiral center/s [90]. This is an amazing finding, since the molecule escapes the pressure of a large period of evolution.

10.5 Conclusion

Careful analyses by means of Dreiding models of the nonbonded interactions present in different conformations of polycyclic molecules containing three, four, or five rings (six-membered) having terminal cis-fused rings may lead to their preferred conformations which might be confirmed by suitable X-ray and/or NMR studies.

10.5.1 Diagenetic Product of β-Amyrin

1,8-Dimethylpicene [Fig. 10.20, structure (**d**)] is the major diagenetic product of β-amyrin.

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Chapter 11
Steroids: Cholesterol and Other Phytosterols

11.1 Introduction

Steroids belong to a class of organic compounds having a common cyclopentanoperhydrophenanthrene nucleus, a fused four-ring system made up of 17 carbon atoms. Most steroids carry a side chain at C17, varying in composition. Sometimes the side chain remains attached to C16 and C17 in the form of a spiroketal ring. The steroids contain a hydroxyl group at C3 (a few contains C3-keto) and extra hydroxyl/s at different position/s, a double bond at C5–C6, and often with aromatized ring A. The term steroid has been first used in 1827. Steroids upon heating with selenium at ~360 °C in a sealed tube generally yield *Diels hydrocarbon*, shown to be 3′-methyl-1,2-cyclopentenophenanthrene derivative (last structure in Fig. 11.1). Steroids are bioactive compounds; many of them behave as hormones and prohormones and drugs and prodrugs and control many important physiological actions. They include *bile acids* in which the terminal carbon atom of the truncated side chain has been oxidized to a carboxylic group. Some examples of common steroidal compounds of plant (P) and animal (A) origins are shown in Fig. 11.1.

11.2 Cholesterol

11.2.1 Introduction: Functions in Human System

Cholesterol and some other sterols, e.g., cholic acid and ergosterol, were isolated in the nineteenth century. However, their structural elucidations were achieved in the twentieth century when chemical knowledge came out of its infancy and was ripe enough for the structural work. In course of time (1936–1992) a number of steroidal sex hormones and more than 30 steroids were isolated. The synthesis of cortisone by Sarett and Kendall and the report by Philip Hench on the anti-inflammatory

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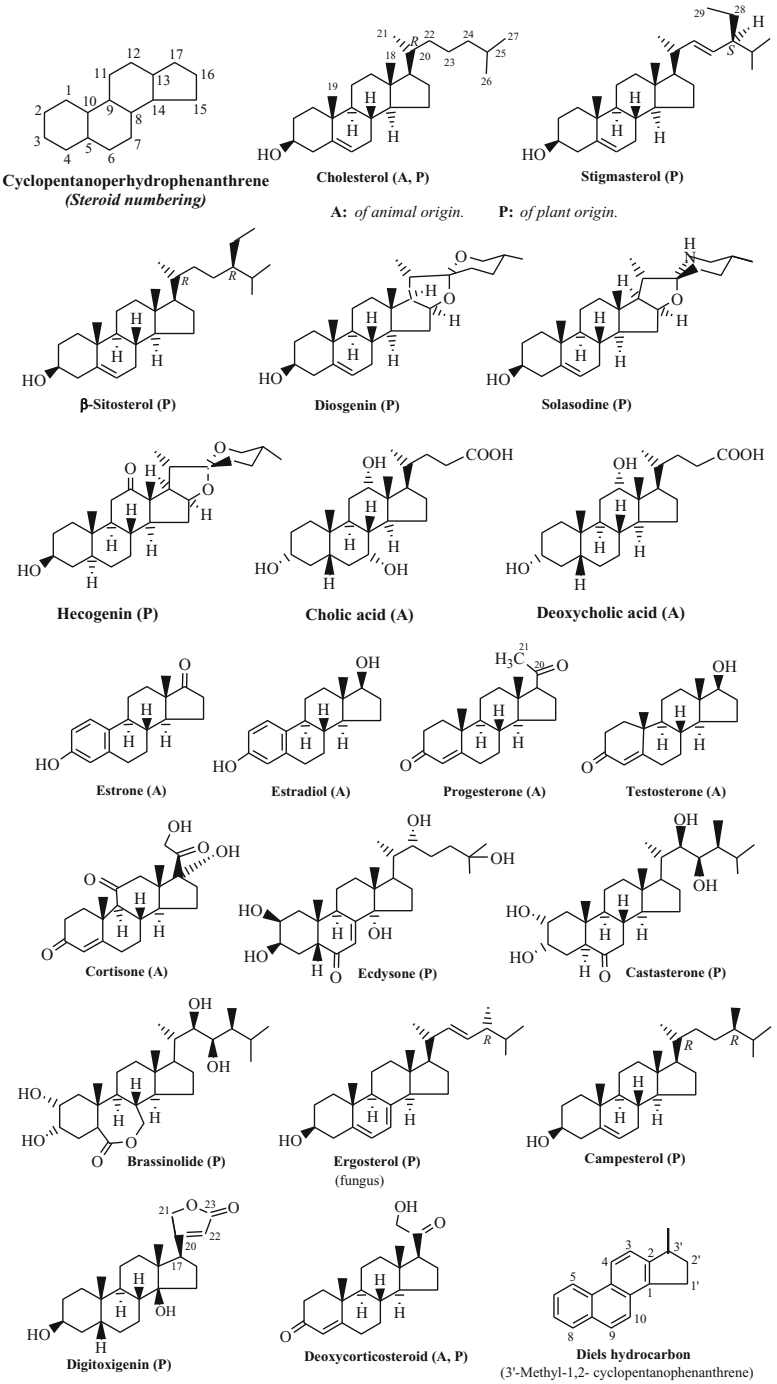


Fig. 11.1 Common steroidal compounds of plant (P) and animal (A) origin

property of cortisone in the treatment of rheumatoid arthritis made the whole field of steroid an attractive area for chemical and biochemical research [1].

"A string of Nobel Prizes signaled contemporary appreciation of the significance of steroid discoveries during these decades. Wieland (1927), Windaus (1928), Butenandt, and Ruzicka (1939) were awarded the Chemistry Prize, while Hench, Kendall and Reichstein shared the Nobel Prize in Physiology or Medicine in 1950 for their pioneering work on cortisone. The same Prize went to Konrad Bloch in 1964. The classic early structural studies of the 1920s and 1930s were followed by the elucidation of steroid stereochemistry, which took a new turn following the introduction of conformational analysis by Derek Barton in his classic *Experientia* paper in 1950. Barton and Odd Hassel were awarded the 1969 Nobel Prize in Chemistry for their work in this area." [1, see also 2 and 3]

Industries also got involved in the discovery and synthesis of steroidal drugs having profound bioactivity and high sensitivity as well as high specificity.

Of all the bioactive steroids mostly occurring in animals cholesterol is the most familiar and abundant one. It is also the first steroid isolated from human gall stones by Conradi in 1775 and thus was the first member of the steroid family to be discovered (see [4]). It derived its name from the Greek version of gallstone—*Chole* (gall bile) and *stereo* (solid) and *ol* for alcohol. Cholesterol is found in tissues of mammalian body, particularly in the spinal chord and brain. Mammals are capable of absorbing cholesterol from dietary sources. It is also synthesized in the body. It serves as the precursor of various steroidal hormones and bile acids. The latter as the sodium salt can act as detergent-like substances that emulsify fats and oils to make them easily digestible.

Improper balance by way of a marked change in the cholesterol content in our system is responsible for some serious diseases. When cholesterol content of the bile acids increases to the extent of making its supersaturated bile acid solution, cholesterol is thrown out of the solution as crystals and forms gallstones in which it remains as a major component.

Defects in maintaining proper cholesterol level in blood serum have serious consequences. Elevated level of serum cholesterol causes atherosclerosis (hardening of arteries due to cholesterol deposits) and coronary artery disease. *When the cholesterol deposits break loose and the resulting thrombus blocks the flow of blood in the heart it causes a heart attack and causes a stroke when the flow of blood in the brain is blocked.*

In order to reduce the cholesterol content of the body system, some inhibitors have been designed which interrupt certain step/s in the biosynthesis of cholesterol. But cholesterol being the precursor of various steroidal hormones is necessary to maintain the homeostasis in the organisms; the desirability of such restriction or "full-scale assault" in its biosynthetic pathway is questioned. Bloch wrote [5]: "Researchers in the field refer to cholesterol as the paradigm of a Janus-faced substance, a villain causing cardiovascular disease on the one hand, yet at the same time, a molecule essential for bodily function."

11.2.1.1 Functions of LDL and HDL

Cholesterol and its esters are transported in the plasma in the form of water-miscible lipoprotein particles. They are characterized by their density as (i) *very low-density lipoprotein* (VLDL, *highest lipid content*), (ii) *low-density lipoprotein* (LDL, *intermediate lipid content*), and (iii) *high-density lipoprotein* (HDL, *least lipid content*). LDL transports cholesterol and its esters from hepatocyte to extrahepatocyte tissues, i.e., from liver to other tissues. An elevated LDL level will transport more cholesterol especially to cell walls of blood vessels (arteries) and forms *atherosclerotic plaques* causing narrowness of the vessel. Therefore, LDL is commonly known as *bad cholesterol*. High-density lipoprotein (HDL) binds and esterifies cholesterol released from peripheral tissues and then transfers cholesteryl esters to hepatocyte tissues, i.e., from the blood vessel walls to the liver or to tissues that use cholesterol to synthesize steroid hormones. Hence, HDL is usually known as *good cholesterol*. Thus, the words good cholesterol and bad cholesterol, signifying HDL and LDL, respectively, in our system, are justified. For a healthy person the HDL/LDL ratio is ~3.5.

11.2.1.2 Occurrence in Animals and Plants

The occurrence and function of cholesterol in animal, especially in human tissues, have been a much discussed topic; thus, most people, even some scientists, believe that cholesterol does not occur in plants. In view of such a belief and also in view of the absence of adequate mention in the biochemistry text books and in the *Dictionary of Natural Products* [6] of the presence of cholesterol in plants, Behrman and Gopalan [7] wrote a paragraph for the next generation of biochemistry texts as stated below. “But plants also contain cholesterol both free and esterified. Cholesterol occurs as a component of plant membranes and as a part of the surface lipids of leaves where it is sometimes the major sterol. The quantity of cholesterol is generally small when expressed as percent of total lipid. While cholesterol averages perhaps 50 mg/kg total lipid in plants, it can be as high as 5 g/kg (or more) in animals.”

It is pertinent to point out that biosynthetic proofs were available for the formation of cholesterol from oxidosqualene via cycloartenol in plants and via lanosterol in animals and fungi. Since its first isolation from plants (algae) by Tsuda [8], reports on the occurrence of cholesterol from various plants are known in the literature [9, 10]. Further, since mammals are capable of absorbing cholesterol from dietary sources, people become more concerned about the intake of vegetable and non-vegetable diets, more especially of **vegetable oils** which contain cholesterol (Chap. 34).

11.2.2 Structural Elucidation. Relative and Absolute Stereochemistry. Conformation

The structural elucidation of cholesterol (**1**) has a long chemical history [11] which includes the brilliant and extensive work of a number of outstanding chemists of those days (1903–1933), of which special mention may be made of Adolf Windaus (dating from 1903), Otto Diels, H.O. Wieland (dating from 1912), E. Abderhalden, Otto Rosenheim, and Harold King. X-ray crystallographic work on steroids by J. D. Bernal led to the revision of the structure initially proposed by Windaus and Wieland for cholesterol.

Cholesterol, $C_{27}H_{46}O$, is a laevorotatory crystalline solid, m.p. 149°, $[\alpha]_D$ –39 (EtOH), –31.8 (Et_2O). The stereostructure (**1**) and its molecular conformation (**1a**) have been established on the basis of the findings shown schematically in Fig. 11.2, stereochemical analysis, and finally by its total synthesis. The steroidal nomenclature is shown in (**1**). A huge amount of experimental data, observations, and often

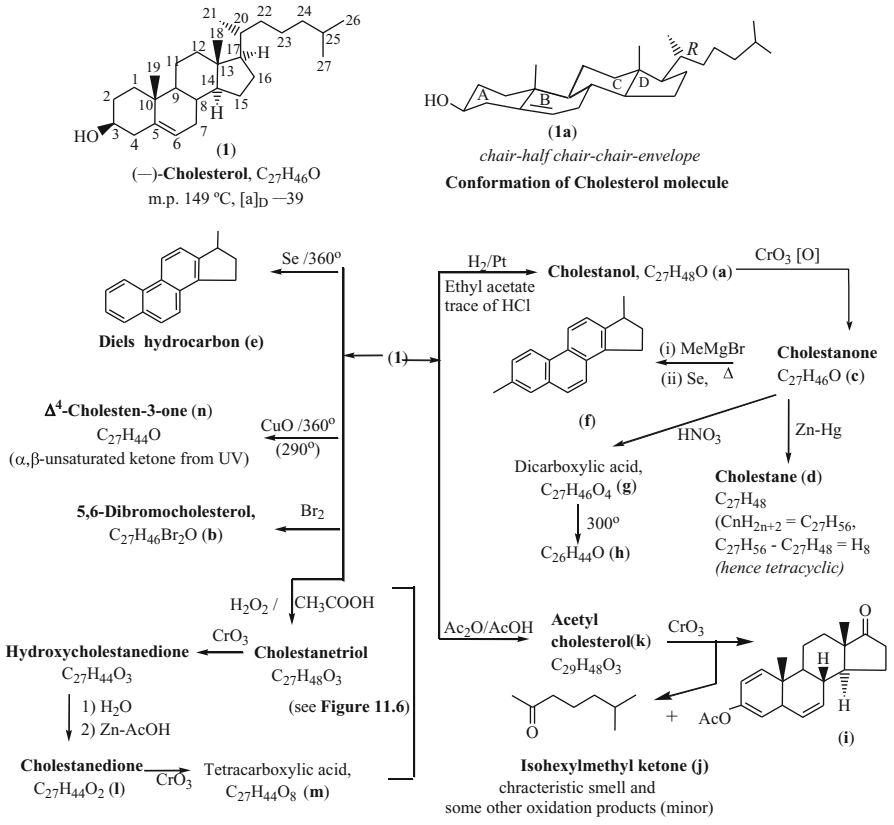


Fig. 11.2 Schematic representation of the reactions of cholesterol (1)

debated conclusions have accumulated during many years of hard work. We have presented here only a very small part of it, helpful in developing the structure.

(i) The nature and location of the hydroxyl group, (ii) presence of a double bond, (iii) the basic carbocyclic skeleton, and (iv) the presence of an isooctyl side chain and its site of attachment in the steroid skeleton have been deduced through the formation of the products (Fig. 11.2).
(ii) Derivatives (**a**) and (**b**) indicate the presence of a double bond.
(iii) Derivatives (**c**), (**f**), (**k**), and (**n**) suggest the presence of a secondary OH group and (**f**) indicates its presence at C3.
(iv) Derivatives (**d**), (**e**), (**f**), and (**i**) suggest that the tetracyclic skeleton of cholesterol has the cyclopentanoperhydrophenanthrene orientation as shown.
(v) Derivatives (**i**) and (**j**) suggest the presence of an isooctyl C_8 side chain located at C17 of the steroid nucleus.

The partial structure of cholesterol may thus be represented as (**1′**) (Fig. 11.3) which takes care of all the carbon atoms, barring two methyls and their locations. Since (**e**) and (**f**) lack these two methyls, they must have been knocked off at the demand of aromatization and hence *they must be angular in disposition*. The methyls in (**e**) and (**f**) originate from the side chain and the Grignard reaction.

Locations of the angular methyls have been decided by the sequence of reactions shown in Figs. 11.4 and 11.5. The keto acid (**p**) (Fig. 11.4) is resistant to esterification, and eliminates CO on treatment with conc. H_2SO_4. These observations are indicative of the tertiary nature of the CO_2H. *Thus the methyl group*

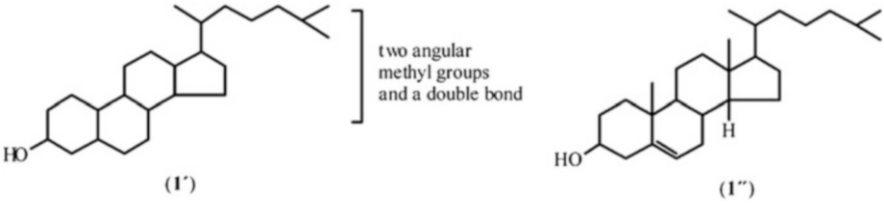


Fig. 11.3 Structures (1′) and (1″)

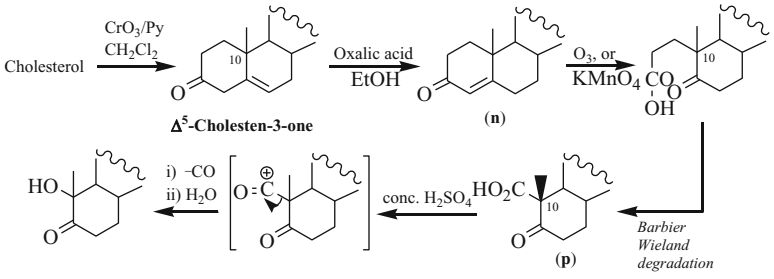


Fig. 11.4 The location of a methyl group at C10

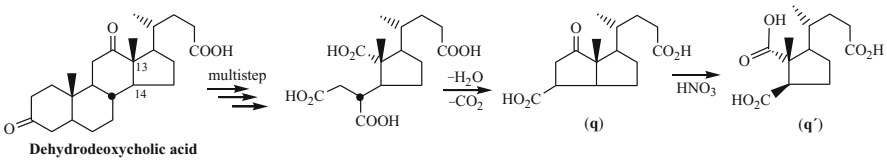


Fig. 11.5 The position of a methyl group at C13

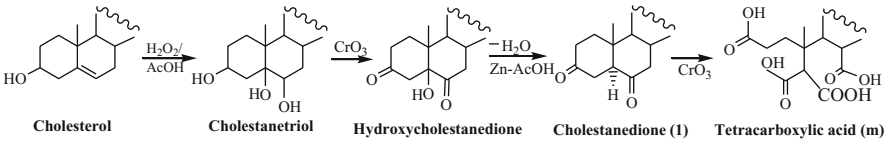


Fig. 11.6 The location of the double bond

must be geminal to COOH, and hence angular, and is attached to C10 of the skeleton.

The position of the second tertiary methyl group has been determined from the degradative methods shown in Fig. 11.5 in which dehydrodeoxycholic acid has been used, being known to be structurally related to cholesterol. Of the three COOH groups of (**q**′), one COOH group is tertiary in nature, being resistant to esterification, suggesting the geminal disposition of the COOH and the methyl group. The methyl may thus be attached either at C13 or C14. The COOH groups at these centers are *trans*, as no anhydride is formed on vacuum distillation. The C/D rings are thus *trans* fused. The *cis* 5,5-bicyclic system is known to be energetically more comfortable than the *trans* system in (**q**). Had there been a hydrogen instead of a methyl at C13, the C13 could have epimerized via enolization with C12–CO group to produce the *cis* system, which was not the case. *Hence a methyl group must be at C13*.

11.2.2.1 Location of the Double Bond

The formation of cholestanedione (**l**) and its subsequent conversion to a tetracarboxylic acid (**m**) suggest the presence of the two keto groups in two different rings and the genesis of (**l**) can be well explained (Fig. 11.6) if *the double bond is present at C5–C6*. Thus cholesterol's non-stereostructure may be expressed as (**1**″) (Fig. 11.3).

11.2.2.2 Configuration at C3

The reactions carried out by Shoppee (1948), as delineated in Fig. 11.7, suggest that 3-OH and 5-COOH must be *cis* disposed in the oxidation product (**r**). The

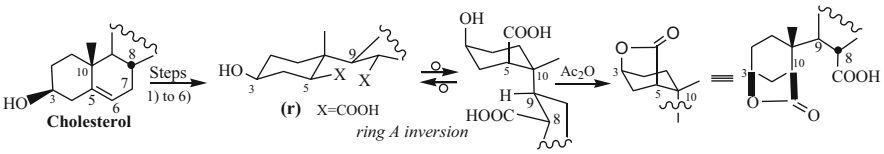


Fig. 11.7 Determination of configuration of cholesterol at C3

conversion of cholesterol to the compound (**r**) involved six straightforward successive steps, viz, treatments with (1) HNO_3 to form the 6-nitro derivative, (2) Zn/AcOH to get the reduced product 5α-6-imino compound via the 6-amino derivative, (3) acid to give the 5α-6-ketone, (4) Br_2, (5) $AgNO_3$ in pyridine to give the 5α-6,7-diketo derivative, and (6) $NaOH/H_2O_2$ to get oxidized to the dicarboxylic acid (**r**). The ring A flips to enable the 1,3-diequatorial relationship to become 1,3-diaxial to form the lactone. Thus, 3-OH group is β-equatorial in this system and A/B rings are *trans* fused.

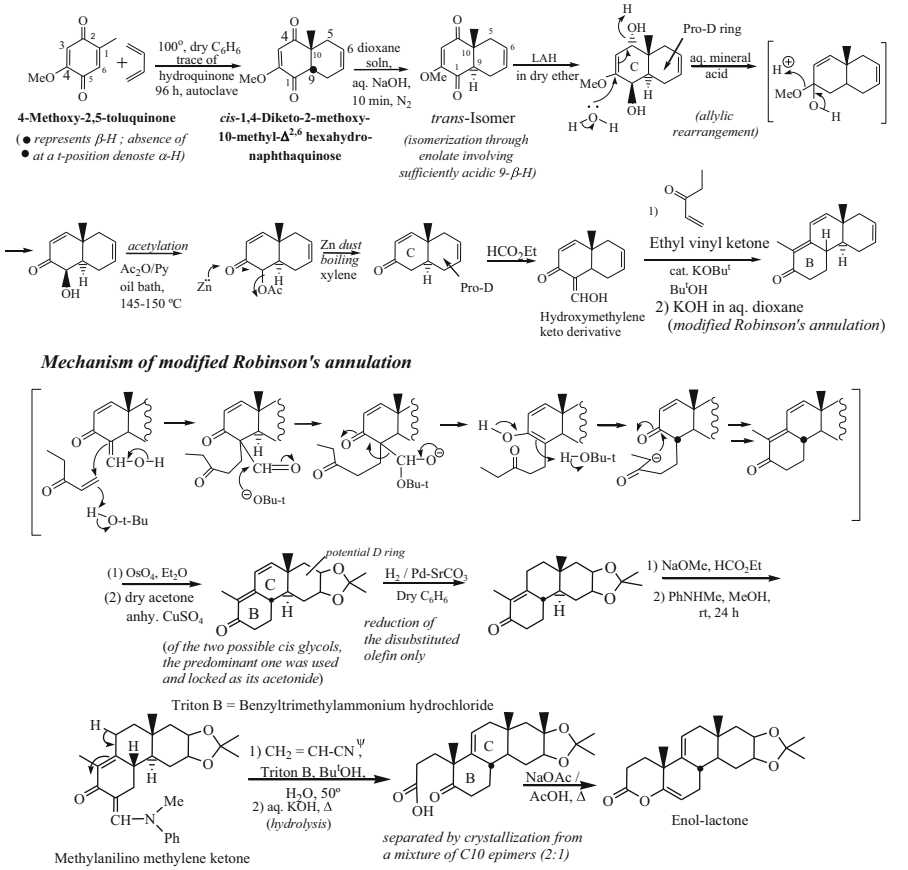
Thus the structure and the relative configuration of cholesterol were elucidated in pre-NMR age by ingenious planning/methods.

11.2.2.3 A/B-, B/C-, and C/D-Ring Fusions. Absolute Configuration

In the 5,6-dihydroderivatives of cholesterol the ring junction A/B may be *trans fused* or *cis fused;* the former is *cholestanol* (5α-steroid), and the latter is *coprostanol* (5β-steroid). The *trans fused* B/C rings offer conformational stability to 5β-steroids due to conformational mobility of ring A. In most of the steroids like sterols, bile acids, and related compounds the C/D rings are *cis fused*. The absolute stereochemistry of cholesterol (**1**) and some other steroids has been established by X-ray crystallographic studies. The absolute configurations of different 5α-cholestanones and 5β-coprostanones are in conformity with the observed Cotton effects in their ORD curves (see Fig. 2.171).

11.2.3 Synthesis of Cholesterol

The total synthesis of cholesterol [4, 12, 13] by Woodward et al. constitutes the first total synthesis of a non-aromatic natural steroid. The synthetic schemes contain the great qualities of the master revealing imagination, planning, and their successful implementation. On the whole, they offer a beautiful microcosm of organic chemistry *securing a permanent place in the history of the synthetic organic chemistry*. The synthesis of cholesterol by Woodward et al., involving more than 30 steps, is outlined in Fig. 11.8, showing mechanistic rationalization, when necessary.



Ψ *Preferential attack of the electrophile from the axial β-face in the low energy pre-chair TS leading to the undesired α-Me epimer (~66%). The desired β-methyl epimer is formed by the attack of the electrophile from the α-face in the higher energy pre-twist TS in less amount (~38%)*

Fig. 11.8 (continued)

Since Woodward did not achieve an asymmetric synthesis, both the natural (–)-cholesterol and its enantiomer, unnatural (+)-cholesterol, are formed in equal amounts leading to the formation of racemic (±)-cholesterol.

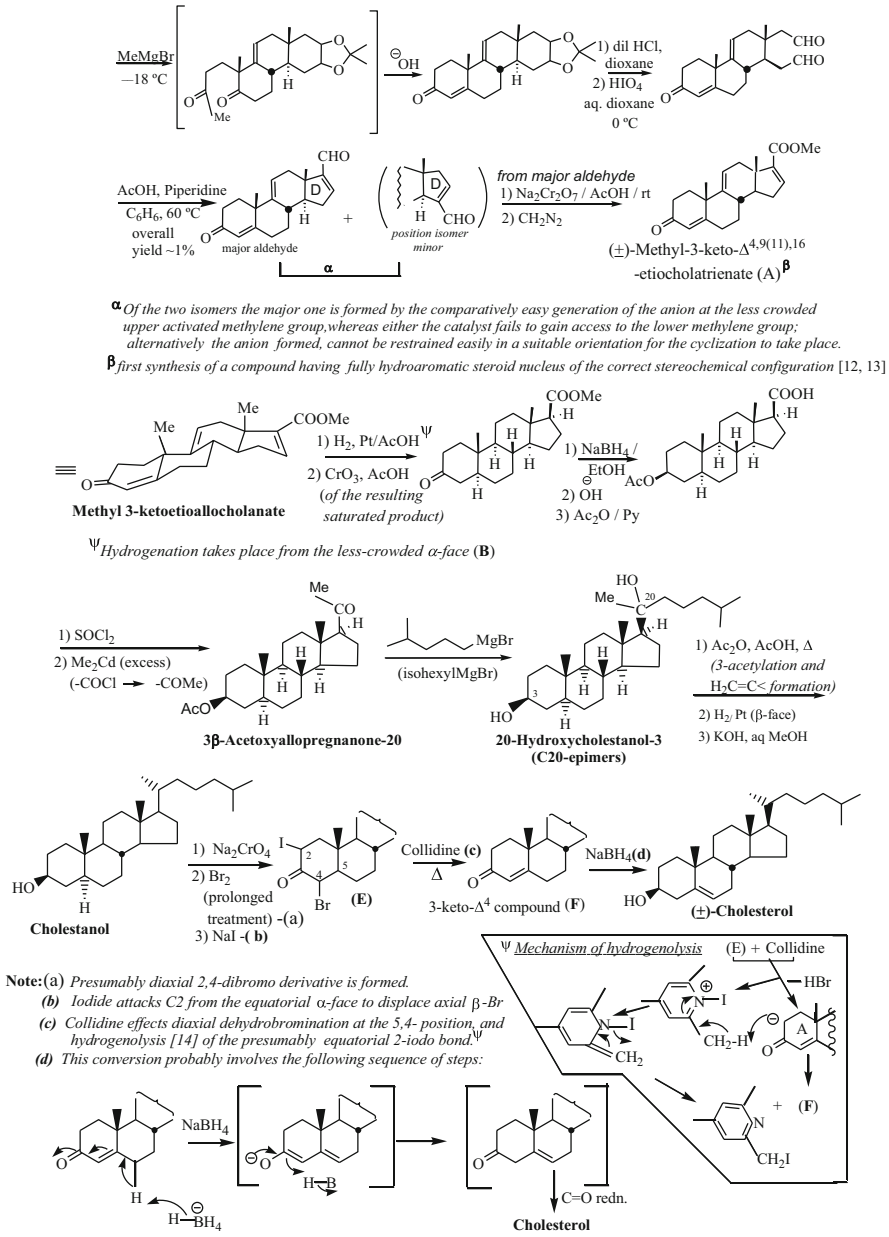
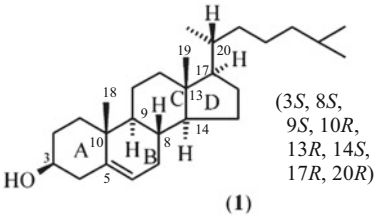


Fig. 11.8 Total synthesis of (±)-cholesterol (Woodward) (1951–1952) [4, 12, 13]

Further, during an achiral synthesis whenever a chiral center is generated, the absolute configuration of the natural molecule is shown, though in each step, the corresponding enantiomer is formed in equal amount. The tabletop construction of cholesterol requires the building up of the carbocyclic system, cyclopentanoperhydrophenanthrene, the placement of the substituents, and the double bond in the later stages. The rings are constructed in the reverse order C → CD → BCD → ABCD. The angular methyls fall on the right places during ring construction. Strategy wise this is a linear multistep synthesis in which each new part is added stepwise leading to the formation of the final product. Even though the yields are good to excellent in individual steps, the overall yield is low.



(3*S*, 8*S*, 9*S*, 10*R*, 13*R*, 14*S*, 17*R*, 20*R*)

(1)

The ring D was initially kept as a six-membered one, but at a later stage was converted to the desired 5-membered ring with a handle via the seco derivative (i.e., D-ring opening). Through the handle by chemical manipulation a C8-hydrocarbon side chain with a chiral center was constructed.

The reactions leading to the total synthesis of (±)-cholesterol as per the above strategy involved more than 30 steps using common laboratory chemicals. In this method *cis-trans* equilibrium is shifted more to *trans* by seedling with the pure *trans* isomer obtained from previous batch and purified through crystallization. A mechanism of the formation of the 3-keto-Δ^4 compound, as shown in Fig. 11.8, has been suggested by Carl Djerassi [14].

Woodward et al. achieved a total synthesis [15] cholestanol from the intermediate (**B**) and another intermediate methyl 3α-acetoxy-$\Delta^{9(11)}$ etiocholenate (**C**) from (**A**) by partial hydrogenation and oxidation. From these intermediates, the paths to progesterone, desoxycorticosterone, testosterone, androsterone, and cortisone have been described previously by other investigators. The saturated keto-ester (**B**) has previously been converted to the Δ^4-compound. The corresponding acid was previously converted to the hormones desoxycorticosterone and progesterone. Thus this reaction sequence constitutes their total syntheses [12, 13].

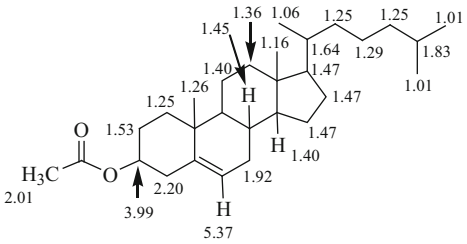
Another classical synthesis of (±)-cholesterol was simultaneously reported in 1951 by Robinson's group [16–18]. It consists essentially of the addition of ring D onto a saturated A-B-C tricyclic diketone derived from 1-methyl-5-methoxy-2-tetralone [16–18].

11.2.3.1 Specification of the Chiral Centers. Conformation

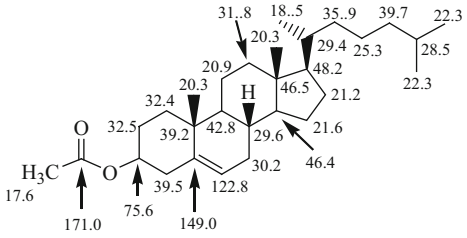
Cholesterol molecule (**1**) contains eight dissimilar stereogenic (chiral) centers and accordingly it may have $2^8 = 256$ stereoisomers. Only few including cholesterol exist in nature due to biogenic feasibility, which also avoids steric encumbrance. The *trans fusions* of B/C and C/D rings confer conformational rigidity to such skeletons, and the *axial* and *equatorial* substituents can be differentiated in chemical reactions. The absolute configurations of the chiral centers of (–)-cholesterol are specified according to CIP rules as 3*S*, 8*S*, 9*S*, 10*R*, 13*R*, 14*S*, 17*R*, and 20*R* (steroid numbering) (structure **1**). This is in conformity with its biosynthesis in the presence of specific enzymes to be discussed in the sequel. The architecture of the ring scaffold, the stereochemistry of the fusion sites and other chiral centers, the specified absolute configuration of each stereogenic center, and the unequivocal conformation of cholesterol are shown in structures (**1**) above and (**1a**) in Fig. 11.2.

11.2.4 NMR Spectral Data of Cholesterol

^{1}H NMR: ($CDCl_3$, δ-values)



^{13}CNMR: ($CDCl_3$, δ-values)



11.2.5 Biosynthesis of Cholesterol in Animals, Fungi, and Plants

The biosynthesis of cholesterol in animals has been discussed along with its biosynthesis in plants to show the similar and dissimilar steps involved in the processes.

The biosynthesis of cholesterol may be expressed in the following compressed form (Fig. 11.9).

Steps (a). The conversion of *R*-(+)-mevalonic acid into squalene has been discussed in detail in Chap. 5.

Step (b). "The discovery of Elias J. Corey and independently of Eugene van Tamelen of squalene epoxidation prior to cyclization was another key element for formulating cholesterol pathway" [5]. The enzyme squalene epoxidase (squalene monooxygenase) converts squalene into 3(*S*)-2,3-oxidosqualene [4] by a regio- and stereospecific epoxidation of the former. The absolute stereochemistry of the latter has been established [19]. It has been shown that oxidosqualene cyclase shows substrate specificity on (2) and not on its 3(*R*)-enantiomer.

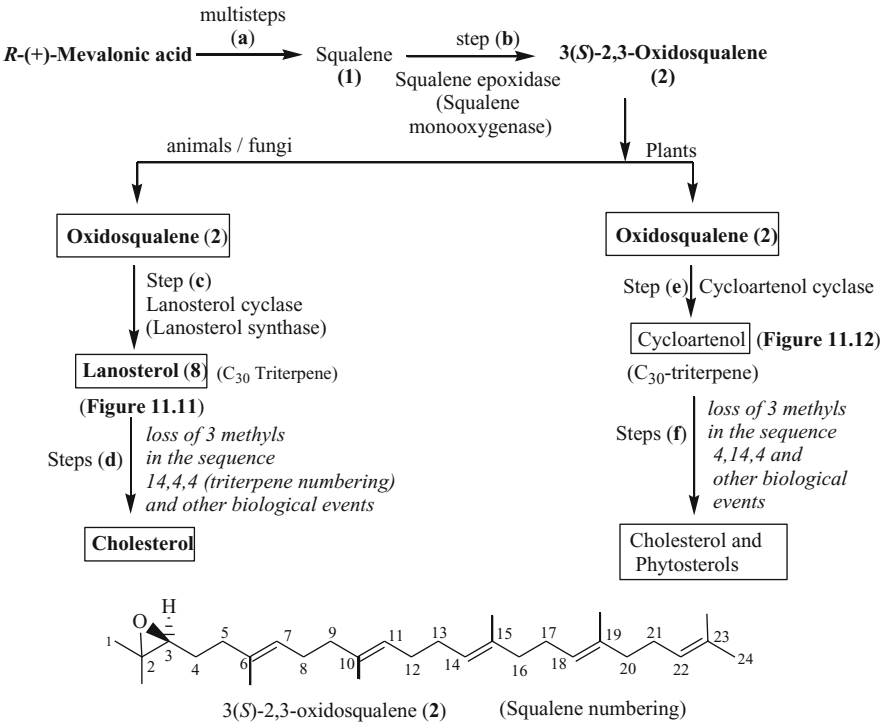


Fig. 11.9 Biosynthetic routes for formation of cholesterol in animals/fungi and plants and phytosterols in plants

11.2.5.1 Formation of Lanosterol in Animals and Fungi

Lanosterol (**8**) is the experimentally supported essential precursor of cholesterol in animal and fungi, and the study of its biosynthesis in the cell has evolved much interest. As a consequence of intensive studies several parallel proposals with thoughts in similar lines as well as some reviews [5, 20–24] came out in print [**Steps (c)**]. The enzymatic folding of (**2**) is assumed to attain preformed chair–boat–chair–open conformation (Fig. 11.10). During cyclization the conformational change is very less and the cyclization is initiated by enzymatic electrophilic addition of a proton to the epoxide which opens up to generate a transient carbocation. The latter is then added to the suitably disposed π bond, and the cation–olefin cyclization takes place. For each cyclization a new carbocation is formed and the substrate being a polyene, cation–olefin cyclization is repeated until it reaches a stopping point which is protosterol C20 carbocation (**4**). Subsequently a series of 1,2 shifts of hydrides and methyls result in the formation of lanosterol C8 carbocation (4″); the latter is quenched through the loss of C9-H as proton to yield lanosterol (**8**).

Extensive study on this conversion (**Step c**) (Fig. 11.10) provides a remarkable example of polyene cyclization, revealing a comprehensive stereochemical course of electrophilic antiparallel addition of carbocation to the olefinic double bond, followed by Wagner–Meerwein type 1,2-migrations of hydrides and methyls, and finally quenching of the carbocation through the loss of an appropriate proton. Incidentally, it should be mentioned that the brilliant work of some stalwarts [25–27] will always have a lasting influence on such studies.

Initially the cyclization was thought to proceed in a concerted nonstop fashion as per Ruziicka's "isoprene rule," while van Tamelen suggested that the cyclization proceeds through discrete carbocation intermediates based on some experimental observations and entropy considerations. However, Corey [28] suggested that during cyclization to form A, B, and C rings, ring C appears first as a

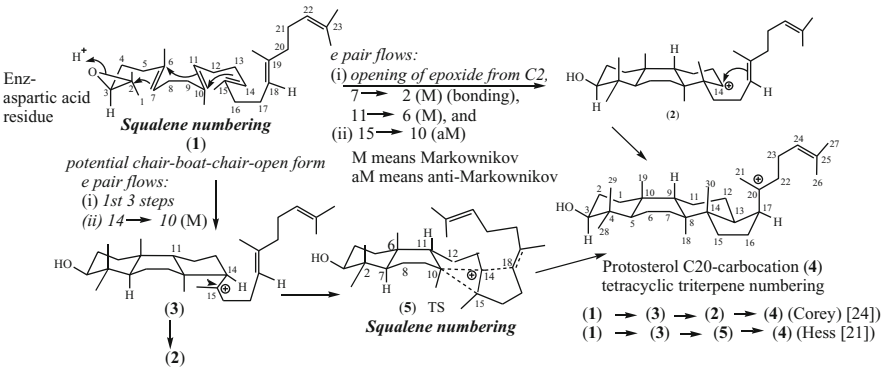


Fig. 11.10 Formation of protosteryl C20 carbocation (4)

5-membered ring (**3**) to avoid energetically disfavored anti-Markovnikov addition needed to effect six-membered ring formation via a secondary carbocation. The five-membered ring thus formed expands to 6-membered ring [6.6.5 (A.B.Pro C (**3**) → 6.6.6 A.B.C) (**2**)] (Fig. 11.10). The ring expansion is then followed by ring D (five membered) formation and a cascade of 1,2-hydride and methyl migrations, and lanosterol (**8**) is finally formed through the loss of H-9 as proton causing the quenching of carbocation (4″) (Fig. 11.11).

At this stage it should be pointed out that Hess Jr. [22] suggested that potential ring C (Pro C, being 5-membered) and ring D are formed in concert (**5**) (Fig. 11.10) in TS prior to the expansion of ring C in the biosynthesis of lanosterol and thus avoids energetically unfavorable ring expansion through a secondary carbocation in preference to a tertiary carbocation.

The absolute configuration at C20 of natural lanosterol is *R*. Had this side chain at C17 been α- and pseudo-equatorial, it would have necessitated ~120° rotation of the side chain around C17–C20 bond prior to H17 migration at C20 to confer *R* configuration at C20. However, this rotation of C17–C20 bond with C_8 side chain is disfavored because of 14β-Me group (severe nonbonding interactions). Further, the rate of hydride migration from C17 to C20 is faster than the rate of bond rotation. All these observations point towards the β- and pseudo-axial orientation of the side chain. This conjecture is supported by trapping the protosteryl carbocation analogue (**7**) and establishment of its structure by synthesis by Corey [28] from (**6**). This configuration of the side chain at C17 allows more stereochemical control of the enzyme on C20 carbocation (**4**) compared to the α-pseudo-equatorial orientation (**4′**).

The points revealed from the above studies on lanosterol biosynthesis are

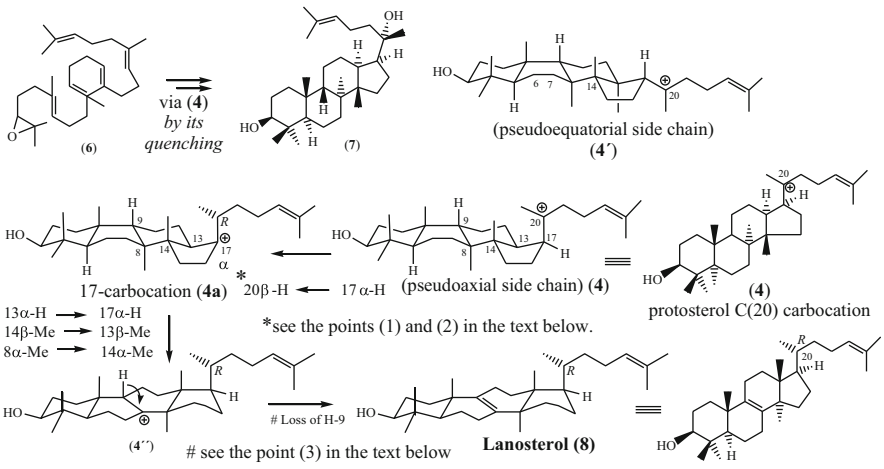


Fig. 11.11 Probable pathway for the formation of lanosterol in animals and fungi

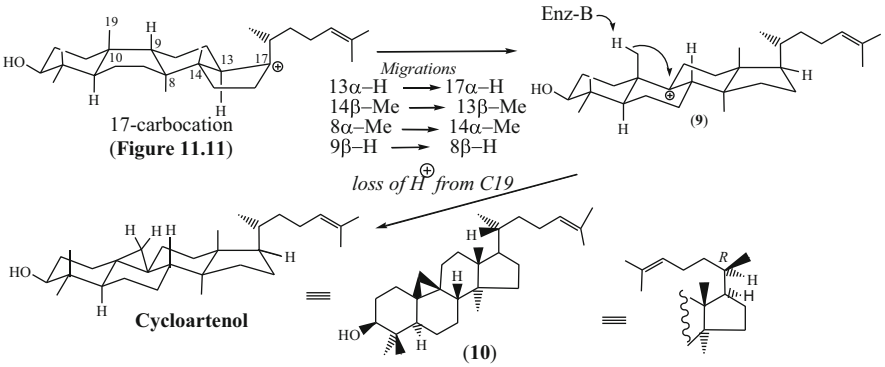


Fig. 11.12 Formation of cycloartenol in plants

(1) The enzyme protects the C20 carbocation from quenching by a nucleophile like water and holds the conformation of the cationic side chain in such a way that on delivery of the hydride the configuration at C20 becomes *R*.
(2) C17-H or H17) migrates first to C20 carbocation prior to other 1,2 spontaneous concerted migrations of hydrides and methyls leading to lanosterol C8 carbocation (**4′**).
(3) Enzyme plays an important role in the elimination of H9 as proton to quench C8 carbocation, completing the biosynthetic process of lanosterol.

11.2.5.2 Formation of Cycloartenol in Plants

In the plant cell in the presence of the enzyme cycloartenol cyclase, the carbocation moves in concert one carbon atom further, compared to lanosterol C8 carbocation (**4″**) and gets a new home at C9 (**9**) **(Steps e)**. The latter is then quenched through the loss of one of the hydrogens as proton from an enzyme-activated C19-Me to form a cyclopropane ring [29]—a characteristic structural feature of cycloartenol (**10**). The enzyme plays a vital role in eliminating such proton (Fig. 11.12).

11.2.5.3 Lanosterol to Cholesterol and Cycloartenol to Cholesterol Conversions (Fig. 11.13)

The biosynthetic conversion (Steps **d**) (Fig. 11.9) requires

(1) *Loss of three angular methyls*
(2) *Change in the following existing structural features :*

(a) *Reduction of the side chain double bond*
(b) *Introduction of a new* $\Delta^{5,6}$ *double bond in place of* $\Delta^{8,9}$ *double bond.*

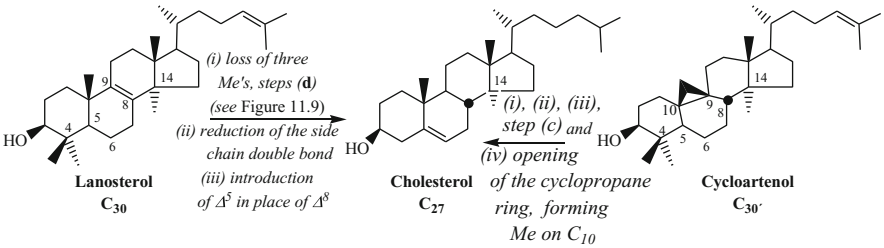


Fig. 11.13 Stages in the bioconversion of lanosterol into cholesterol (in animals and fungi), and of cycloartenol into cholesterol (in plants)

The biogenetic conversion path (**e**) (Fig. 11.9) followed in plants requires in total

(a) *Loss of the three angular methyls*
(b) *Opening of the cyclopropane ring generation C_{19}-Me on C_{10}*
(c) *Reduction of the side chain double bond and*
(d) *Creation of a double bond at $\Delta^{5,6}$.*

The three angular methyl groups are lost in the sequence 14, 4, 4 from lanosterol and 4, 14, 4 from cycloartenol. In between, phenomena like reduction, isomerization, and introduction of a new double bond by desaturation, etc., occur. The sequence of demethylation has been settled in both the cases through the isolation of selectively demethylated or oxygenated methyl sterols or products. In phytosterols the extra carbon atom/s are delivered by AdoMet (SAM) (*S*-adenosyl-1-methionine) at C24 and the π-facial diastereoselectively will leave the stereochemical information at the newly generated chiral center.

11.2.5.4 Biosynthetic Conversion of Lanosterol to Cholesterol

Biosynthetic conversion of lanosterol to cholesterol (Fig. 11.14) in animals and fungi starts with 14α-Me demethylation catalyzed by Lanosterol-14-demethylase (cytochrome-P-450 monooxygenase). By two successive oxidation reactions ($CH_3 \rightarrow CH_2OH$–CHO) and finally by Baeyer–Villiger type of oxidation, the original 14α Me is eliminated as HCOOH. Elimination of other methyls takes place as COOH → CO_2 (with the help to 3 keto (3-OH → 3-CO) group, which is then reduced to 3β-OH.

The established association of high plasma cholesterol level, especially of LDL with plaque formation and development of artery blockage and coronary disease, stimulated the interest in the biosynthesis of cholesterol with a view to intercepting the formation of biosynthetic intermediate/s by identifying the relevant enzymes and inhibiting the enzyme/s to control the endogenous formation of cholesterol. In this effort the inhibitor/s of HMG-CoA reductase (Chap. 5, footnote 1), squalene epoxide cyclase, and demethylating enzymes of lanosterol have been discovered.

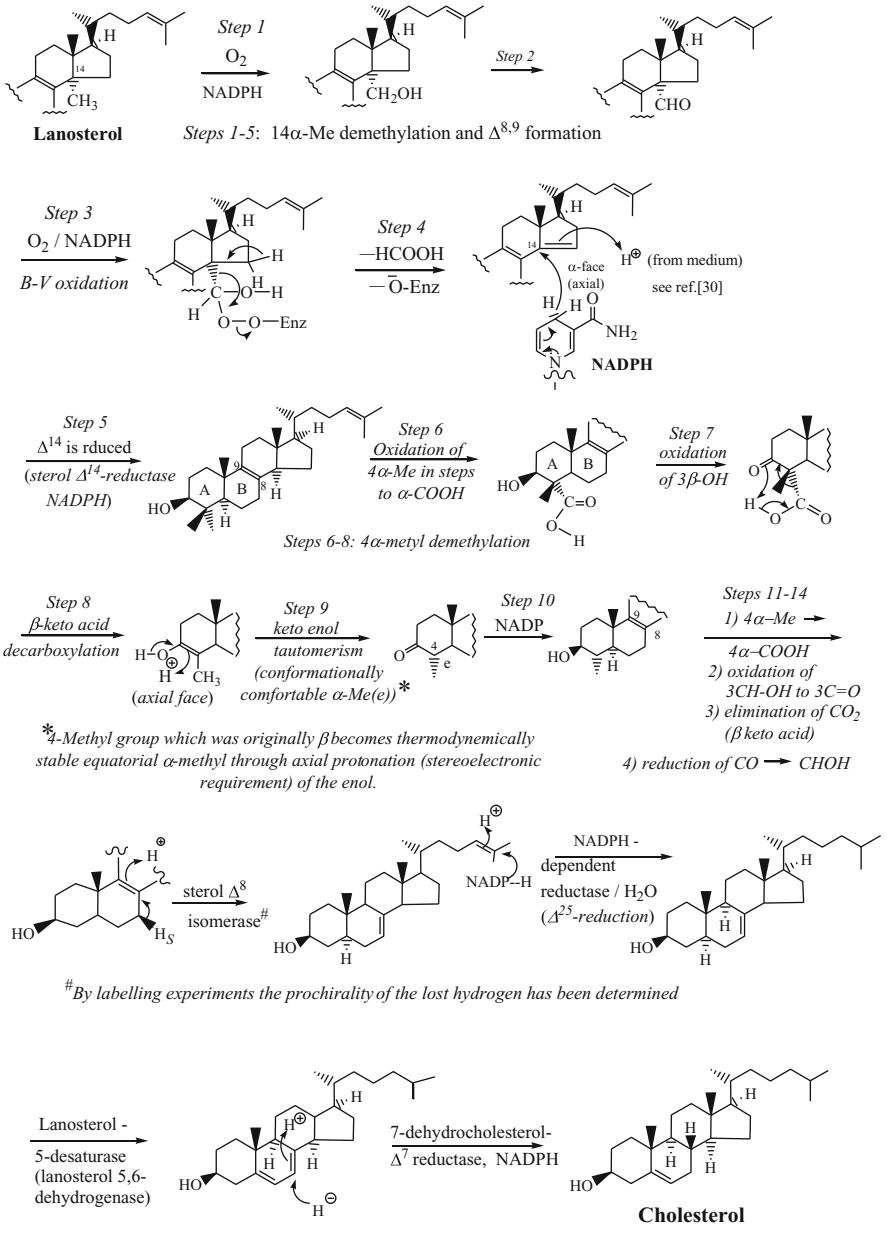


Fig. 11.14 Biogenetic conversion of lanosterol to cholesterol (in animal and fungi)

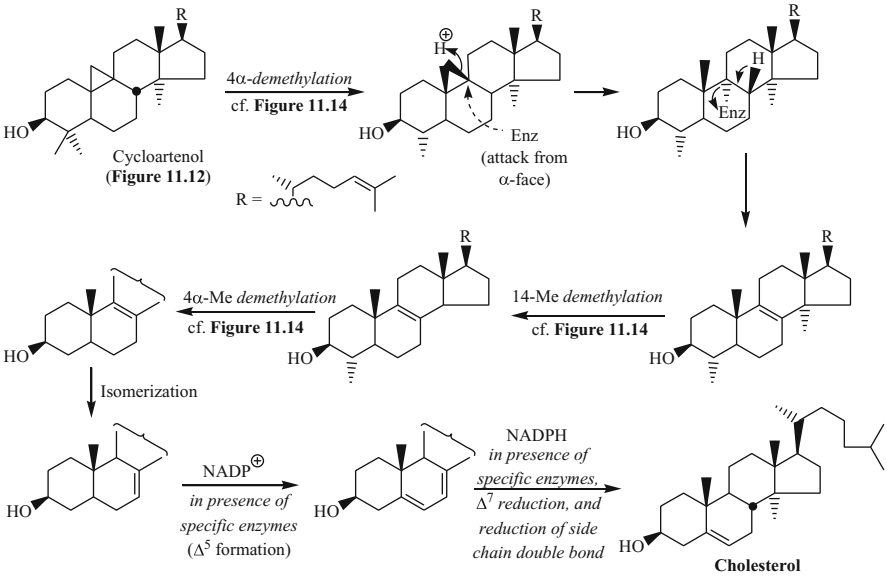


Fig. 11.15 Probable sequence of biogenesis of cholesterol from cycloartenol in plants

11.2.5.5 Biosynthesis of Cholesterol from Cycloartenol in Plants

Probable sequence of steps in the biogenesis of cholesterol from cycloartenol is outlined in Fig. 11.15. This proposal based on the present knowledge of biosynthesis of cholesterol via lanosterol in animals and fungi (Figs. 11.11 and 11.14) and phytosterols from cycloartenol is delineated in these Figures. However, it seems from the literature that chronology of the steps for plant cholesterol, in the absence of any circumstantial evidence, cannot be defined in definite terms.

11.2.5.6 Biogenetic Conversion of Cycloartenol to Other Phytosterols (Fig. 11.16)

Unlike the animal membranes, the plant membranes contain a mixture of sterols, some of which are sitosterols (24*R*)-24-ethylcholesterol, stigmasterol(24*S*)-24-ethylcholesta-5,22-dienol, (24*R*)-24-methylcholesterol, 24-epicampesterol, and campesterol. Sitosterols are involved in membrane reinforcement.

The biosynthesis of phytosterol (Fig. 11.17) starts with the methylation at Δ^{24} of the side chain. The latter is considered to be the obligatory location for first alkylation. The addition of the methyl group is governed by the enzyme which dictates the π-facial diastereoselectivity of alkylation and plays a vital role for being nucleophilic toward electrophilic SAM.

Lanosterol is nowhere present in the plant system. The side chain variation using Δ^{24} is shown in Fig. 11.17. Biogenesis of these phytosterols has been studied [31–33] and the enzymes have been identified.

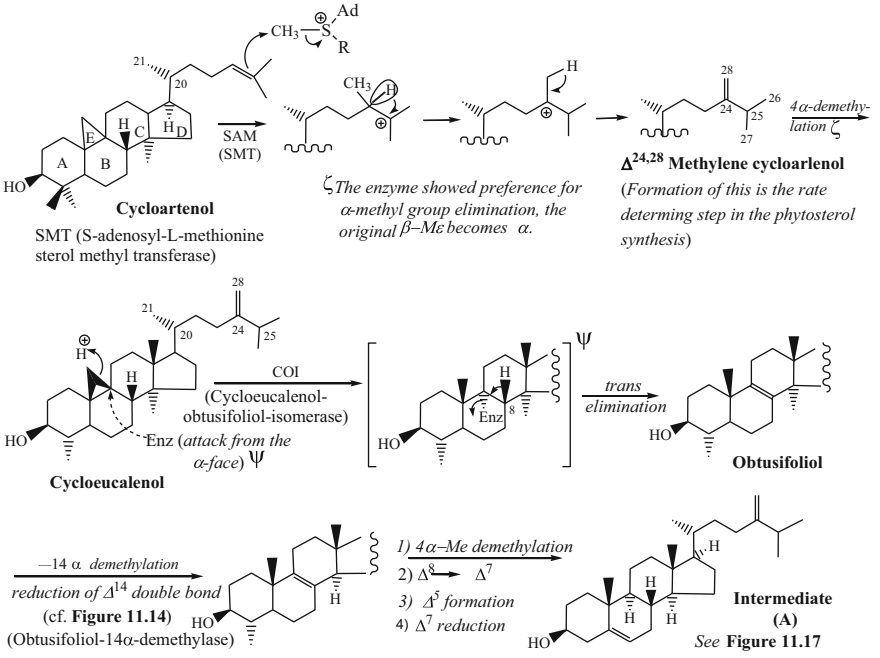
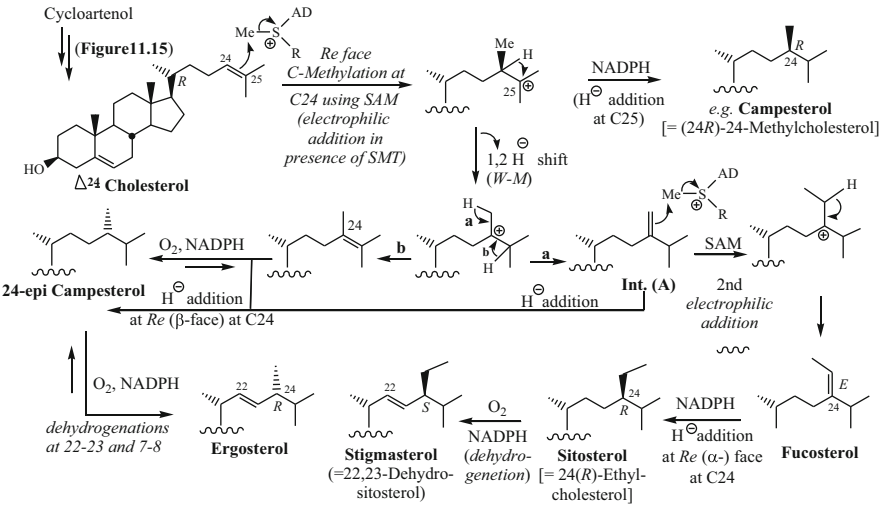


Fig. 11.16 Biogenetic modifications of the side chain and cyclopropane ring of cycloartenol to form phytosterols



Note: *For full structures of the phytosterols see* **Figure 11.18**

Fig. 11.17 Bioformation of different phytosterols by side chain variation using Δ^{24}-cholesterol

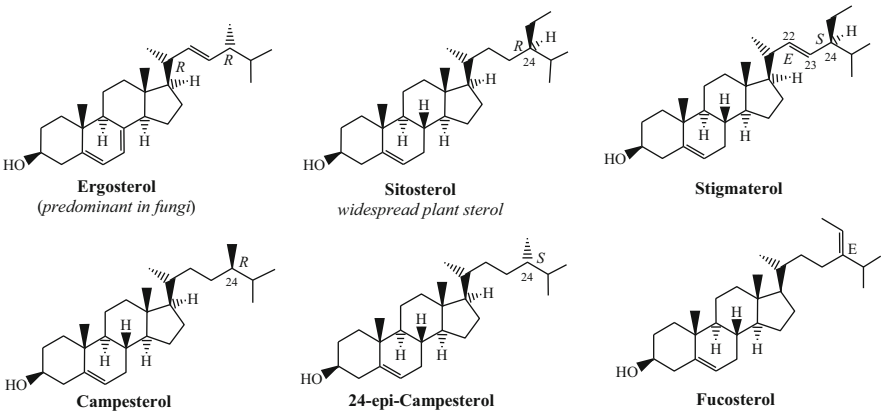


Fig. 11.18 Some phytosterols

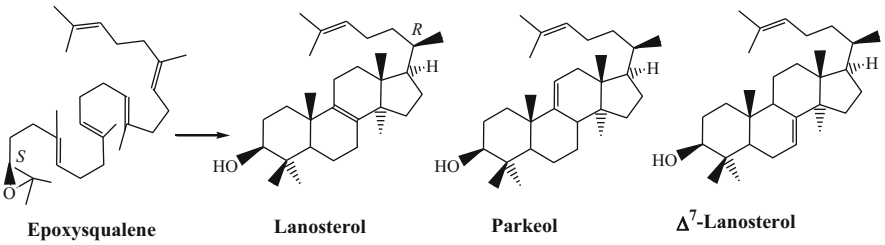
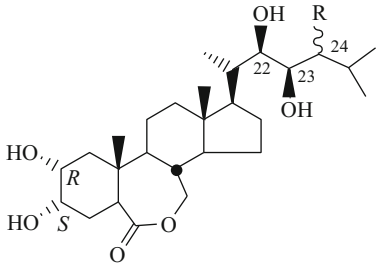


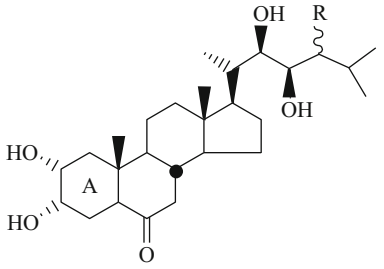
Fig. 11.19 Mutation of cycloartenol synthase lanosterol and allied compounds

Plant cell membranes produce phytosterols elaborating different variations of side chains (Fig. 11.17), which include campesterol [=(24*R*)-24-methylcholesterol], 24-epicampesterol, ergosterol [=(24*R*)-24-methylcholesta-5,22-dienol], sitosterol [=(24*R*)-24-ethylcholesterol) and stigmasterol [=(24*S*)-24-ethylcholesta-5,22-dienol] [31], and fucosterol (=24*E*-ethyledenecholesterol). The structures of these phytosterols are shown in Fig. 11.18. The biosynthesis of sitosterol (=24-*R*-ethylcholesterol) and stigmasterol (=22,23-dehydrositosterol) in plant cells has demonstrated that their isoprene units are supplied exclusively from the mevalonate (MVA) pathway [31, 32]; MVA is located in the cytoplasm.

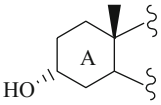
The specific deprotonation by these two different enzymes (lanosterol synthase and cycloartenol cyclase) is responsible for different pathways for cholesterol formation in animals and plants. However, recent studies [34] showed that mutagenesis at specific site of cycloartenol synthase allows lanosterol biosynthesis (Fig. 11.19). A novel site-specific mutant His477Asn was uncovered that produces 88 % lanosterol and 12 % parkeol, while His477Gln mutant produces 73 % parkeol, 22 % lanosterol, and 5 % Δ^7-lanosterol; however, in plants no lanosterol biosynthesis takes place in the absence of lanosterol synthase.



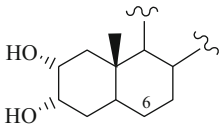
a) R=β-Me (24*S*), Brassinolide
b) R=β-Et (24*S*), Homobrassinolide
c) R=H, 28-Norbrassinolide
d) R= $=CH_2$, Dolicholide
e) R= $CH=CH_2$, Homodolicholide



Castasterone, R= as in **a)**
Ethylbrassinone, R = as in **b)**
Brassinone, R = as in **c)**
Dolichosterone, R = as in **d)**
Homodolichosterone R = as in **e)**
24-Epicastasterone R = α-Me (24*R*)



2-Deoxycastasterone (Typhasterol) (3*R*) and its 3 epimer (3β–OH, 3*S*) (Teasterone)



6-deoxycastasterone
6-deoxydolichosterone
6-deoxyhomodolichosterone

Fig. 11.20 Some natural brassinosteroids

11.3 Brassinosteroids [35–40]

11.3.1 Introduction. Some Brassinosteroids

Brassinosteroids represent a new class of phytohormones and its first member named brassinolide **1** has been isolated in extremely small yield (4 mg from 40 kg bee collected pollens) of rape seed plant (*Brassica napus*, Cruciferae/Brassicaceae) [35, 36]. Isolation of brassinolide confirms the role of steroids as hormones in plants and demonstrates an evolutionary conservation between plants and animals. It is a powerful growth-promoting agent and the increase in the growth is due to both cell elongation and cell division. For this duel effect of brassinolide, its discovery appears to be more important to plant physiologists and biochemists. It contains an unprecedented seven-membered B-ring lactone and its structure has been elucidated as (2R,3S,22R,23R,24S)-2,3,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5-α-cholestan-6-one (Fig. 11.20) from chemical, spectral, and X-ray

analysis. Till 1991, more than 60 brassinosteroids have been isolated from a wide variety of plants [37, 38]. A few representatives of brassinosteroid family are shown to possess the following structural characteristics [36, 39, 40] (Fig. 11.20).

All the brassinosteroids are (2*R*,3*S*,22*R*,23*R*)-tetrols except in *typhasterol* and *teasterone* where only one 3*R* or 3*S* OH group is present. Brassinosteroids carrying *lide* in the suffix contain a 7-membered-B-ring lactone with differently substituted side chain. Compounds of this class with 6-oxo group also contain the same type of side chains.

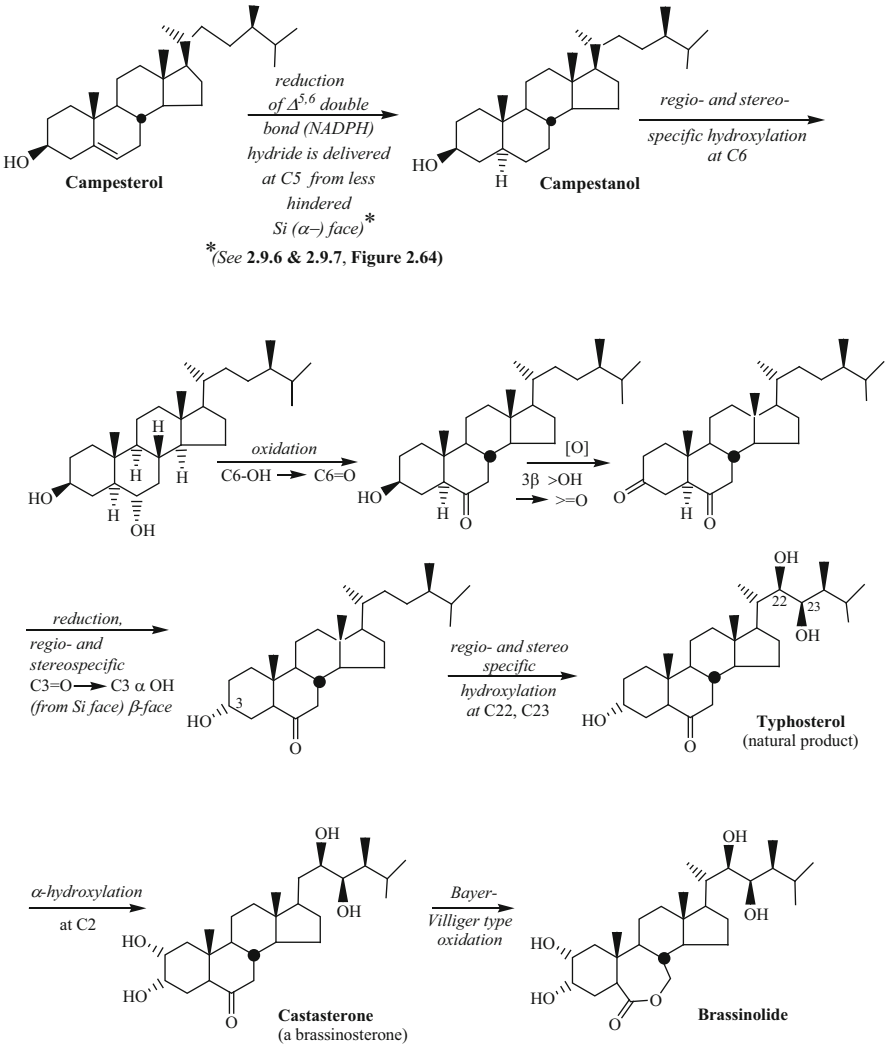


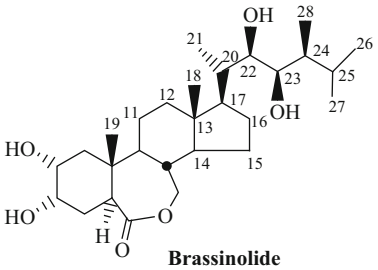
Fig. 11.21 Biosynthesis of brassinosterone and brassinolide

11.3.2 Biosynthesis

From the relationship of brassinolides and the brassinosterones, it is obvious that in the final step Baeyer–Villigar oxidation takes place and brassinosterones are the immediate precursors of the corresponding brassinolides. From label experiments it has been shown that campesterol is converted to a brassinosterone and finally to a brassinolide (Fig. 11.21). Further the side chain functionalization takes place during the formation of the 6-oxo-compound.

In view of their low natural content in plants, and their importance as agricultural agents, much effort has been directed toward the total and semisynthesis of brassinosteroids. However these are multistep processes and the yields are not encouraging for commercial use. The challenging stereostructures of brassinosteroids have attracted the attention of synthetic chemists and a number of syntheses have been reported. The first synthesis of brassinolide has been reported in 1980 by two groups of workers [41, 42].

11.3.3 Spectral Data of Brassinolide [35, 36, 38]



Brassinolide

$C_{28}H_{48}O_6$, mp. 274–275 °C, $[\alpha]_D$ + 16

IR (KBr) ν_{max} 3450, 1,690 cm^{-1}, (CH_2Cl_2), 1,720 cm^{-1}

^{1}H NMR (C_5D_5N): δ 0.72 (*s*, 3H), 1.03 (*d*, 6H, J = 6.3 Hz), 1.04 (*s*, 3H), 1.10 (*d*, 3H, J = 6.4 Hz), 1.13 (*d*, 3H, J = 6.5 Hz)

^{13}C NMR (CD_2Cl_2–CD_3OD) (9:1): δ 10.3, 11.9, 12.1, 15.6, 20.8, 21.0 (CH_3), 68.3, 68.4, 71.0, 73.7, 74.8 (4*CH*OH, 1 CH_2–O–), 177.6 (lactone carbonyl)

MS (FD): m/e 463 (M+1 – H_2O)$^+$, 445 (M+1 – $2H_2O$), 409, 379, 361, 349, 101, 71 (side chain fragmented species)

The structure of brassinolide was completely established by its X-ray crystallographic analysis [35, 36].

11.4 Other Bioactive Steroidal Compounds

11.4.1 Ecdysones [43, 44]

11.4.1.1 Introduction. Structures

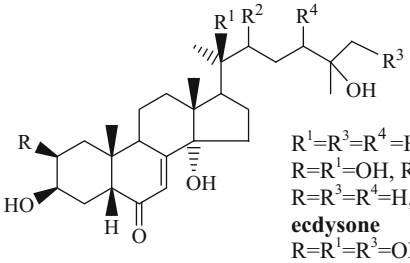
Ecdysones (Fig. 11.22) are a group of moulting hormones necessary for the larval development (cf. *Sect.* 1.6.2.2) of the insects. Insects cannot synthesize sterols, the biogenetic precursors of ecdysones. They take food which supplies the ecdysones or sterols (cholesterol, sitosterols, etc.). The sterols are converted into ecdysones in their system. Thus they are of dietary origin. Ecdysis (Greek, shedding), the entomological term for moulting, has been used to name this class of moulting hormones. Higher plants elaborating ecdysones play important role in the metabolism of phytophagous insects.

11.4.1.2 Biogenesis

The probable events (1–7) in the biogenesis of ecdysones from cholesterol are outlined in Fig. 11.23.

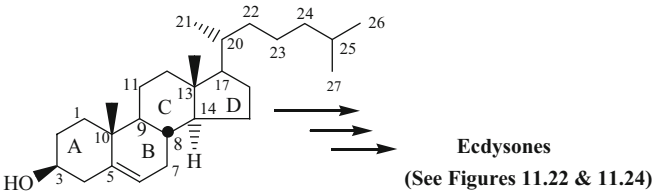
The hydroxylation is a very common biosynthetic phenomenon in ecdysones. The sequence of the biological events (1–7) is speculative and may be different. It depends on the enzyme specificities. The side chain containing an extra methyl, e.g., β-sitosterol chain, may be modified. Other sterol side chains may also undergo similar hydroxylations during their conversion to ecdysones.

Some other examples of ecdysones [43, 44] are given in Fig. 11.24.



$R^1=R^3=R^4=H$, R=OH, R^2=α-OH, Ecdysone (**α-Ecdysone**)
R=R^1=OH, R^2=α-OH, $R^3=R^4$=H, Ecdysterone (**β-Ecdysone**)
R=$R^3=R^4$=H, R^1=OH, R^2=α-OH, **2-Deoxy-20-hydroxy-ecdysone**
R=$R^1=R^3$=OH, R^2=α-OH, R^4=H, **Inokosterone**
R=R^1=OH, R^3=H, R^2=βOH, R^4=Me, **Makisterone-A**

Fig. 11.22 Structures of some ecdysones

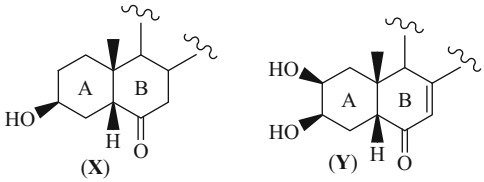


Cholesterol

(1) Reduction of $\Delta^{5,6}$ bond to yield cis fused A/B rings

(2) Hydroxylation at C6⟶ regiospecific oxidation to C6>=O or

(3) *α-epoxidation at $\Delta^{5,6}$, trans opening – delivery of hydride from β-face making A/B cis fused, followed by regiospecific oxidation of C6>CHOH; operations (1-2) or 3, converts A/B rings to* (**X**).



(**X**) (**Y**)

(4) Dehydrogenation at C6-C7 ($2NADP^+ \longrightarrow 2NADPH$)

(5) In most of the cases β-hydroxylation at C2; after operations 4-5 A/B rings are converted to (***Y***).

(6) Selective hydroxylation/s at C20, C22, C25, C26/C27 elaborating the following side chains is ecdysones,

(7) A compulsory α-hydroxylation at C14, and hydroxylation at C25 in most ecdysones

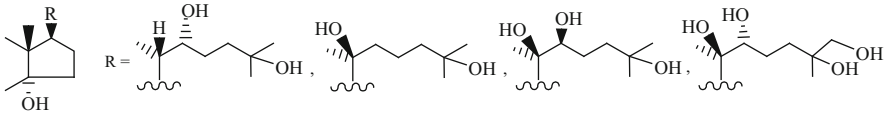


Fig. 11.23 Probable steps in the biogenesis of some ecdysones from cholesterol and hydroxylation patterns in the side chain

11.4.2 Diosgenin: Diosgenin-Derived Steroidal Drugs

Diosgenin (Fig. 11.25), $C_{27}H_{42}O_3$, m.p. 204–207, $[\alpha]_D$ + 129 ($CHCl_3$), an important steroidal derivative, occurs mainly as the glycosides and rarely in the free state in the rhizomes of the plants belonging to the genus *Dioscorea* (Dioscoreaceae). Dioscine, a common glycoside, is diosgenin-rhamno-rhamno glucoside. Other sources of diosgenin where it occurs in the free state are *Costus speciosus* (Zingiberaceae), *Kallstroemia pubescens*, and *Trigonella-foenum-graecum* (Leguminosae). Diosgenin has been the steroid drug precursor of choice. It is converted to 16-DPA (16-dehydropregnenolone acetate and then into various steroidal drugs (Fig. 11.25)—corticosteroids. Mexico is the largest producer of diosgenin.

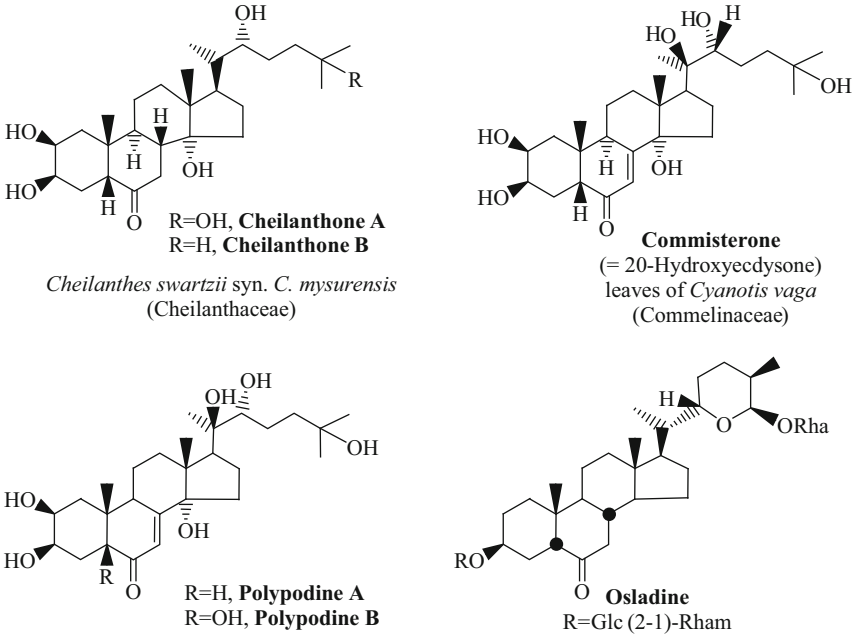


Fig. 11.24 The structures of some other ecdysones

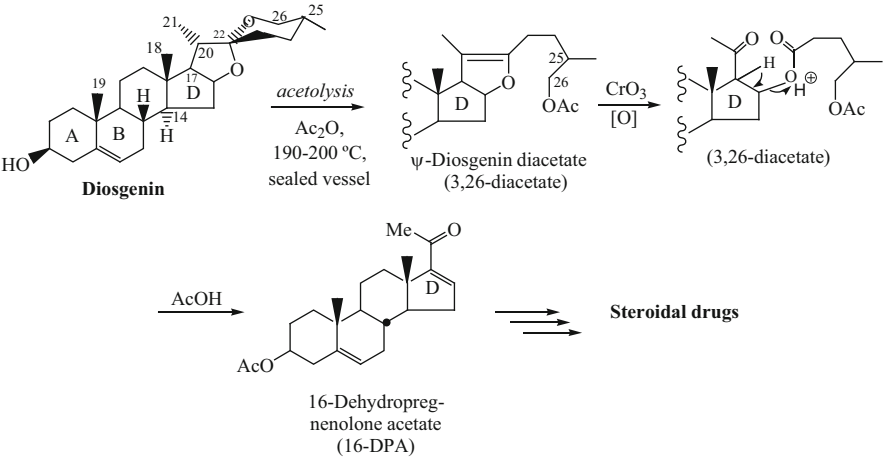


Fig. 11.25 Diosgenin and derived steroidal drugs

11.4.2.1 Spectral Data of Diosgenin

IR (KBr) ν_{max} 3,460 (OH)

^{1}H NMR ($CDCl_3$) δ: 0.79 (*s*, C27 Me and C18-Me), 0.98 (*d*, J = 6.4 Hz, C21 Me), 1.03 (*s*, C19-Me), 4.40 (*q*, C16 H), 5.31 (brd, C6-H)

^{13}C NMR ($CDCl_3$) δ: C1 (37.3), C2 (31.6), C3 (71.6), C4 (42.3), C5 (140.6), C6 (121.3), C7 (32.6), C8 (31.4), C9 (8.501), C10 (36.6), C10 (36.6), C11 (20.4), C12 (34.8), C13 (40.3), C14 (56.5), C15 (31.8), C16 (80.7), C17 (62.1), C18 (16.3), C19 (19.4), C20 (14.6), C21 (14.5), C22 (109.3), C23 (31.4), C24 (28.8), C25 (30.3), C26 (66.8)

MS: *m/z* 414 (M^+), 342, 139, 115.

11.4.3 Cardioactive Glycosides

The aglycone part of the cardioactive glycosides is having steroidal structures and is characterized by A/B and C/D *cis* fused rings; sugar molecules forming glycosidic bonds with 3β-OH group; a β-hydroxyl at C14; and an unsaturated γ- or δ-lactone on C17β. The most well known is **digitoxin** which occurs in the genus *Digitalis*. In Hellebrigenin having the δ-lactone on C17β., an additional β-OH at C5 and more oxidized C10-Me as C10-CHO are present (Fig. 11.26). The aglycone part improves the action of the heart. The sugar part of the glycoside is responsible for the solubility of the drug. These basic structures arise biosynthetically by metabolism of cholesterol effecting modifications of side chains.

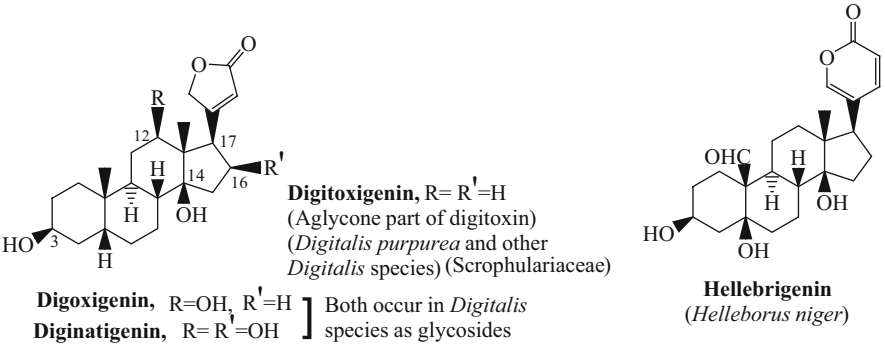


Fig. 11.26 Structures of the aglycone parts of two cardiac glycosides, digitoxin, and hellebrigenin

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Chapter 12
Carotenoids: GGPP-Derived Polyisoprenoid (C_{40}) Coloring Pigments

12.1 Introduction

Carotenoids (carotenes, tetraterpenoids) are biogenetically GGPP derived widely distributed and highly conjugated coloring pigments which absorb light between 400 and 500 nm. The color of carotenoids ranges from deep red to light yellow and sometimes even extends to purple, depending on the environment of the extended conjugated system. The name carotene is derived from carrots (edible roots) *Daucus carota* (Umbelliferae) in which these polyene pigments were first found by Wackenroder in 1832 [1], and the word termination "ene" was proposed by Hofmann in 1866 for unsaturated compounds [2]. Carotenes impart yellow to reddish color to carrots. They are widespread and distributed in flowers (daffodils, marigold), fruits (orange, tomato, pumpkin, red pepper or paprika), algae, fungi, photosynthetic bacteria, fall-coloration of deciduous plants, and also in animals—imparting natural coloration to birds, reptiles, amphibians, fishes, and various invertebrates [3]. During the ripening of fruits and bright yellow-orange coloration of leaves in autumn (fall color), the chlorophyll pigments break down in chloroplast thylakoid membrane, and the otherwise masked color of carotenoids starts revealing their brilliant bright yellow to orange colors. Further, during ripening biosynthesis of some new carotenoids also occurs.

Chlorophylls are the primary light harvesting pigments, and carotenoids are important accessory light-harvesting molecules that transfer energy to the reaction center during photosynthesis. They protect plants from the damage caused by oxygen especially during fall, when chlorophyll starts degrading and is not able to absorb light energy. Hence, carotenes minimize or stop the photooxidative damage. They quench the triplet excited state of photosynthesizers as well as the excited singlet state of oxygen. Thus, plants lacking carotenoids are damaged and killed quickly on exposure to light and oxygen, compared to the plants having their presence. The light harvesting molecules chlorophyll **a**, chlorophyll **b**, and carotenoids remain arranged in the highly organized way around the reaction center. These accessory pigments increase the efficiency of the system by transferring the energy to the

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reaction center through a mechanism of resonance energy transfer. Similar light harvesting combination also exists in photosynthetic bacteria as energy donors. Carotenoids being fat soluble are also known as lipochromes or chromolipids.

12.2 Structures of Carotenoids

Currently more than 800 structurally unique natural carotenoids are known, each of which can form further *cis-trans* isomers [4]. Carotenoids containing oxygen functions such as OH, OMe, epoxy, carboxy, aldehyde, etc. are collectively known as xanthophylls. Apocarotenoids are structurally close to carotenoids but contain carbon atoms less than C_{40}. Dietary vitamin A deficiency is a severe nutritional problem for children in developing and underdeveloped countries. β-Carotene and lycopene are precursors of vitamin A. Attempts have, therefore, been made to genetically introduce these carotenoids or their precursor *(15Z)-phytoene* in seed tissues of transgenic rice plants. Carotenoids with $C_{45,}$ C_{50} units are also known. Structures of a few familiar carotenoids are shown in Fig. 12.1.

Most natural acyclic carotenoids are having *all-E*-configuration of the double bonds (X-ray). The presence of some specific Z isomers has also been established. Some examples (**2**)–(**6**) are cited in Fig. 12.2. Prolycopene (**6**), the poly-*Z*-isomer of lycopene and the main pigment of Tangarine tomato fruits (*Lycopersicon esculentum* var. "*Tangella*" (Solanaceae)), has been shown to possess (7*Z*, 9*Z*, 7′*Z*, 9′*Z*) stereochemistry from spectroscopic data and comparison with synthetic model compounds. In the same way the other co-occurring pigments—the poly-*Z*-isomeric carotenoids (**2**)–(**5**) have been shown to possess (15*Z*) (15*Z*, 9′*Z*), (9*Z*, 9′*Z*), (9*Z*, 7′*Z*, 9′*Z*) stereochemistries, respectively [5] (Fig. 12.2). The remaining double bonds in the pigments have the *E*-geometry. Sterically hindered as well as *cis* isomers are found in much lesser number (Fig. 12.2).

12.3 Spectral Properties

IR ν_{max} Diagnostic peaks for (*Z*)-double bond (~730–780 cm^{-1}) and (*E*)-double bond (~960 cm^{-1}).

NMR, especially ^{13}C NMR spectra reveal the detailed structural information of carotenoids. The number of peaks in noise-decoupled spectra suggests the homogeneity as well as whether the molecule is symmetrical ($\mathbf{C_2}$) in the event of which half of the carbon atoms will appear in the noise-decoupled ^{13}C NMR spectra of the compound under investigation. Since the accurate assignments of the sp^2 carbons are not always easy, the advantage of the distinct ^{13}C NMR peaks of vinyl methyls and vinyl methylenes on (*Z*) and (*E*) configuration has been used [6]. To generalize these data, many open chain polyenes having C_{15}, C_{20}, and C_{30} chains with (*E*) and (*Z*) as well as (*E, Z*) stereochemistries of the double bonds have been synthesized

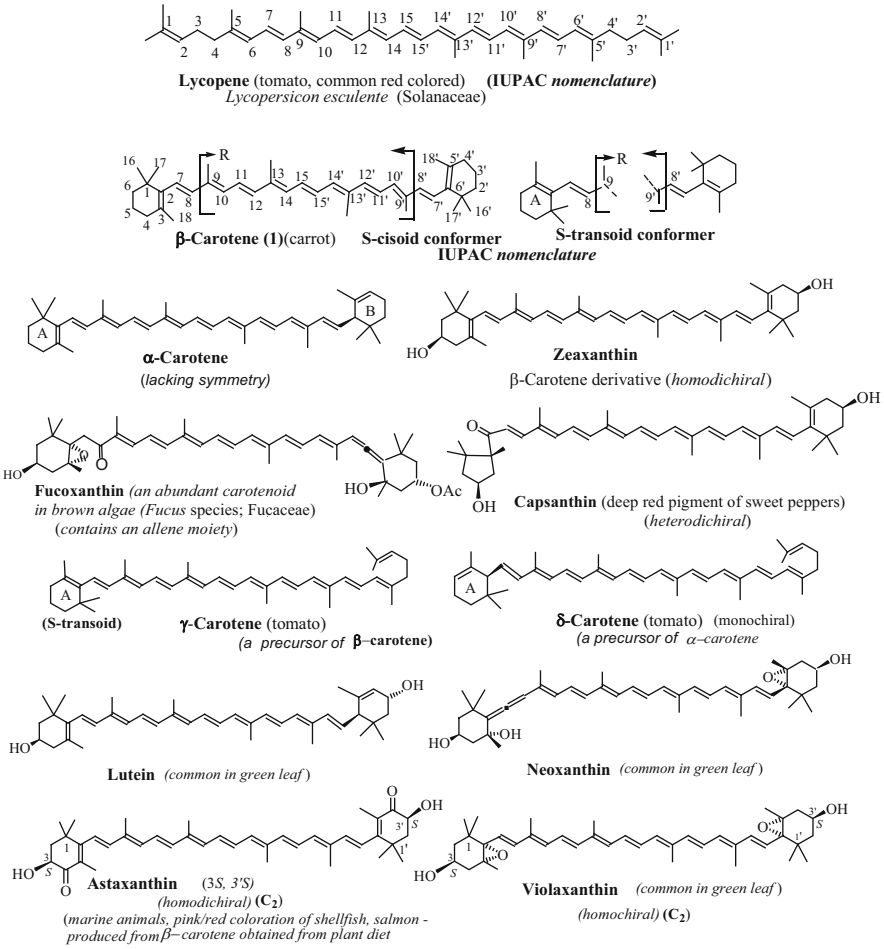
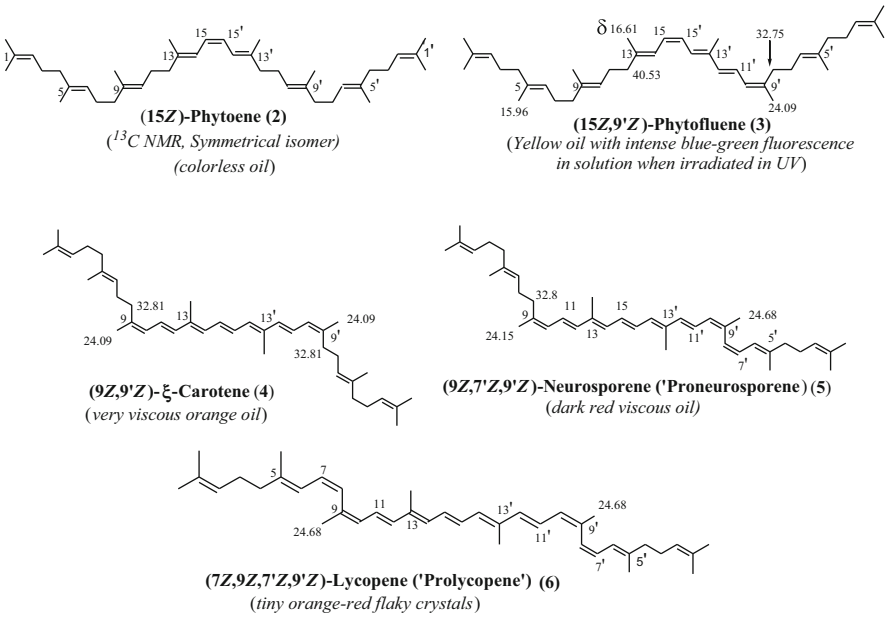


Fig. 12.1 Structures of some *all–E*-carotenoids

and the ^{13}C NMR data have been recorded and generalized [6]. Some data with structures have been given for natural carotenoids in Fig. 12.2 and for model synthetic compounds (Fig. 12.3).

12.4 β-Carotene and Lycopene

β-Carotene and lycopene are most familiar of all carotenoids. The structure of β-carotene (**1**) (a symmetrical C40 polyolefin), based on its degradation products (Fig. 12.4), its synthesis (Figs. 12.5 and 12.6), and the synthesis of lycopene (Fig. 12.7) (another symmetrical C40 polyolefin) have been discussed in brief.



Note : *The system of numbering carotenoids is recommended by IUPAC*

Fig. 12.2 Carotenoids with *Z*-configurations at specific location from tangerine, tomato fruits and some of their diagnostic ^{13}C peaks (Sect. 12.3) [5]

Based on these degradation products, the structure of β-carotene has been shown to be (**1**) (Fig. 12.1) which has been confirmed by its synthesis. Several syntheses of β-carotene have been reported. The synthesis [7] by Isler et al. (1957) which gave a high yield has been outlined later (see Fig. 12.5).

β-Carotene, lycopene, zeaxanthin, astaxanthin, and violaxanthin, shown in Fig. 12.1, and the carotenoids (**4**), and (**6**), shown in Fig. 12.2, belong to the $\mathbf{C_2}$ point group possessing a C_2 axis passing through the midpoint of C15-C15´ bond and orthogonal to the polyolefin chain. The carotenoid (**2**) (Fig. 12.2) also belong to $\mathbf{C_2}$ point group**;** however, its C_2 axis passing through the midpoint of C15–C15´ bond lies in the plane of the polyolefin chain.

12.5 Synthesis of β-Carotene

The synthesis by Isler (1957) is outlined later (see Fig. 12.6).

There are other methods of joining two or three smaller molecules to form the C40 skeleton, *e.g.*, C16+C8+C16→C40 (symmetric synthesis), whereas C25+C15→C40 (unsymmetrical synthesis). Retinal (C20 aldehyde)

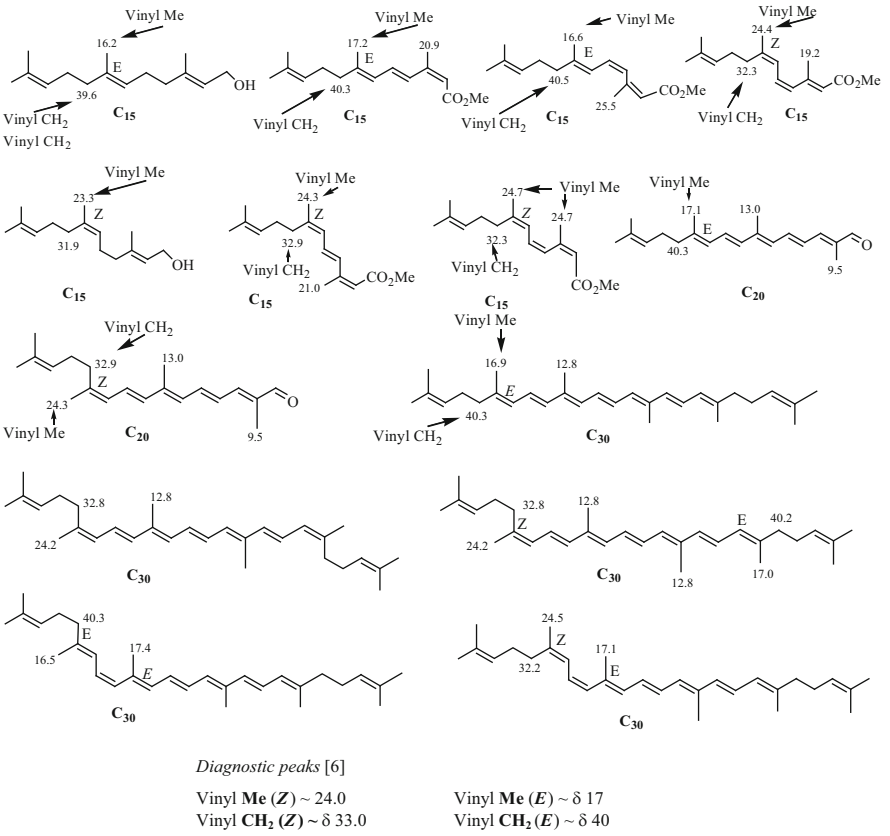


Fig. 12.3 Diagnostic peaks for vinyl methyl and vinyl methylene carbons of model compounds [6]

(see Fig. 12.6), when subjected to intermolecular McMurry coupling by treatment with $LiAlH_4$–$TiCl_3$ underwent reductive dimerization (C20+C20) (symmetric reaction) to yield β-carotene in 85 % yield [8].

Industrial syntheses [7, 9] of β-carotene have been accomplished by (i) BASF by coupling of β-retinyltriphenylphosphonium chloride (C20) and retinal (C20) (*cf.* Figs. 12.6 and 34.8), and (ii) Roche by condensing two units of β-C19-aldehyde with acetylenedimagnesium bromide (C2 unit) (C19+C2+C19) and conversion of the diol formed to the all-*trans* β-carotene. (iii) Both (i) and (ii) involve symmetric synthesis.

12.6 Conversion of Vitamin A to β-Carotene

β-Carotene is generally prepared by a multistep route from vitamin A, as outlined in Fig. 12.6.

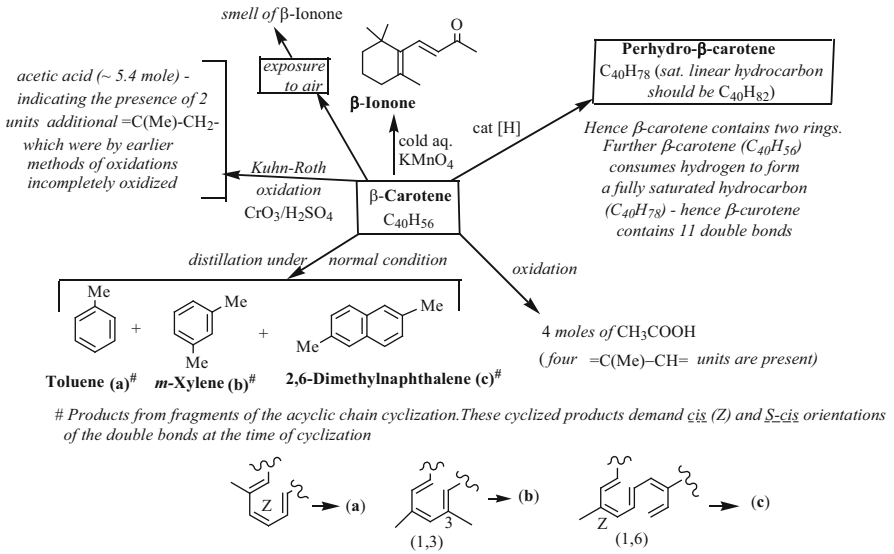


Fig. 12.4 Degradation products of β-carotene

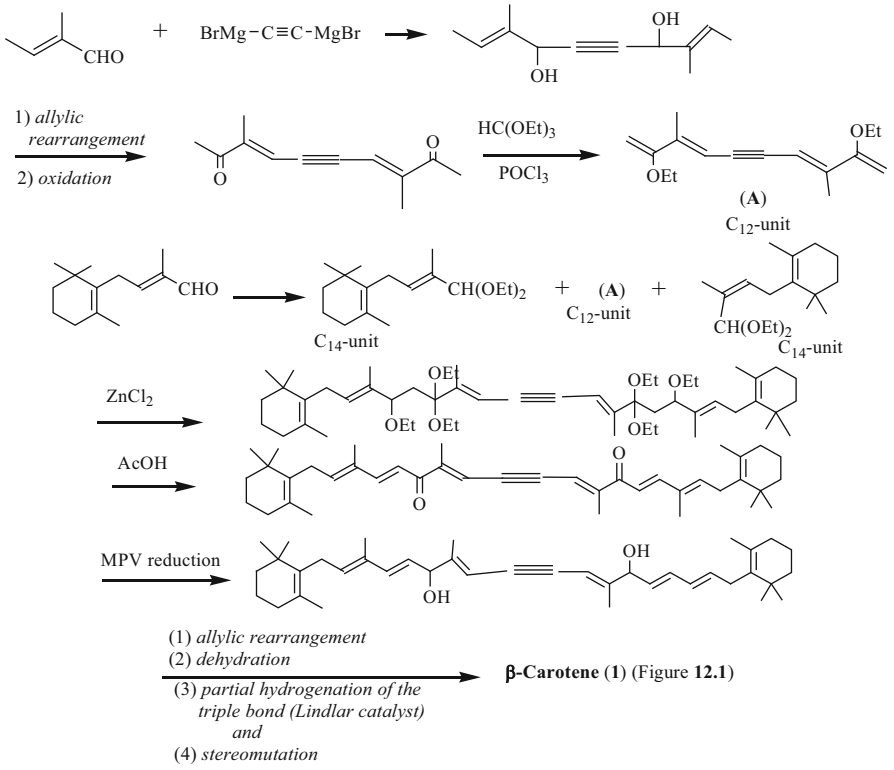


Fig. 12.5 Synthesis of β-carotene (1) by Isler (1957)

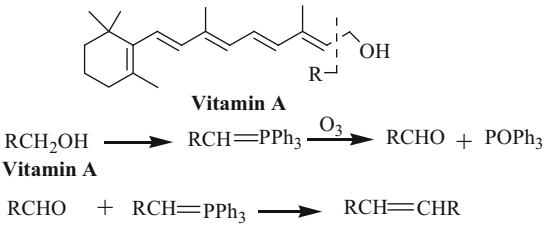


Fig. 12.6 Conversion of vitamin A to β-carotene

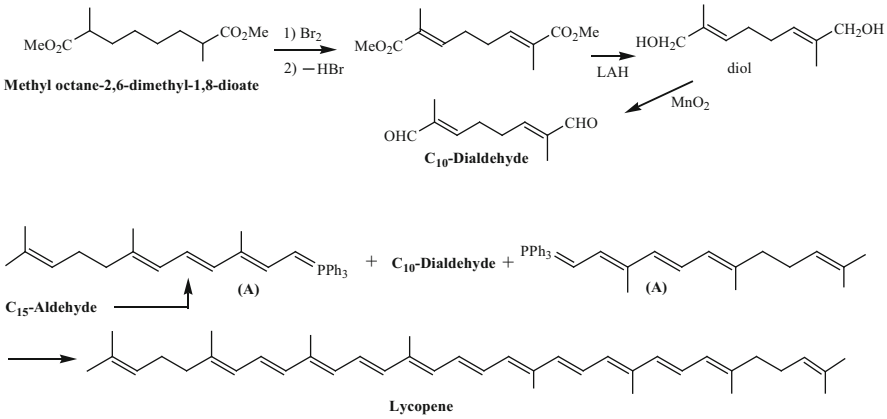


Fig. 12.7 Synthesis of lycopene

12.7 Synthesis of Lycopene

Of the several syntheses of lycopene (the red tomato pigment), the synthesis by Weedon (1965) by employing Wittig's reaction is outlined in Fig. 12.7.

Synthesis of a dialdehyde (C_{10}-unit):

12.8 Biosynthesis [10]

Most carotenoids are tetraterpenes (C_{40}). Two C_{20} units originating from geranylgeraniol are joined by tail-to-tail to form a chain of 32 carbon atoms bearing 8 methyl side chains. Often the basic C_{40} skeleton is retained or only slightly modified by cyclization at one or both ends (Figs. 12.1 and 12.2). Biosynthesis of squalene from FPP via presqualene has been discussed in Chap. 5 (Fig. 5.20). Biosynthesis of phytoene (C_{40}) from GGPP via prephytoene follows analogous mechanism like squalene from FPP. It then undergoes desaturation in stages, each

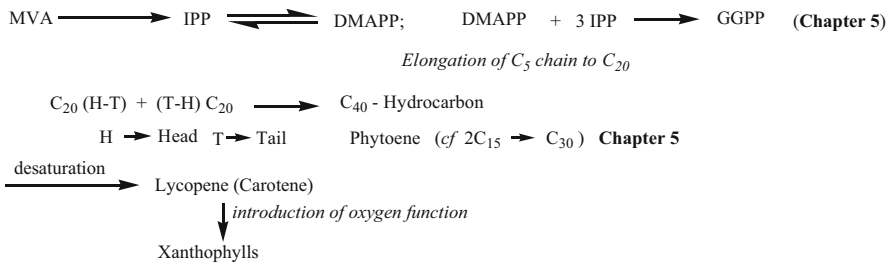


Fig. 12.8 Schematic expression of carotenoid biosynthesis

of which removes two hydrogen atoms and extends the conjugation of polyene by two double bonds on either side. Four such desaturations of two H atoms convert phytoene to lycopene. The latter undergoes cyclization at both or at any one terminal to form cyclocarotenes (β, α, γ, δ-carotenes) (Fig. 12.1). Epoxidation and hydroxylation occur at later stages to yield a special class of carotenes called xanthophylls. The steps in the process could be shown schematically in Fig. 12.8.

The genesis of C_{40}-linear chain from two molecules of GGPP is shown in Fig. 12.9.

Unlike squalene in which at the terminal step the carbocation is quenched by the donation of a hydride from NADPH, the carbocation in carotenoid biosynthesis is quenched through the loss of the adjacent prochiral hydrogen. Label experiment with (5*R*)-(5-$^3H_1$)-MVA suggests the formation of (15*Z*)-phytoene as the true intermediate in the biosynthesis of carotenoids. Since carotenoids with all *E*-double bonds are formed from (15*Z*)-phytoene, (15*Z*→15*E*) isomerization is an essential step in lycopene (*all E*) biosynthesis. Further, during desaturation the prochiral hydrogen that is lost has also been identified. The exact mechanism of desaturation is not known but each newly generated double bond holds *trans* relationship. The stepwise desaturation reactions take place in the following sequence: phytoene → phytofluorene (7,8,11,12,7′,8′ hexahydro-ψ,ψ-carotene) → ζ-carotene (7,8,7′,8-′-tetrahedro ψ,ψ-carotene) → and neurosporene (7,8-dihydro-ψ,ψ-carotene) → lycopene. Because of the geometry of the molecule and long extended conjugation, the scope for cyclization, involving polyene, is not likely except at the termini. Here, squalene-like folding behavior under influence of different cyclases is not expected. Hence, the main chain of the carotenoids remains linear, and the terminal parts undergo cyclizations and oxidations to generate different carotenoids, as outlined in Fig. 12.10.

12.9 Uses

Natural carotenoids are precursors of vitamin A and also important photosynthetic light-harvesting pigments. β-Carotene is commercially important as a food coloring agent. In 1995 commercial preparation of β-carotene to the scale of 500 tons per year was planned [11].

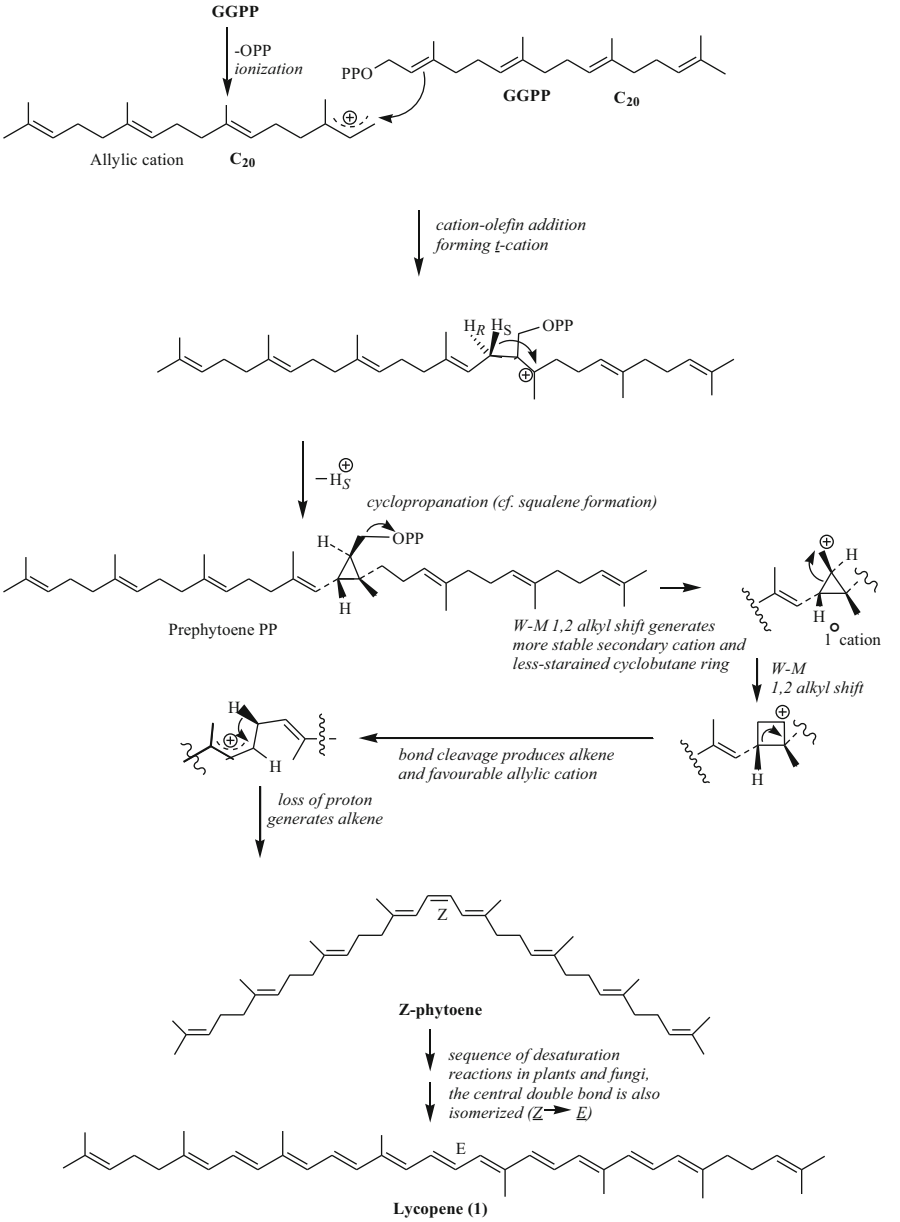


Fig. 12.9 Biosynthesis of the C_{40}-polyene lycopene

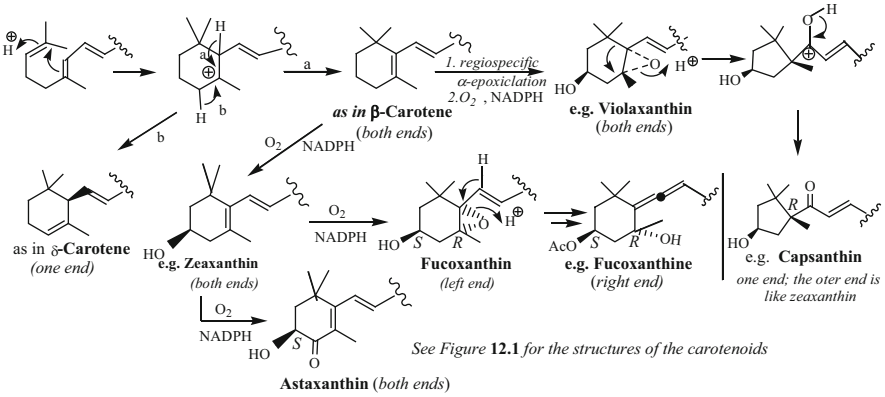


Fig. 12.10 Formation of various types of terminal rings of carotenoids

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Chapter 13
Shikimic Acid Pathway

13.1 Introduction and Biosynthesis of C_6-C_3 Moieties via Shikimic Acid

Of the identified biosynthetic paths, **shikimic acid pathway** plays a very important role in providing precursors of a large number of aromatic compounds of diverse skeletal patterns and substitutions. **Chorismic acid**, an important branching point product in the shikimic acid pathway also serves as an important precursor. Natural products with **C_6–C_1** or **C_6–C_3** or **C_6–C_3–C_3–C_6** unit as their complete skeletal carbon content or partial skeletal carbon content like **C_3–C_6** of C_6–**C_3–C_6** system utilize these precursors.

The vital role of (–)-shikimic acid in the metabolic pathway was discovered by B. D. Davis. The compound was first isolated in 1885 by Eijkman from the fruits of *Illicium religiosum*, (syn. *I. anisatum*) (Fam. Illiciaceae) in a surprising good yield (~20 %). The Japanese name of this plant is shikimi-no-ki from which the compound derived its name. The structure and absolute configuration of (–)-shikimic acid were established during 1930s—more than 50 years after its first isolation, by a series of degradation reactions and by correlation with 2-deoxy-D-arabinohexanoic acid. The work was mainly due to Harman Otto Laurenz Fischer, the eldest son of Emil Fischer (Appendix A, A-12).

13.1.1 (–)-Shikimic Acid

Shikimic acid, $[\alpha]_D$ −184 (H_2O) is (3*R*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid (Fig. 13.1), In the plant cells (–)-shikimic acid gets its skeletal carbons (C_6–C_1) from phosphoenol pyruvate (PEP) and D-erythrose-4-phosphate (Fig. 13.1). The PEP is derived from 2-phosphoglyceric acid (2-PGA) through the elimination of the elements of water, while D-fructose-6-phsophate delivers its –$COCH_2OH$ group to D-3-phosphoglyceraldehyde through the participation of the

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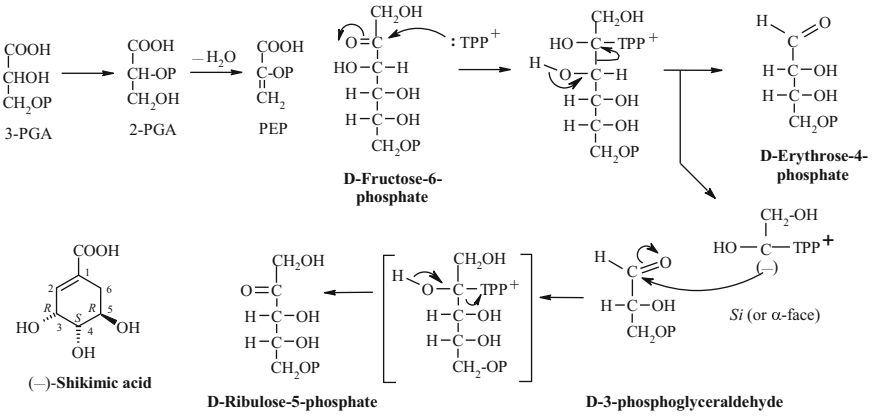


Note: *OP represents phosphate, OPP pyrophosphate or diphosphate, and TPP thiamine pyrophosphate* (see Chapter **3**).

Fig. 13.1 Formation of PEP, D-erythrose-4-phosphate, and D-ribulose-5-phosphate

prosthetic group TPP (thiamine pyrophosphate) (see Fig. 3.8) of the transketolase, keeping its remaining four carbon atoms as in D-erythrose-4-phosphate configuration (Fig. 13.1). The stages 1 to 4 involved in the formation of (–)-shikimic acid are discussed in the sequel.

13.1.1.1 Stage 1. Formation of 3-deoxy-D-arabinoheptulosonic acid 7-phosphate (DAHP)

Enzyme involved: DAHP synthetase. PEP and D-erythrose-4-phosphate combine to form a C_7-saccharide phosphate identified as 3-deoxy-D-arabinoheptulosonic acid 7-phosphate (DAHP). The obvious choice for such $C_3 + C_4$ condensation yielding a C_7-saccharide is aldol condensation, a central reaction in the biosynthetic processes. The process is initiated by the nucleophilic attack on phosphorous of P–O–C bond as shown in Fig. 13.2. However, *studies with P–^{18}O–C labeled PEP show that the iP liberated contains almost quantitative amount of ^{18}O* and the release of iP is slow. Thus, it is concluded that the cleavage of O-C bond of P–^{18}O–C and not P–O bond is operative. To explain these observations a number of suggestions have been put forward. One logical proposition that has been suggested involved the sequence of reactions shown in Fig. 13.3.

PEP is reversibly conjugated to the enzyme by Michael addition. The latter then binds the second substrate D-erythrose-4-phosphate (**A**). In the bound form the O–P and EnzS undergo exchange of positions through crossed 1,2-shifts. The enolate form of the pyruvate, generated through the elimination of phosphate, is now ready for aldol condensation with D-erythrose-4-phosphate (**A**). The resulting product

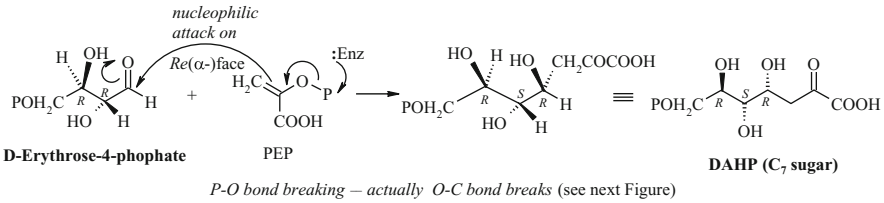


Fig. 13.2 Formation of DAHP (*P–O bond cleavage*)

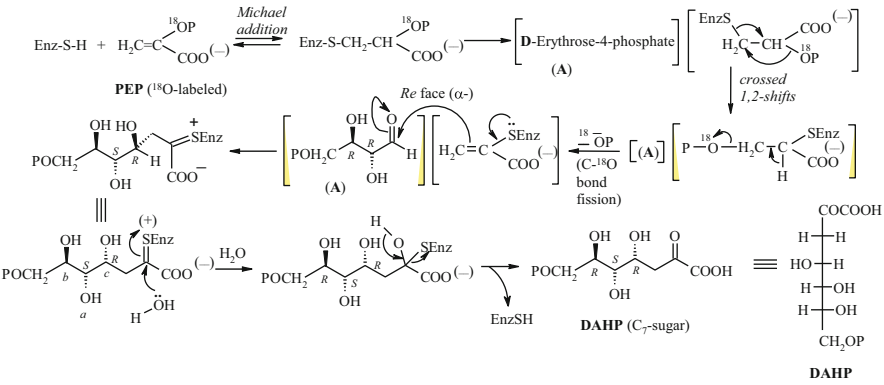


Fig. 13.3 Formation of DAHP (*C–O bond cleavage*)

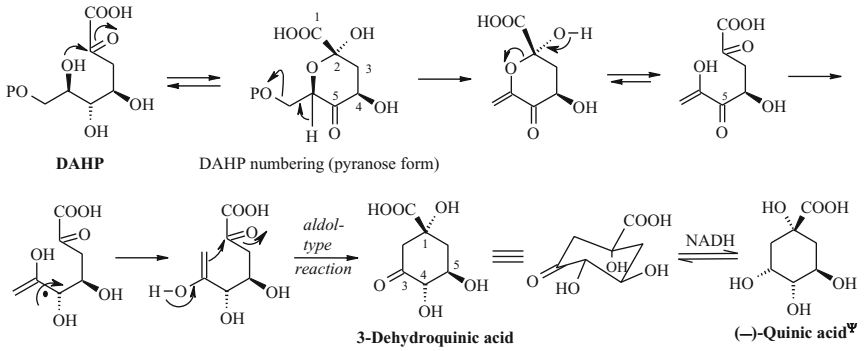
then undergoes hydrolysis to form DAHP (Fig. 13.3). This mechanistic approach includes the O–C (of P–O–C) bond fission, and not P–O bond fission, and the kinetics of the reaction is consistent with slow release of phosphate due to kinetic isotope effect.

13.1.1.2 Stage 2. Formation of 3-Dehydroquinic Acid

Enzyme involved: 3-dehydroquinate synthetase (synthase). In the conversion of DAHP to 3-dehydroquinic acid, the most reasonable mechanism would be the formation of the pyranose form of the C_7-sugar, oxidation at C_5, followed by elimination of the phosphate, opening up of the pyranose ring, reduction at C_5 destabilizing the enol, and consequent cyclization to give 3-dehydroquinic acid (Fig. 13.4).

13.1.1.3 Stage 3. Formation of 3-dehydroshikimic Acid

Enzyme involved: 3-dehydroquinate dehydratase. Elimination of the elements of water from 3-dehydroquinic acid leads to the formation of dehydroshikimic acid; the direction of elimination is governed by the formation of an α,β-unsaturated



Note: *Each reaction is catalyzed by different specific enzymes.*
Ψ(–)-Quinic acid commonly occurs in nature in free form, as esters or in combination with alkaloids such as quinine

Fig. 13.4 Conversion of DAHP to 3-dehydroquinic acid

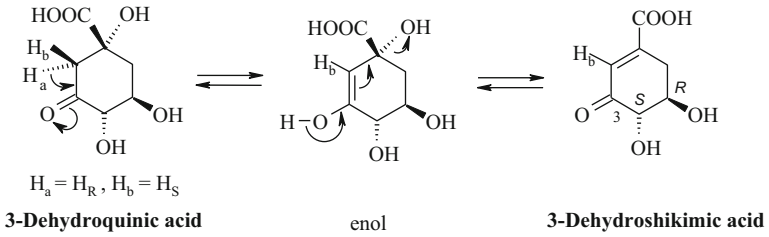
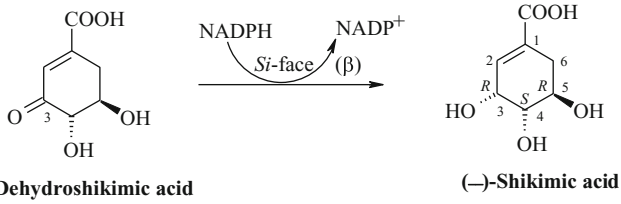


Fig. 13.5 Formation of 3-dehydroshikimic acid



(3*R*,4*S*,5*R*)-3,4,5-Trihydroxy-1-cyclohexene-1-carboxylic acid

Fig. 13.6 Formation of (–)-shikimic acid

ketone system. In this process $H_a = H_R$ is lost (as evident from labeling experiment), claiming the process to be a *cis* elimination, which is not consistent with the stereoelectronic requirement of such 1,2 elimination. This enzymic dehydration being in contrast to what we find in the ordinary acid/base catalyzed *trans* E_2 elimination; the elimination is suggested to proceed via enolization (Fig. **13.5**). This is followed by reduction of the CO group of dehydroshikimic acid by NADPH in the presence of the enzyme to form shikimic acid, as shown in Fig. **13.6** (Stage 4).

13.1.1.4 Stage 4. Formation of (–)-shikimic acid from 3-dehydroshikimic acid (Fig. 13.6)

Enzyme involved: shikimate-NADPH-oxoreductase.

13.1.2 Formation of Chorismic Acid and Prephenic Acid

The conversion of (–)-shikimic acid to chorismic acid is a very important biological event since it is the branching point of shikimic acid pathway, and chorismic acid serves as the precursor for C_6–C_3 (cinnamic acid, coumarins), C_6–C_3–C_3–C_6 (lignans) compounds, and also the C_3–C_6 part of the C_6–C_3–C_6 (flavonoids) compounds and various important aromatic amino acids.

13.1.2.1 Chorismic Acid

Enzymes involved: (i) 5-enolpyruvylshikimate-3-phosphate synthetase, (ii) chorismate synthetase. The conversion of shikimic acid to chorismic acid involves few steps, and starts with the regiospecific phosphorylation at C3-OH, followed by regiospecific etherification at C5-OH of shikimic acid with a C_3-unit delivered by phosphoenol pyruvate (PEP) (Fig. 13.7). The enzyme responsible for ether formation is 5-enolpyruvylshikimate-3-phosphate synthetase (EPSP synthase). Synchronous nucleophilic attack at C2 by 3-phosphoshikimic acid and protonation at C3 of PEP give an intermediate, 5-phosphoenolpyruvate of shikimic acid 3-phosphate (Fig. 13.7). In shikimic acid formation one molecule of each of D-erythrose-4-phosphate and PEP (Fig. 13.2) are necessary, while chrorismic acid formation requires one more molecule of PEP (Fig. 13.7).

Conversion of 5-enolpyruvylshikimic acid 3-phosphate (EPSP) to chorismic acid. This conversion takes place through 1,4-elimination. Labeling experiment shows that H_R is eliminated which claims the 1,4-elimination process to be *trans*, while stereoelectronic requirements demand a 1,4 *cis* elimination. It was, therefore, suggested that the elimination may take place in two steps. A combination of S_N2 substitution and E_2-elimination may take care of the stereoelectronic requirement (Fig. 13.8).

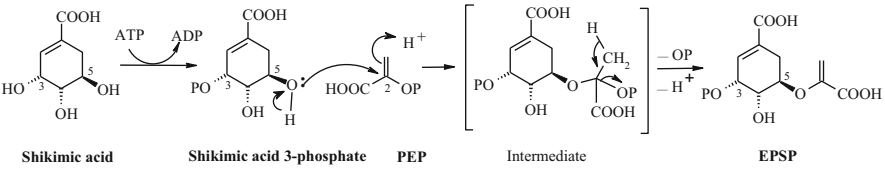


Fig. 13.7 Formation of 5-enolpyruvylshikimic acid 3-phosphate (EPSP)

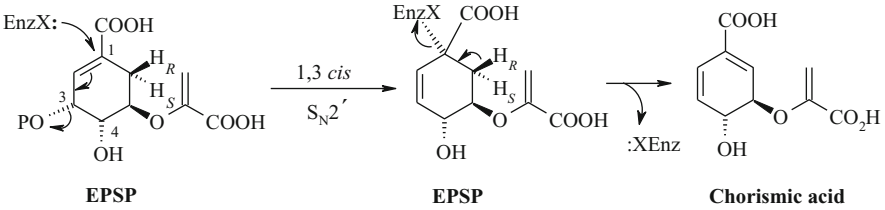


Fig. 13.8 Formation of chorismic acid from EPSP

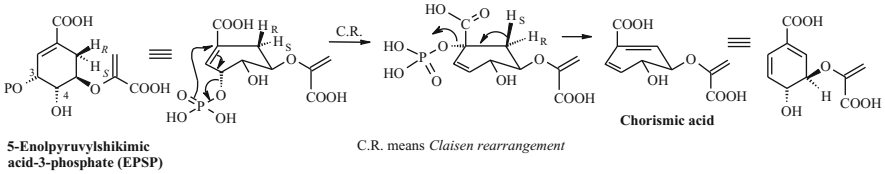


Fig. 13.9 Formation of chorismic acid from EPSP (alternative path)

A nucleophilic attack on C1 by enzyme (on the α-face) takes place, which pushes the double bond and in effect eliminates the *cis*-phosphate group located at C3. This is then followed by *trans* E_2-elimination involving H_R and the enzyme nucleophile (Fig. **13.8**).

An alternative mechanism is shown in Fig. 13.9. The formation of chorismic acid from 5-enolpyruvylshikimic acid 3-phosphate can also be conceived through the participation of C3-phosphate by way of its transfer on C1 from α-face (Fig. 13.9).

13.1.2.2 Prephenic Acid

The identified enzyme chorismate mutase catalyzes the conversion of chorismic acid to prephenic acid—a remarkable bond reorganization process involving Claisen rearrangement ([3,3]sigmatropic shift)—a type of pericyclic reaction occurring in the metabolic pathway (Fig. 13.10). Interestingly, from an optically active substrate an achiral compound possessing a plane of symmetry is formed. Thus, the PEP-derived three-carbon side chain becomes directly bonded to the cyclohexadiene ring and the basic C_6C_3 carbon skeleton of phenylalanine and tyrosine is formed. The enzyme increases this pericyclic reaction ~10^6 times faster than that of the corresponding thermal reaction. It has been observed that Claisen rearrangement reactions are accelerated on going from nonpolar to aqueous solvent. Thus, chorismic acid undergoes Claisen rearrangement 100 times faster in water than in methanol.

Studies with isotopically labeled chorismic acid showed that this enzyme catalyzed reaction proceeds via a *concerted but asynchronous pericyclic transition state*

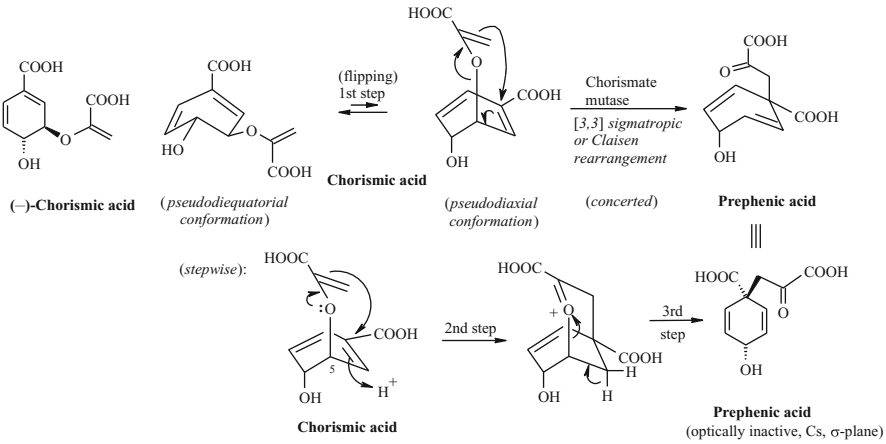


Fig. 13.10 Formation of prephenic acid from chorismic acid (both concerted and stepwise mechanisms)

with C–O bond breaking occurring considerably before the C–C bond formation, both taking place on the same β-face (suprafacial).

Some recent experiments using model compounds and quantum chemistry calculations suggest a three-step enzyme mechanism that involves the complexation of the unstable pseudodiaxial conformer of chorismic acid in its half chair-like geometry, allowing the spatial confinement of the reactive termini, followed by C–C bond formation and then C–O bond breaking (Fig. 13.10) in this rearrangement.

13.1.3 Aromatic Amino Acids: Phenylalanine and Tyrosine

Monogastric animals (like swine, chicks, and human beings) are incapable of synthesizing some essential amino acids which include some aromatic amino acids like phenylalanine, tyrosine, and tryptophan. These are essential for protein synthesis; monogastric animals use plant products as the source of these amino acids.

Biosynthetic pathways of the important aromatic amino acids—anthranilic acid, *p*-aminobenzoic acid (PABA), and L-tryptophan from chorismic acid are outlined with mechanism in Fig. 13.11. The evidence suggests that the (i) aromatic ring, (ii) the C2 and C3 of indole part, and (iii) the alanine side chain of tryptophan are derived from (i) anthrantic acid, (ii) D-ribose, and (iii) L-serine, respectively. Anthranilic acid, although a precursor of L-tryptophan, may also be produced by metabolism of the latter. Both these compounds serve as building blocks for a variety of alkaloids (see Chap. 15).

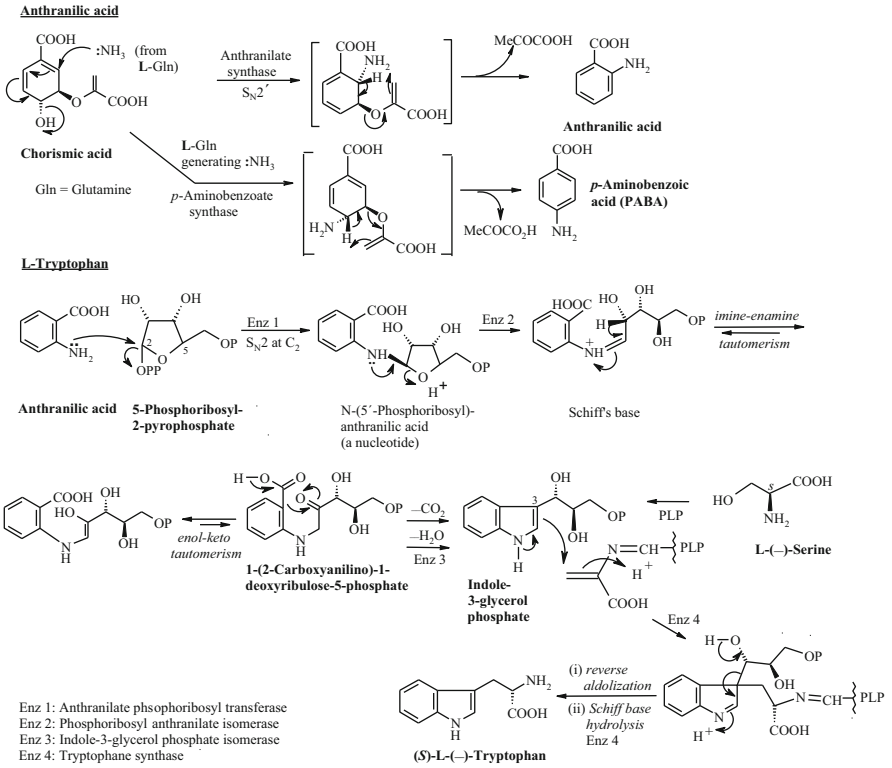


Fig. 13.11 Biosynthesis of anthranilic acid, *p*-aminobenzoic acid, and tryptophan from chorismic acid

Biosynthesis of L-phenylalanine and L-tyrosine from prephenic acid. These two C_6C_3 building blocks are the precursors of a wide range of natural products of plant origin. Their biosynthesis from prephenic acid in the presence of appropriate enzymes through transamination of keto acid ⇆ L-amino acid of the intermediates phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid, respectively, has been annotated in Fig. 13.12. Depending upon the organism and the presence of specific enzymes, the sequence of aromatization (involving decarboxylative dehydroxylation) and transmination may be reversed. Thus, prephenic acid may undergo transamination to give the corresponding L-amino acid called L-arogenic acid, followed by decarboxylative aromatization with the loss of OH to give L-phenylalanine. On the other hand, L-arogenic acid may undergo decarboxylative aromatization with the loss of hydride to NAD^+ to give L-tyrosine (Fig. 13.12). Incidentally, it may be mentioned that *in human L-phenylalanine is converted to L-tyrosine, though L-phenylalanine is not synthesized in the system.*

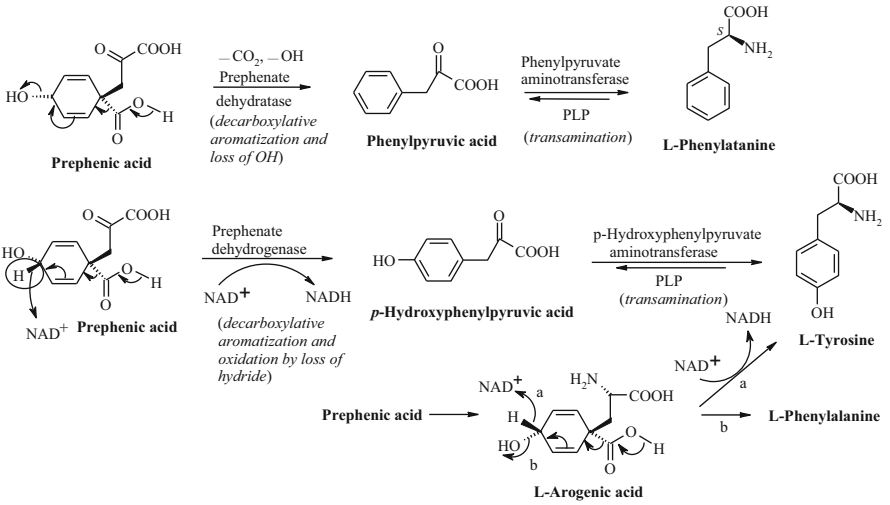


Fig. 13.12 Biosynthesis of L-phenylalanine and L-tyrosine from prephenic acid

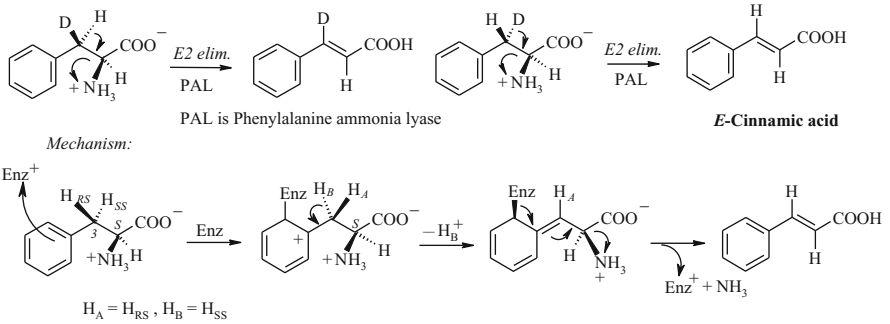


Fig. 13.13 Mechanism of biosynthesis of *E*-cinnamic acid from L-phenylalanine

13.1.4 Formation of trans*-Cinnamic Acid from L-Phenylalanine*

Two protons at C-3 of phenylalanine are diastereotopic. The enantiotopic protons are designated as H_R (***pro-R***) or H_S (***pro-S***) (Fig. 2.64, Sect. 2.9.7) whereas diastereotopic protons at C3 of phenylalanine are designated as H_{RS} and H_{SS}, by adding the *S* configuration of C2 to the subscripts of C3 protons (Fig. 13.13) (*vide* 2.9,11, Fig. 2.67, Ex. 1). The enzyme phenylalanine ammonia lyase (PAL) catalyzes the conversion of L-phenylalanine to *trans*-cinnamic acid by an E2 *anti-elimination* of the amino group and the H_{SS}. This stereochemical course has been demonstrated using labeled L-phenylalanine (Fig. 13.13).

The obvious choice for such conversion is the E2-elimination as shown. However, it has been shown that the prosthetic group of the enzyme binds at the *ortho* position of the side chain generating a carbocation which collapses through stereospecific loss of $3H_{SS}$ ($3H_B$) (2.6.2). This is followed by the release of enzyme and elimination of NH_2 group (Fig. 13.13).

Involvement of Cinnamic Acid as Carbon Source of C_6–C_3–C_3–C_6 (Lignans) and C_6–C_3 (Coumarins) and C_6–C_3 Part of C_6–C_3–C_6 (Flavonoids) Skeletons
Hydroxylation of the aromatic rings is a very common biological event and occurs on the aromatic rings at various stages and by different means on the operative substrates, e.g., 4-hydroxylase can transform cinnamic acid to 4-hydroxycinnamic acid, known as 4-coumaric acid. More hydroxyl groups are introduced regiospecifically with the right performing enzyme/s. Variously substituted cinnamic acids can be activated as the corresponding coenzyme A ester (see Fig. 13.15) for further participation.

13.1.5 Biosynthesis of Coumarins

Coumarins get their skeletal carbons (C_6–C_3) from *E*-cinnamic acid formed in the shikimic acid metabolic pathway in the plant cells. The biological steps needed for the conversion of *E*-cinnamic acid to coumarin skeleton are hydroxylation, glucosylation, isomerization ($E \rightarrow Z$), deglucosylation (enzymatic hydrolysis), and lacatonization (Fig. 13.14) in the presence of suitable enzymes. The exact chronology of these events is not known with certainty. The hydroxylation occurs in the presence of O_2 and NADPH, being catalyzed by a suitable enzyme.

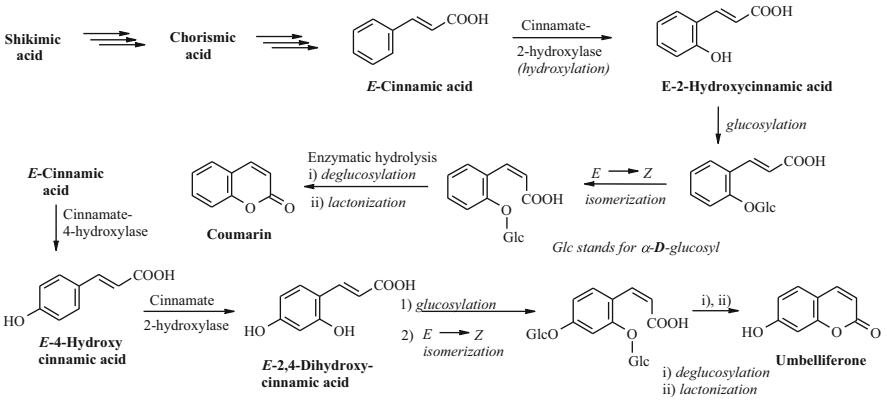


Fig. 13.14 Biosynthesis of coumarin and umbellferone [Mechanism of $E \rightarrow Z$ isomerization (last but 2 steps) is still obscure]

13.1.6 Biosynthesis of Phenylpropanoids $(C_6–C_3)_x$ (Lignins) and $(C_6–C_3–C_3–C_6)$ (Lignans) of Different Skeletal Patterns from Cinnamyl Alcohols via Cinnamic Acids

The first dimeric phenylpropanoid compound structurally elucidated by Schroeter has been guaiaretic acid, isolated from the heartwood of *Guaiacum officinalis*. Haworth introduced the term lignan for dimeric phenylpropanoid compounds. The monomeric phenylpropanoid unit is generated from cinnamic acid which in turn is formed from phenylalanine, a shikimic acid pathway metabolite.

Cinnamic acid undergoes sequential hydroxylation and methylation reactions forming substitution patterns typical of shikimate pathway metabolites. Some of the more common natural cinnamic acids are shown in Fig. 13.15. Hydroxylated cinnamic acids are converted into the corresponding cinnamyl alcohols. At some stage the hydroxyl group or groups are methylated or methylenedioxylated (Sects. 3.6.1 and 3.6.2) prior to entering into the lignan skeleton formation. All these substituted cinnamic acids, cinnamic aldehydes, and cinnamyl alcohols can be represented as (**1**) (**2**), and (**3**) (Fig. 13.15).

Oxygenation patterns are not necessarily elaborated at the cinnamic acid level. Coenzyme A esters and aldehydes through which the cinnamic acids are converted to the corresponding cinnamyl alcohols (Fig. 13.15) may also serve as substrates for aromatic hydroxylation and methylation, being catalyzed by specific enzymes.

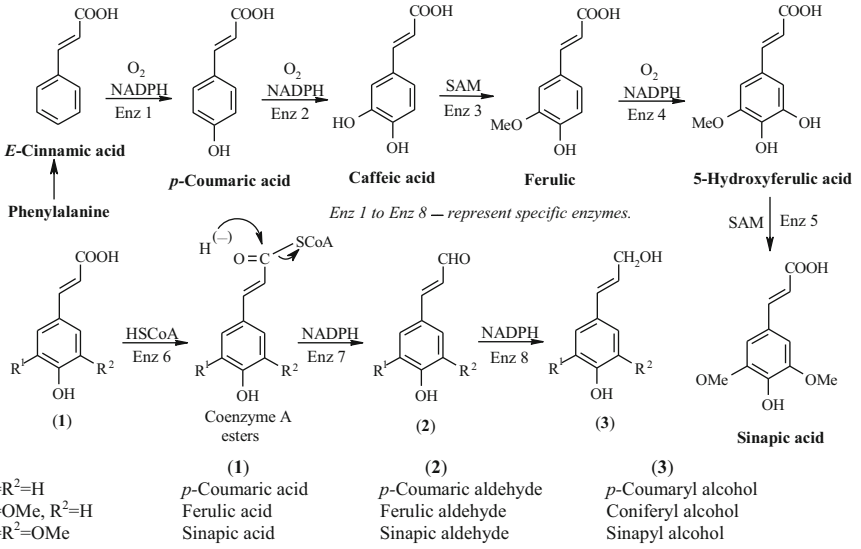


Fig. 13.15 Biosynthesis of oxygenated cinnamic acids and cinnamyl alcohols

13.1.6.1 Lignins

The different cinnamyl alcohols, e.g., *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, depending on the plant type, undergo phenolic oxidative couplings to form the most abundant plant polymers **lignins**, strengthening materials for the plant cell walls. Thus, the lignins represent vast reservoirs of aromatic materials in plants. These are mostly inaccessible because of the difficulties faced to release these metabolites.

13.1.6.2 Lignans. Biosynthesis

The lignans are formed from the dimerization of substituted cinnamyl alcohols through resonance stabilized radical–radical coupling known as *homogenesis*, being opposite to *homolysis*. The new σ-bond is formed by the contribution of one electron from the radical center of each monomer. Biosynthesis of several lignans (**1**) to (**9**) of varied skeletal patterns is depicted in Fig. 13.16. Radical pairings thus provide a range of dimeric systems containing reactive quinonemethides susceptible to nucleophilic attack from proximate OH groups in the system or by external water molecules. The radical pairing and nucleophilic attack being catalyzed by specific enzymes are stereocontrolled and thus lead to the formation of enantiomerically pure chiral lignans.

The products of phenylpropanoid coupling have a range of structural systems of different skeletons. They are not restricted to 8-8′ linked moieties as contemplated earlier, and the lignans with other types of linkages were termed *neolignans*. Since only a relatively small number of distinct skeletal forms are known to occur, the term **lignan** is conveniently used to include all skeletal types involving precise linkages, e.g., 8-8′, 8-7′, 8-5′, 8-O-4′, 5-5′, 3-O-4′, 7-1′, 8-O-7′, 1-5′, 2-O-3′, and 3-O-3′, and also some complex linkages as in chrysophyllan 1A. Several common lignans having some of these linkages and their plant sources are included in Table 13.1.

13.1.7 Biosynthesis of Some Common Phenolic Acids via Shikimic Acid and via Chorismic Acid

Biosynthesis of some common phenolic acids like **gallic acid, protocatechuic acid, PABA, *m*-hydroxybenzoic acid, and resorcylic acid** from shikimic acid and dehydroshikimic acid may be conceived, being catalyzed by an appropriate enzyme in each step, as delineated in Fig. 13.17.

Chorismic acid serves as the precursor of salicyclic acid, *p*-hydroxybenzoic acid, and 2,3-dihydroxybenzoic acid (Fig. 13.18). The specific enzymes that catalyze the different conversions are mentioned in the figure. Salicylic acid is found in plants,

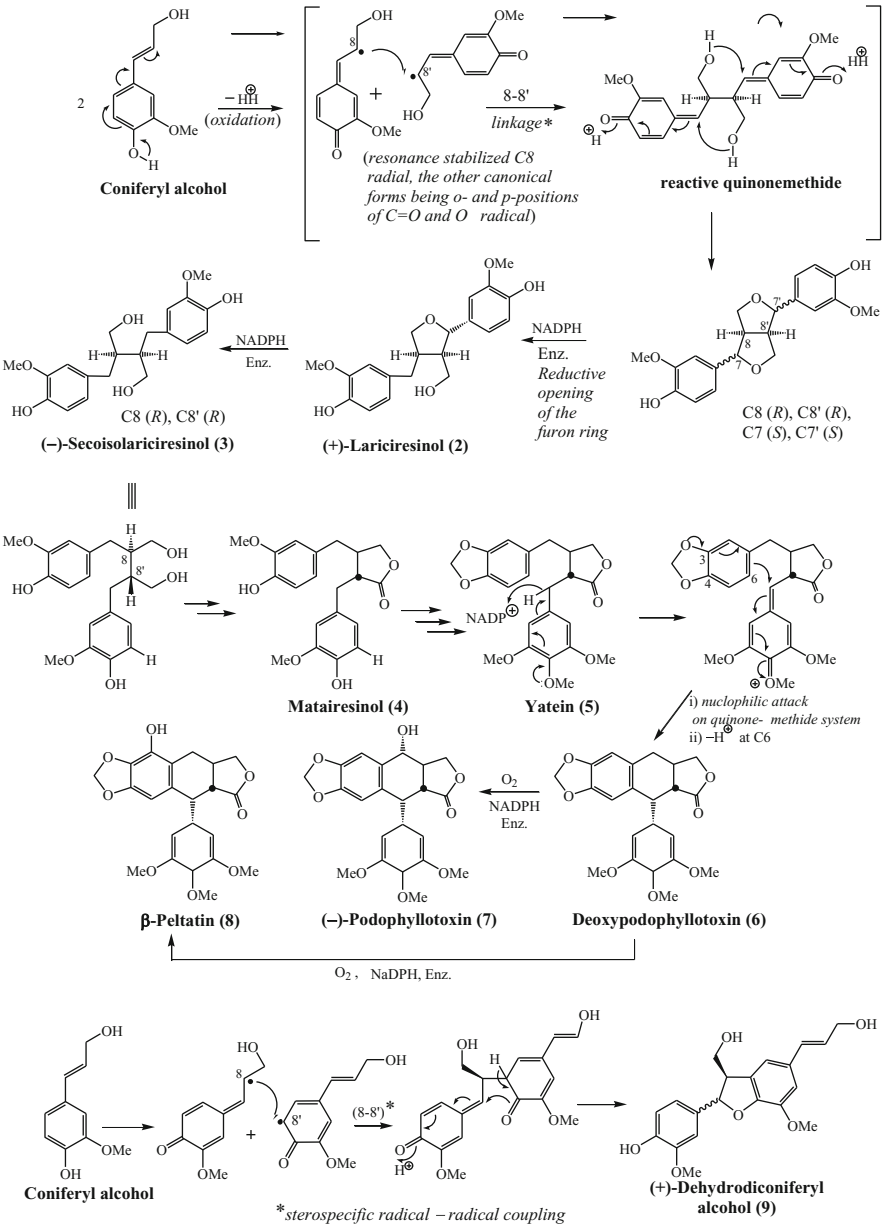
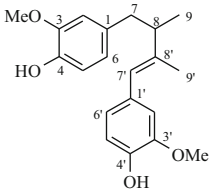
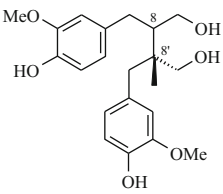
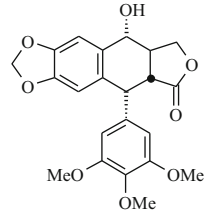
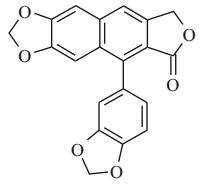
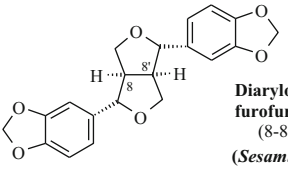
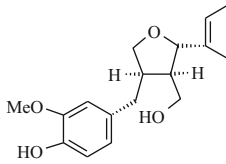
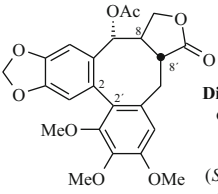
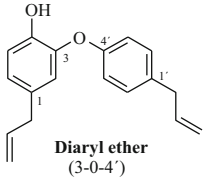
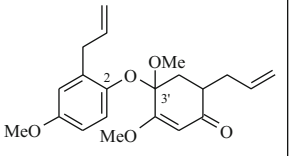
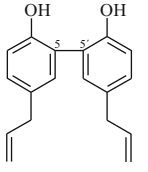
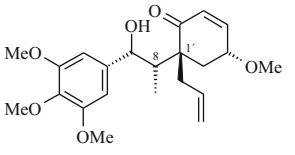
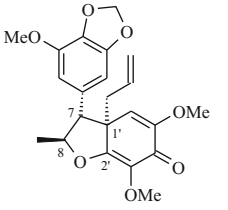


Fig. 13.16 Biosynthesis of the lignans (**1**)–(**9**) from coniferyl alcohol

Table 13.1 Some structural variations of lignans arising out of different linkages and their plant sources

Lignan	Skeletal Pattern (Linkage) (Plant source)	Lignan	Skeletal Pattern (Linkage) (Plant source)
 **(–)-Guaiaretic acid, $[\alpha]_D$ –94**	**Dibenzylbutene** (8-8′) (*Guaiacum officinale*)	 **(–)-Secoisolariciresinol, $[\alpha]_D$ –32 (Me_2CO)**	**Dibenzylbutane** (8-8′) (*Podocarpus spicata*) (+)-variety occurs in *Linium usitatissimum*
 (–)-Podophyllotoxin, $[\alpha]_D$ –109 (EtOH)	**Aryltetrahydro-naphthalene** (8-8′) (*Podophyllum hexandrum*)	 **Justicidin E**	**Arylnaphthalene** (8-8′) (*Justicia precumbens*)
 **(+)-Sesamin**, $[\alpha]_D$ +78.4 ($CHCl_3$)	**Diaryloctahydro-furofuran** (8-8′) (***Sesamum indicum***)	 **(+)-Lariciresinol**	**Diaryltetrahydrofuran** (8-8′) (*Araucaria augustifdia*) (–)-Lariciresinol occurs in *Daphne tangutea)*
 **(–)-Steganacin**, $[\alpha]_D$ –114 ($CHCl_3$)	**Dibenzocyclo-octadiene** (8-8′) (*Staganotaenia araliaceae*)	 **Diaryl ether** (3-0-4′) (***Sassafras randaiense***) **Isomagnolol**	 ***Aryl methyl ketal*** (2-0-3') **Lancilin** (***Aniba lancifolia***)
 **Magnolol**	**Biaryl** (5-5′) (*Magnolia virginiana*)	 (–)-Megaphone (8-1′ linkage) $[\alpha]_D$ –23 (EtOH, *c* = 0.15) (*Aniba megaphylla*)	 Chrysophyllan 1A (Complex linkages: 7-1′, 8-0-2′) (*Licaria chrysophylla*)

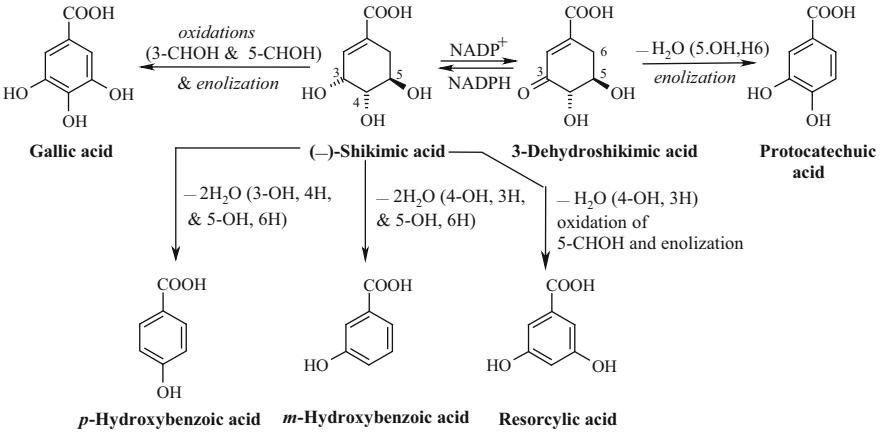


Fig. 13.17 Biosynthetic sequence of some common phenolic acids from shikimic acid

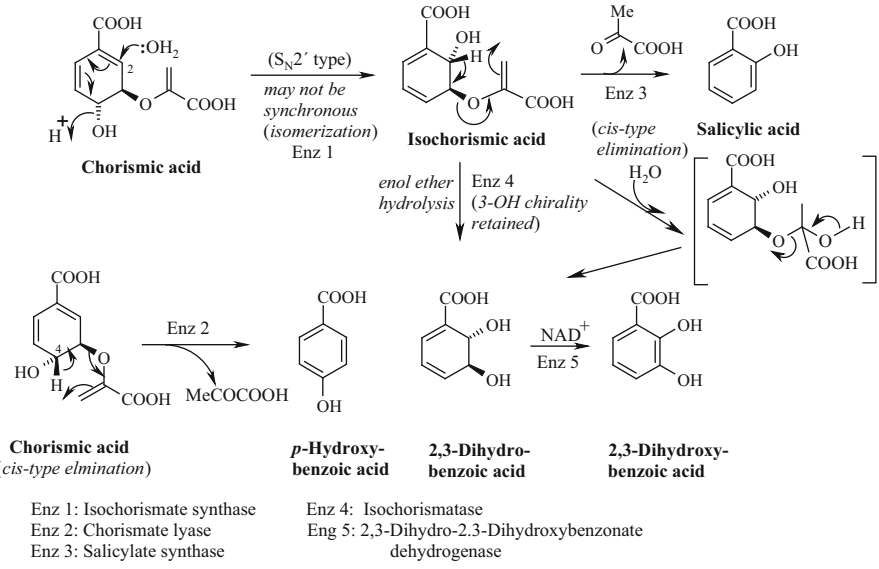


Fig. 13.18 Biosynthesis of some phenolic acids from chorismic acid and isochorismic acid

but the other two aromatic acids are found in microorganisms. Chorismic acid undergoes S_N2'-type nucleophilic α-attack by H_2O at C2 leading to the elimination of α-OH at C4 (this step in the presence of enzyme may not be synchronous) producing isochorismic acid. The latter undergoes *cis*-elimination of 2β-H and pyruvic acid, probably via a 6-membered cyclic transition state, to produce **salicylic acid.** Likewise, chorismic acid undergoes *cis*-type elimination of 4β-H and pyruvic

acid to form ***p*-hydroxybenzoic acid**. Again, isochorismic acid loses the C_3-unit as pyruvic acid by enol ether hydrolysis via Markonikov addition of H_2O at the double bond (retaining the 3-O bond chirality), followed by 2,3-dehydrogenation to produce **2,3-dihydroxybenzoic acid**.

13.1.8 (–)-Shikimic Acid As A Synthon: Synthesis of Oseltamivir Phosphate

Influenza, a viral disease, can be treated by inhibiting the activity of neuraminidase enzymes of influenza virus. The drug/prodrug blocks the release of this virus from the infected cells. Oseltamivir phosphate (GS-4104), a prodrug for influenza, was discovered at Gillead Sciences (GS); its ester group is hydrolyzed in vitro to form the free acid, which acts as the drug.

Oseltamivir was used successfully to combat the influenza epidemic caused by H5N1 (hemagglutinin subtype 5) and neuraminidase (subtype 1) virus strain ("*bird flu*") in Southeast Asia in 2005.

Oseltamivir discovered by Choung U. Kim and his collaborators at Gilead Sciences in 1995 was first synthesized from (–)-shikimic acid via an epoxy compound, the key intermediate of the process. This intermediate was converted to oseltamivir phosphate (generic name) (*tamiflu*®—trade name), involving costly azide chemistry. Later on, the prodrug has been synthesized by several groups.

Initially sources of shikimic acid was limited and hence naturally occurring (–)-quinic acid (Fig. 13.4) was used by some groups as the starting material. However, later on, (–)-shikimic acid was available in large scale from the extracts of Chinese *star anise* (*Illicium verum*) (Illiciaceae) or ginko leaves, as well as by fermentation using a genetically engineered *E. coli* strain.

Here we delineate the later semisynthesis of oseltamivir phosphate ("*tamiflu*") by Roche in Fig. 13.19, starting from (–)-shikimic acid via the key epoxy compound (without involving costly azide chemistry, as was done by earlier groups), to make the production cost-effective [1, 2].

The conversion of (**A**) to (**B**) (Fig. 13.19) was performed without isolation of the intermediates. The domino sequence as explained by the probable mechanistic pathway not so obvious, is quite interesting, and is shown in Fig. 13.20.

Currently, *tamiflu* is synthesized starting from (–)-shikimic acid and is marketed by Roche. However, (–)-shikimic acid is still a limited resource in comparison to the massive demand. Development of alternative synthetic approaches starting from simple materials has drawn extensive attention from the chemists and its synthesis from readily available starting materials with enhanced efficiency has become a challenge. Eight well-known research groups published its synthesis from simple starting materials, only in 2006 and 2007. The concise synthesis by the research group of Barry M. Trost published in 2008 required just eight steps from commercially available starting materials with an overall yield of 30 %, which to

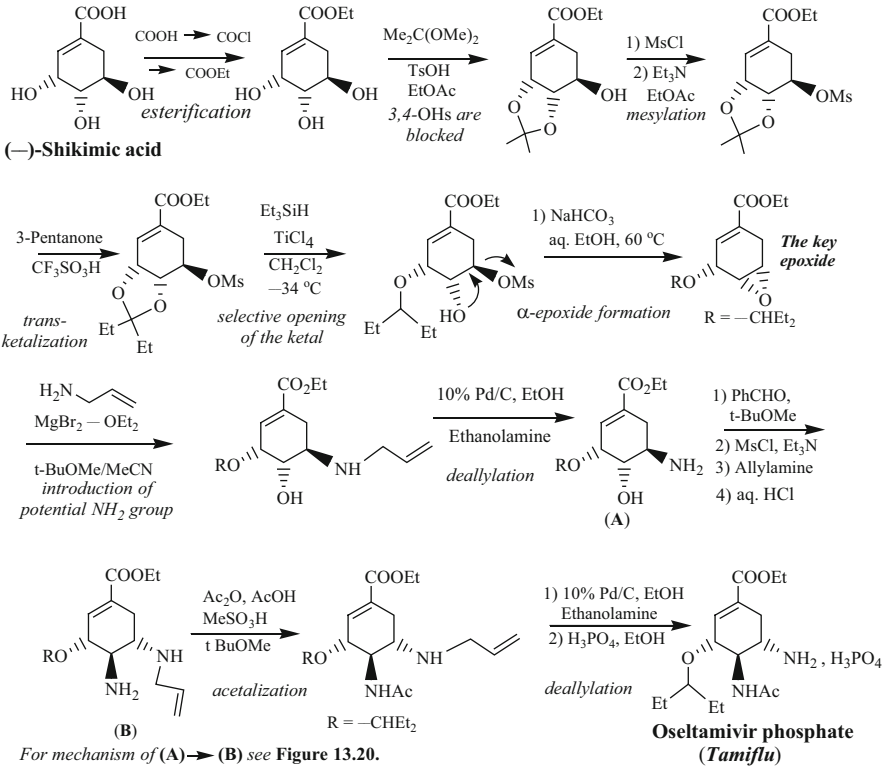


Fig. 13.19 Roche synthesis of oseltamivir phosphate (***tamiflu***®) from (–)-shikimic acid [1, 2]

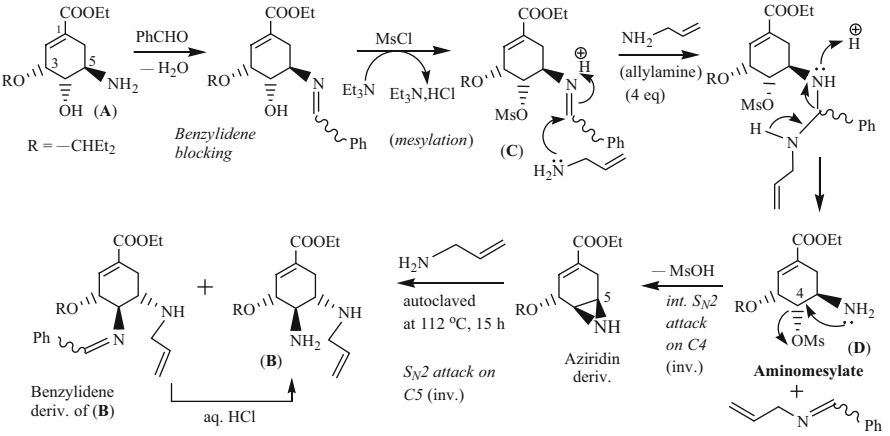


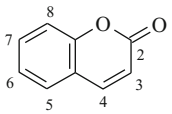
Fig. 13.20 Proposed mechanistic rationale for the domino reaction of compound (**A**) to form compound (**B**)

their knowledge represents the shortest synthesis to date. Very recently, an enantioselective synthesis of *tamiflu* has been disclosed [3]. However, the inclusion of these elegant syntheses is beyond the scope of this section.

13.2 Coumarins

13.2.1 General Introduction, Structure

Coumarins get their skeletal carbons (C_6–C_3) from *E*-cinnamic acid formed in the (–)-shikimic acid metabolic pathway in the plant cells. The biological steps needed for the conversion of *E*-cinnamic acid to coumarin has been discussed (Fig. 13.13).



Coumarin possesses the basic skeletal core structure 5,6-benzo-α-pyrone (≡ 5,6-benzo-2-pyrone ≡ 2*H*-chromene-2-one ≡ 2*H*-1-benzopyran-2-one ≡ 2*H*-benzo[b]-pyran-2-one). It may be conveniently regarded as the lactone of *cis-o*-hydroxycinnamic acid. It was first isolated by A. Vogel from *Tonka* beans as early as 1820 [4]. Tonka tree is locally known as *cumaru*/*coumarou* to *Tupis* who live in the valleys by the sides of several rivers, especially of Amazon in Brazil. Processed Tonka beans are used in tons every year for perfumery and flavoring. Natural forests of this tree exist in Venezuela, Colombia, the Guianas, and Brazil, particularly along tributaries of the Ornico River in Venezuela where they reach a height of 100-150 ft. [4]. The botanical name of the plant *Coumarouna odorata* (Syn. *Dipteryx odorata*) is derived from its native name, and the entire class of compounds bearing the basic skeleton of coumarin is known as coumarins.

Prior to the isolation of the simple coumarin, another coumarin, named daphnin, was isolated from *Daphne alpina* (Thymelaeaceae) in 1812. However, its structure, not known at that time, was elaborated to be 8-hydroxy-7-0-β-D-glucosylcoumarin [5–9] in 1930. Coumarins are widely distributed in plants and are prevalent in the plants belonging to Rutaceae, Umbelliferae (Apiaceae), Compositae (Asteraceae), Leguminosae, and Moraceae families. A number of review articles and a book on natural plant coumarins have appeared [5–9]. Plants elaborate mono- to poly-oxygenated coumarins, of which the most common one is umbelliferone (=7-hydroxycoumarin). In fact, umbelliferone is often regarded as the parent, in both structural and biogenetic sense, of a vast majority of the structurally more complex coumarins having an oxygen atom at the 7-position. The interesting part of coumarin substitution is the occurrence of one or more C_5-units arising out of

γ,γ-dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP). These two C_5-units amazingly undergo all possible structural modifications that one can chemically conceive of [5–9] (Fig. 13.21). They occur as side chains through C-alkylation, 3,3′-sigmatropic Claisen (Rainer Ludwig Claisen, 1851–1930, German Chemist) rearrangement, or as ether (O-alkylation) (Chap. 3). Quite often a C_5-unit is locked in the form of a ring. C_{10}-, C_{15}-Terpenoid chains as such or their modified units are also known to be present as side chain or as a part of a ring [5–9]. A few examples are shown in Fig. 13.21. Coumarins with umbelliferone skeleton include extensive series of C_{10}- and C_{15}-terpenoid ethers. Dihydrofuranocoumarins and dihydropyranocoumarins are more frequently found

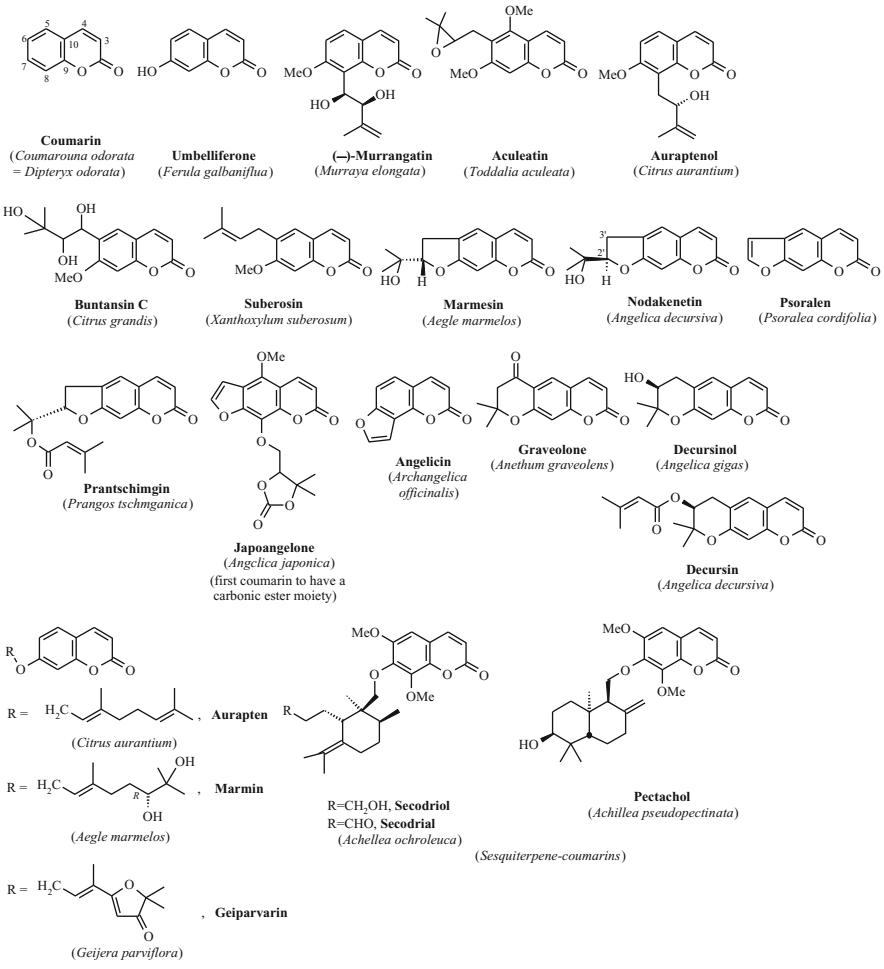


Fig. 13.21 Some natural coumarins with varied types of structural modifications of C_5, C_{10}, or C_{15} side chains

in Umbelliferae/Apiaceae, compared to Rutaceae, while psoralene, a linear furanocoumarin frequently occurs both in Rutaceae and Umbelliferae plants. Angelicin, an angular furanocoumarin, occurs more frequently in Umbelliferae plants [10]. *It has been shown by labeling experiment that the prenyl part of the prenylated umbelliferone originates via the non-mevalonate pathway* [11].

13.2.2 Spectral Properties of Some Natural Coumarins

IR and UV: Coumarins typically exhibit a 6-membered α,β-unsaturated lactonic carbonyl absorption (strong) peak at ~1725 cm^{-1} in their IR spectra Little variation of this peak is sometimes caused by the substitution on the coumarin nucleus. Coumarins absorb ultraviolet light at ~320 nm (log ε ~4) [5–9] due to the α,-β-unsaturated-δ-lactonic carbonyl. All coumarins exhibit strong absorption in the UV region. Upon addition of a few drops of a dilute alkali solution to a solution of a coumarin having phenolic OH at C5 and/or C7 position (e.g., umbelliferone), the UV absorption for this carbonyl undergoes significant bathochromic shift due to the formation of the corresponding phenolate anion stabilized by resonance with the carbonyl function. Linear furanocoumarins display UV absorption maxima at 325–350 nm, but angular furanocoumarins lack this absorption [12]. This has been widely used to identify the furanocoumarins, prior to the advent of ^{1}H NMR spectroscopy.

^{1}H and ^{13}C NMR: When the pyrone part of the coumarin nucleus is unsubstituted, H3 and H4 appear as a pair of clean doublets at (δ 61–6.4) and (δ 7.3–8.5), respectively, with coupling constants (9.5–10 Hz) in their ^{1}H NMR spectra. The above ranges cover the substitution effect (e.g., a OCH_3 at C5 causes the downfield shift of H4 because of the C5–O bond anisotropic effect appears at a lower field). An electron releasing substitution at C7 will be responsible for an upfield shift of H3 because of the resonance effect. The coupling constant of furan protons (H2′ and H3′) is around 2 Hz, close to that of meta-coupled protons, while the coupling constants of *ortho* protons lie between 8 and 9 Hz.

The ^{1}H and ^{13}C NMR spectral data [13, 14] for a few common coumarins are given in Fig. 13.22. Thus, the ^{1}H NMR spectra allows one to identify the linear or angular furanocoumarins unambiguously.

Mass fragmentation pattern depends on the substitution. However, elimination of carbon monoxide takes place from the pyrone ring and in cases of O-prenyl ethers, prenyl groups are eliminated leaving the phenol. In fact O-prenylated simple coumarins when sublimed under high vacuum using a liquid nitrogen trap lead to the formation of the corresponding phenolic coumarins. Further, in cases of polyoxygenated coumarins, the predictability of the mass fragmentation pattern is low.

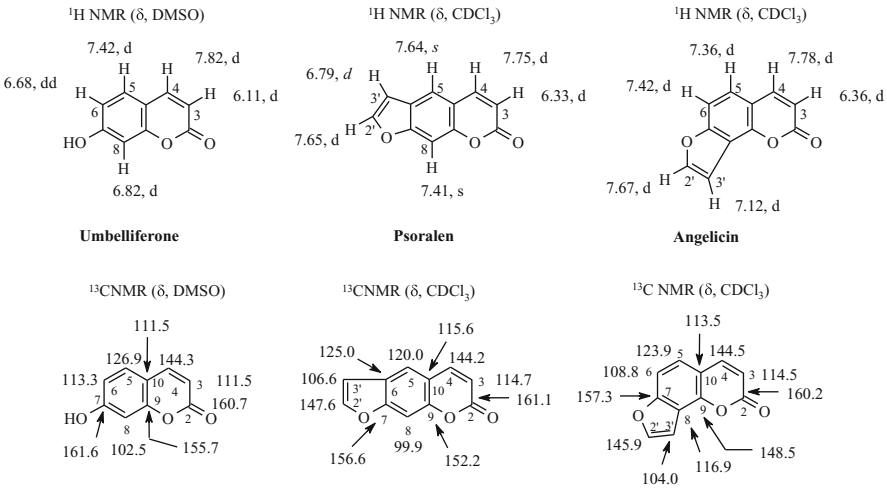


Fig. 13.22 Chemical shift values in the ^{1}H NMR spectra [7] and the ^{13}C NMR spectra [10] of some coumarins

13.2.3 Synthesis: Coumarin, Substituted Coumarins, 4-Hydroxycoumarins, and Dihydrocoumarins

Initially, a few generalized syntheses of coumarins were known in the literature involving Perkin (Sir William Henry Perkin, a British chemist, 1838–1907), Knoevenagel (Emil Knoevenagel, a German chemist, 1865–1921), Reformatsy (Sergius Reformasky, a Russion chemist, 1860–1934), and von Pechmann (Hans von Pechmann, a German chemist 1850–1902) reactions.

However, in view of the ever-increasing applications and uses of coumarins (Sect. 13.2.6) much efforts have been given to the synthesis of variously substituted coumarins and to evolve new as well as modified methodologies for their synthesis.

13.2.3.1 Perkin Reaction

Coumarin was first synthesized by Perkin using salicyldehyde and acetic anhydride in the presence of sodium acetate. This reaction is an example of aldol condensation, and the reaction is known as Perkin reaction [15]. The probable mechanism is shown in the Fig. 13.23.

13.2.3.2 Knoevenegal Reaction

A simple one-pot synthesis of coumarins has been developed using dipotassium *o*-methoxybenzylidenemalonates in 60–80 % yields [16]. The malonates are prepared

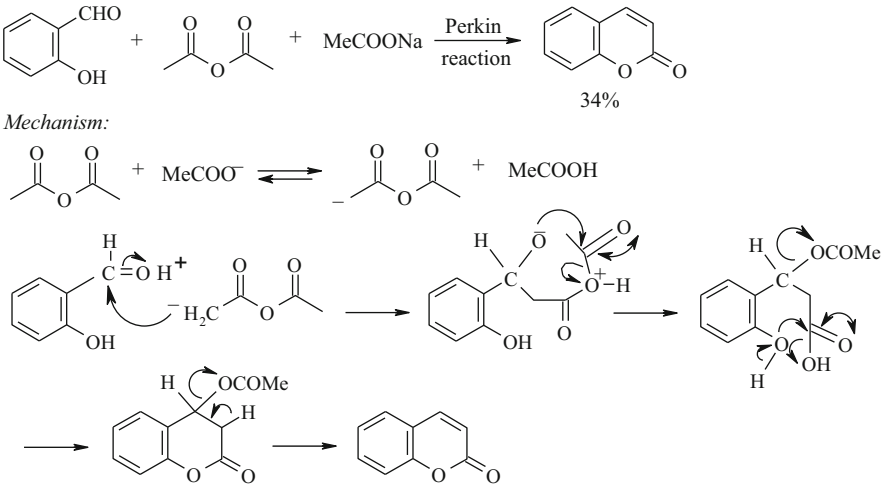


Fig. 13.23 Synthesis of coumarin (Perkin reaction) (1869)

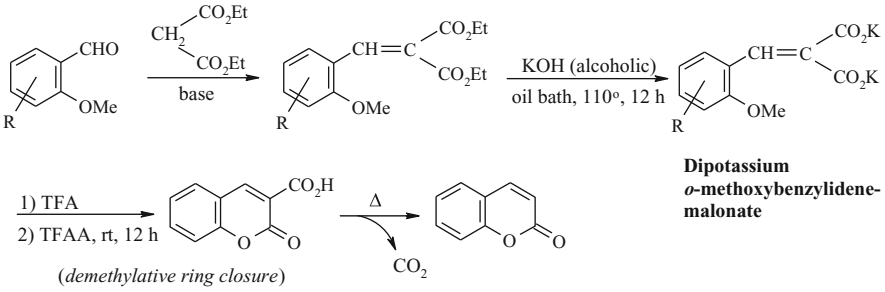


Fig. 13.24 Synthesis of coumarin using Knoevenagel reaction (1891)

from suitable *o*-methoxybenzaldehyde and diethylmalonate under Knoevenagel condition (Fig. 13.24).

13.2.3.3 Pechmann Reaction [17–19]

This widely supplied method uses phenol, ethyl acetoacetate, and 75 % H_2SO_4 (Fig. 13.25). The latter serves both as a solvent and a condensing agent. The condition is drastic and the yield depends on substitution of phenol and sometimes on β-ketoesters.

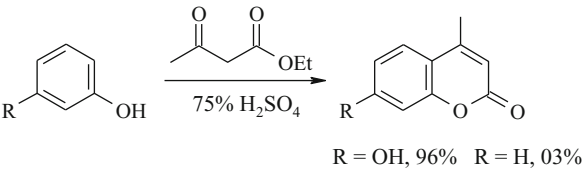


Fig. 13.25 Synthesis of 4-substituted coumarins by von Pechmann reaction (**1884**)

13.2.3.4 Modified Pechmann Methods

Through years Pechmann catalyst has been modified with an aim to improve the yield, independent of substitution effect. A number of Pechmann catalysts has been reported in the literature as follows:

I. In one such modification, Lewis acidic chloroaluminate ionic liquid has been employed [20] in place of conventional acid catalyst (Fig. 13.26). The ionic catalyst plays a dual role in serving also as solvent. The time period for the reaction is dramatically reduced. The most interesting part of this reaction is the formation of coumarin from phenol in a fairly good yield (40 %) compared to the original Pechmann method (03 %).

II. Pechman reaction between seven phenols and methyl acetoacetate in the SO_3H-functionalized trifluoromethane sulfonate imidazolium ionic liquid [MBsIm][CF_3SO_3] under solvent-free condition has been reported to give the corresponding seven 4-methylcoumarins in 61–95 % yield [21]. The condensation of substituted resorcinols and methyl acetoacetate in this ionic liquid to form substituted 4-methylumbelliferones in 90–95 % yields is shown in Fig. 13.27. Of the four non-chloroaluminate acidic ionic liquids studied this ionic liquid has proved to be the most active catalyst. No reaction is observed in the absence of an ionic liquid [21].

III. In another modification sulfamic acid, H_2NSO_3H (SA), a mild solid acid has recently been used as a catalyst. Sulfamic acid is present as zwitterions units ($H_3N^+ - SO_3^-$) and is known to effect transesterification. The initial step in the reaction is transesterification when the phenolic ester is formed out of phenol and ethyl acetoacetate; the phenolic ester then undergoes intramolecular cycloadditon (Fig. 13.28). The best result is obtained when SA:resorcinol (phenol):ethyl acetoacetate are taken in the ratio (0.5:1.0:1.0 molar ratio). The synthesis of seventeen coumarins by this method has been reported (60–90 % yields, 20–80 min) [22].

IV. $BiCl_3$ has been used as a Pechmann catalyst in the synthesis of 4-substituted coumarins in excellent yields and in solvent-free condition [23] (Fig. 13.29).

Pechmann reaction also uses *p*-TsOH [24] in a solvent-free condition of coumarin synthesis (Fig. 13.30).

V. Zirconyl chloride octahydrate [25] (1 mol%) has been used as a Pechmann catalyst either neat or in a small volume of ethanol as solvent, for synthesis of coumarins in moderate to good yields (Fig. 13.31). Ten compounds were reported.

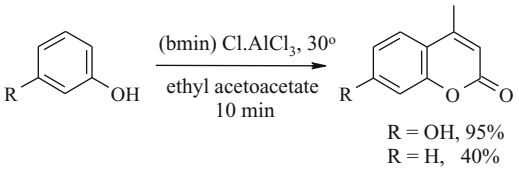


Fig. 13.26 Synthesis of 4-substituted coumarin by modified Pechmann method (i) (**2001**)

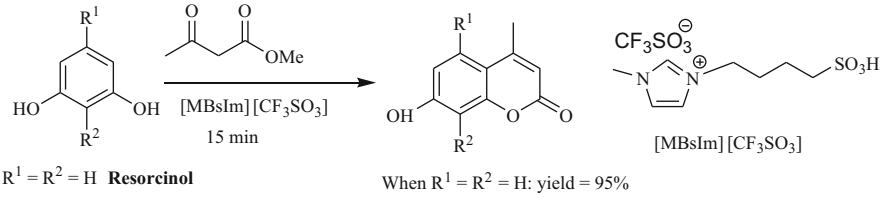


Fig. 13.27 Synthesis of 4-substituted coumarins by modified Pechmann method (ii) (2005)

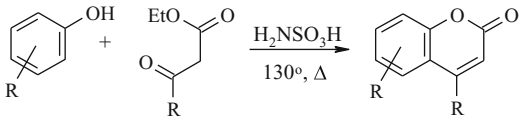


Fig. 13.28 Synthesis of 4-substituted coumarins by transesterification method (iii) (**2004**)

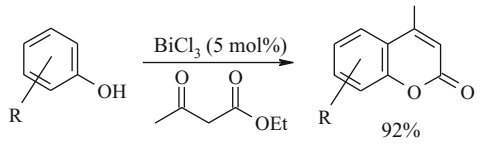


Fig. 13.29 Synthesis of 4-substituted coumarins by using $BiCl_3$ as a Pechmann catalyst (**2005**)

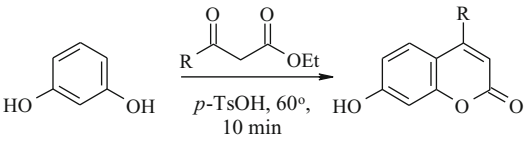


Fig. 13.30 Synthesis of 4-substituted coumarins by using TsOH as a Pechmann catalyst (**2002**)

VI. An efficient synthesis of 7-aminocoumarins was carried out by microwave irradiation on the reactants (m-aminophenol and methyl-2-ketosuccinate) on solid support (graphite/montmorillonite K10) [26] (Fig. 13.32). Graphite microwave interaction causes strong thermal effect which is associated with the acidic catalyst role of clay.

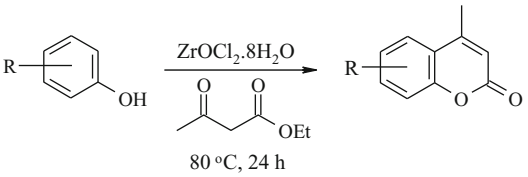


Fig. 13.31 Synthesis of 4-substituted coumarins using zirconium catalyst (**2006**)

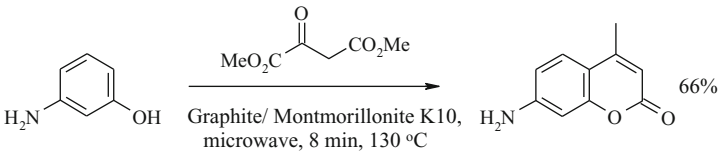


Fig. 13.32 Synthesis of substituted coumarin by modified Pechmann method on solid support (**2001**)

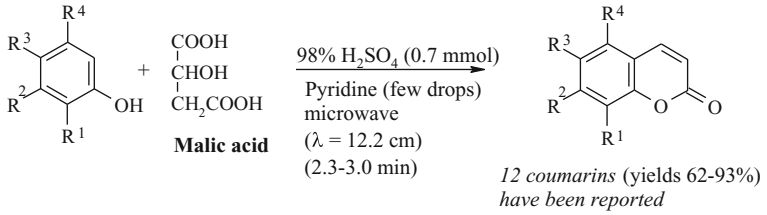


Fig. 13.33 Synthesis of coumarins by a microwave-assisted method

VII. A simple, quick, and advantageous microwave-assisted synthesis of coumarins with variously substituted benzene ring and unsubstituted pyrone ring has been achieved [27]. The reaction is generalized in Fig. 13.33. Twelve compounds have been synthesized by this method.

VIII. A novel one-pot synthesis of coumarins has been reported by B.M. Trost in 1996. Phenols on treatment with a propiolate in the presence of Pd(0) as the catalyst forms coumarins in a one-pot reaction in good yields [28]. This is also a modification of the Pechman condensation. The major drawbacks of the latter stem from the requirement of stoichiometric amounts of strong Brönsted or Lewis acids at high temperature, thus frequently limiting its scope. The present modified method involves the replacement of the typical β-ketoester with an alkynoate and use of Pd(0) catalyst in mild condition (Fig. 13.34).

Compounds (**6**), (**9**), (**11**) perform poorly in Pechmann condensation. The electrophilic addition of Pd on phenol initiates the reaction (*method A*). In *method B* formic acid reduces Pd(+2) to Pd(0) species which serves as the actual catalyst. The aryloxypalladium hydride undergoes *cis*-addition to the alkyne to produce *E*-cinnamic esters; their known ease of E → Z

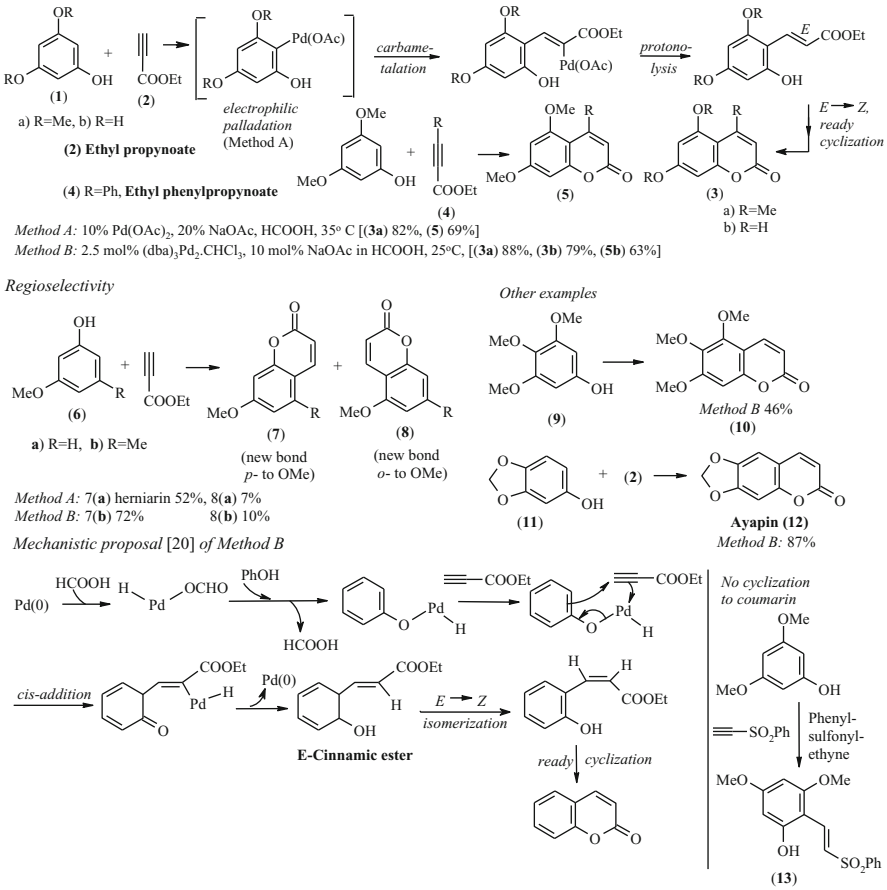


Fig. 13.34 A novel mild method of synthesis of coumarins by Pd(0)-catalyzed addition (Trost) (**1996**)

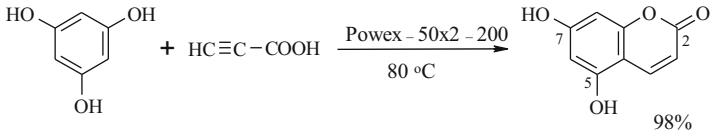


Fig. 13.35 A convenient synthesis of 5,7-dihydroxycoumarin (**1999**)

isomerization followed by ready cyclization accounts for the formation of coumarins rather than the E-cinnamates. When phenylsulfonylethyne is used instead of (**2**), the expected *E*-alkene derivative (**13**) which cannot cyclize is obtained.

IX. A microwave-assisted solid acid catalyzed rapid and environmentally friendly synthesis of 5,7-dihydroxycoumarin in excellent yield has been reported [29] (Fig. 13.35).

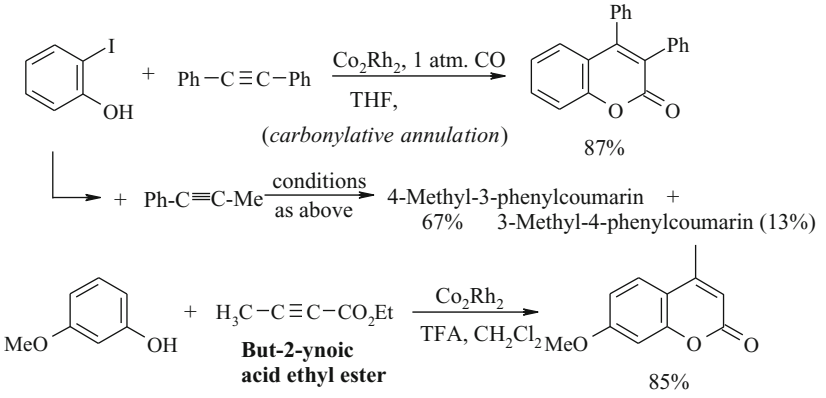


Fig. 13.36 Synthesis of substituted coumarins by the Co_2Rh_2 nanoparticles-catalyzed annulation of alkynes (**2004**)

13.2.3.5 Use of Nanoparticles as Catalysts

In view of the rapid development of transition metal nanoparticles, their uses as catalysts in the various synthetic methodologies have been realized. Recently, cobalt-rhodium (Co_2Rh_2) heterobimetallic nanoparticles catalyzed method for the synthesis of coumarins has been reported [30] (Fig. 13.36). The catalyst could be recovered and reused for nearly five times keeping its catalytic activity intact. This catalyst produces coumarins in very good yield (79–89 %) from phenols and but-2-ynoic acid ethyl ester (Fig. 13.36).

13.2.3.6 A Versalile, High-Yield Coumarin Synthesis via Claisen Rearrangement

H. Rapoport developed [31] a new, versatile, high-yield synthesis of coumarin by application of Claisen rearrangement of allyl or propargyl ethers. The steps are outlined in Fig. 13.37. In this synthesis two oxidative steps, *viz*., (i) oxidation of the hemiacetal to a lactone and (ii) dehydrogenation to an α.β-unsaturated δ-lactone system are involved, which reduced the yield.

One oxidation step was eliminated by using α,α-dioxygenated allyl aryl ether as the substrate for Claisen rearrangement. The ultimate C2 of the coumarin synthesized would thus be at the correct oxidation stage. Thus, triethyl orthoacrylate and guaicol were refluxed in toluene containing a catalytic amount of propionic acid to give the 2,2-diethoxychroman by Claisen rearrangement of the intermediate α,-α-diethoxyallyl aryl ether (Fig. 13.38), formed by exchange between the acyclic orthoester and the phenol. The chroman upon hydrolysis with 10 % aq. HCl followed by Pd/C dehydrogenation produced 8- methoxycoumarin in 71 % overall yield. This new route to coumarin was applied to *o*-cresol (**4**), 2-methyl-4-

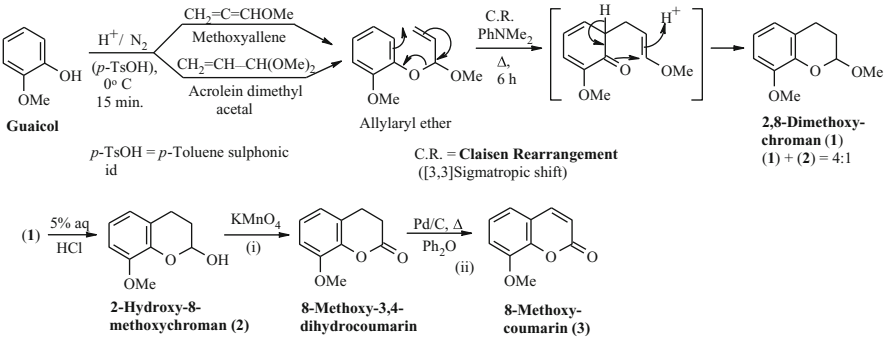


Fig. 13.37 8-Methoxycoumarin (**3**) synthesis starting from guaicol (Rapoport) (1982)

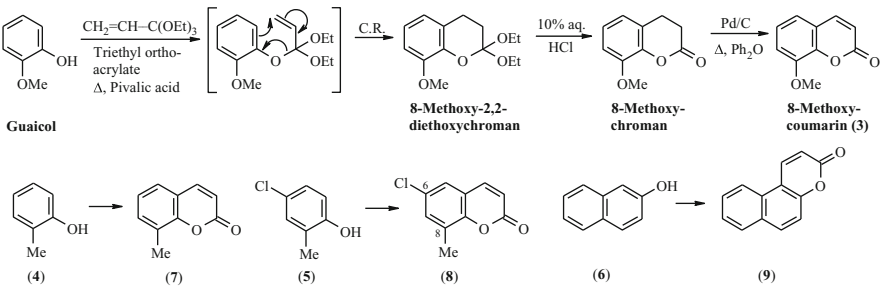


Fig. 13.38 Synthesis of coumarins from phenols and triethyl orthoacrylate (Rapoport (1982)

chlorophenol (**5**), and β-naphthol (**6**) (which give essentially no coumarin in the Pechmann reaction) to afford the corresponding coumarins, 8-methylcoumarin (**7**), 6-chloro-8-methylcoumarin (**8**), and compound (**9**) (formed by cyclization at the *peri* position) in 76 %, 50 %, and 65 % overall yields, respectively (Fig. 13.38).

In order to eliminate the final oxidation (dehydrogenation) step, a substrate with a three-carbon side chain at a higher oxidation state (a propiolate instead of an acrylate) was brought in. Thus, the coumarin is formed with the right oxidation state at C2 as well as at C3 and C4. For such a substrate, triethyl orthopropiolate was selected for the C_3-unit of the coumarins (Fig. 13.39). Guaicol upon refluxing with triethyl orthopropiolate in toluene containing pivalic acid gave products (**10**) and (**11**) along with 8-methoxycoumarin (**3**). Refluxing in *p*-cymene (160 ° C) containing pivalic acid produced a mixture of (**3**) and (**11**), which could be further converted to (**3**), the overall yield of which was 73 %. Thus, use of the propiolate simplified the synthesis by eliminating both the oxidation steps (i) and the dehydrogenation step (ii) (see Fig. 13.37) and increasing the yield [31].

This method was readily extended to the synthesis of 4-substituted coumarins by using triethyl 2-butynoate (obtained by treatment of triethyl orthopropiolate with BuLi and MeI) instead of the propiolate. Treatment of guaicol with triethyl-2-butynoate in refluxing *p*-cymene (160°) containing pivalic acid produced 75 % of

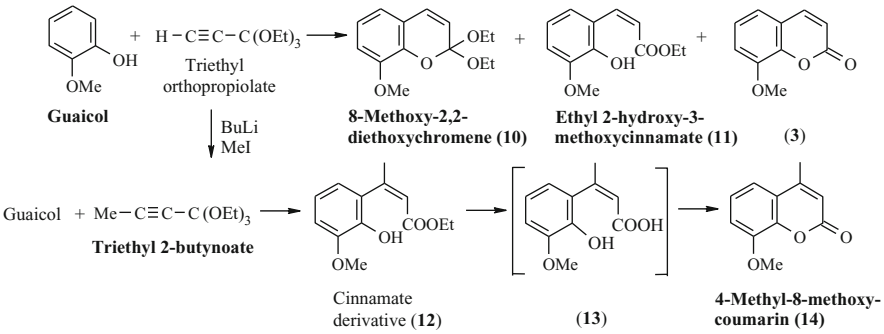


Fig. 13.39 Synthesis of coumarins from phenols and triethyl orthopropiolates Rapoport (1982)

(**12**) which was converted to (**14**) via (**13**) in 49 % overall yield (Fig. 13.39) [31]. This new coumarin synthesis appears to be a general method applicable to a range of substitution pattern and groups. The methods proceed from readily available starting materials and give coumarins with various substitutions in the benzene and pyrone nuclei [31].

13.2.3.7 Some Other Methods of Coumarin Synthesis

(a) A new method for the synthesis of coumarins has been developed using a suitable bulky aryl cerium reagent (**A**) [32], which reacts easily to enolizable *t*-butyl acetoacetate to yield an adduct. The latter in the presence of an acid undergoes deblocking, deesterification, lactonization, and finally dehydration to generate coumarin 3,4-double bond (Fig. 13.40).
(b) A general procedure is reported where a suitable aryllithium is generated by metalation of a protected phenol with *n*-butyllithium and is allowed to add in a conjugated manner (Michael addition) to diethyl ethoxymethylenemalonate [33]. On acid hydrolysis the addition product generates the coumarin derivative (Fig. 13.41). This method is an important compliment to the traditional Pechmann synthesis, since it gives an isomeric coumarin.

13.2.3.8 Synthesis of 4-Hydroxycoumarins

(a) C-Carbonylation of 2′-hydroxyacetophenones with CO in the presence of sulfur and bases afforded 4-hydroxycoumarins (4-hydroxy-2-oxo-2*H*-1-benzopyrone) in good to excellent yields. This is the first example of a *sulfur-assisted C-carbonylation with CO* (Fig. 13.42) [34]. 4-Hydroxycoumarins are important synthons for biologically active molecules (Sect. 13.2.9.5).
(b) Similar carbonylation of 2′-hydroxyacetophenones has also been studied with CO and Se to give 4-hydroxycoumarins in quantitative yield (Fig. 13.43) [35].

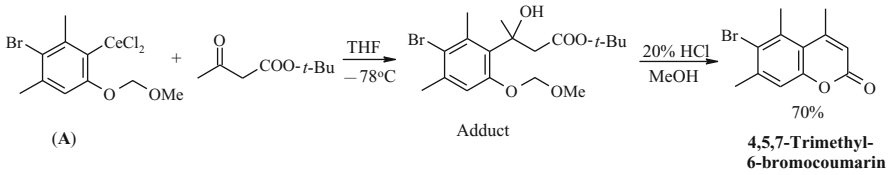


Fig. 13.40 Synthesis of 4,5,7-trimethyl-6-bromocoumarin using a bulky aryl cerium reagent [29] (1989)

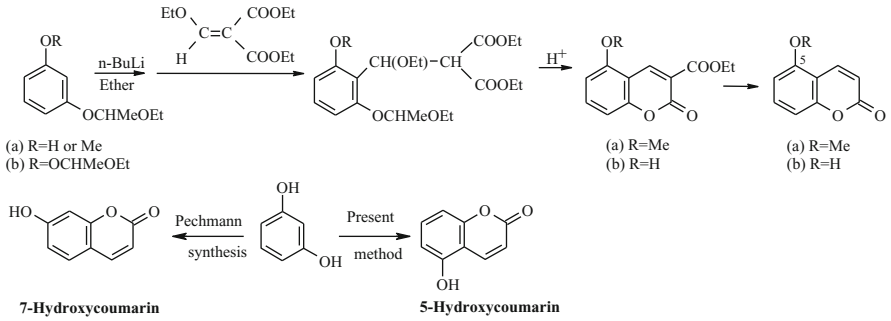


Fig. 13.41 Synthesis of 5-hydroxycoumarin

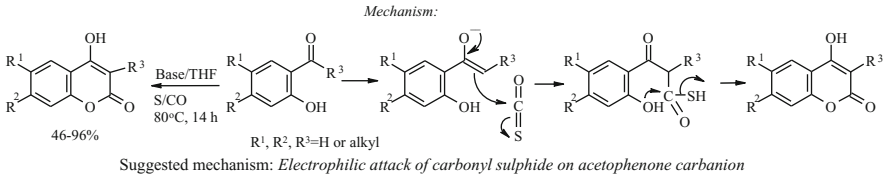


Fig. 13.42 Facile synthesis of 4-hydroxycoumarin by S-assisted carbonylation

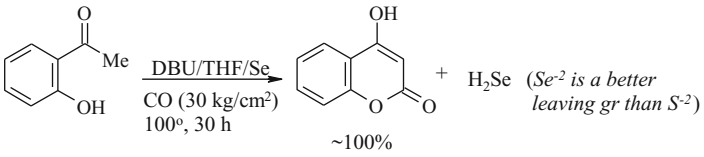


Fig. 13.43 Facile synthesis of 4-hydroxycoumarins by Se-assisted carbonylation

13.2.3.9 Synthesis of Dihydrocoumarins (Chroman-2-Ones)

It is difficult to hydrogenate the coumarin double bond (C3=C4) exclusively to yield dihydrocoumarin by traditional catalytic hydrogenation. During double bond hydrogenation most of the coumarins undergo hydrogenolysis (opening of the

lactone ring with or without reduction of the double bond) and the yield is not appreciative. An extensive review [36] on reduction of coumarin double bond with an aim to synthesize chroman-2-ones (dihydrocoumarins) has been reported. A few methods are stated below.

(a) A number of dihydrocoumarins has been synthesized in good to excellent yield by a gold (III) catalyzed functionalization [37] of aromatic C–H bond with primary alcohol triflate or methane sulfonate esters by constructing a C–C bond (Fig. 13.44).
(b) Coumarins with an EWG (Electron withdrawing group) at C3 have been converted to the corresponding dihydrocoumarin by hydroboration [38] (Fig. 13.45).
(c) Hantzsch 1,4-dihydropyridine [39], used as a model compound of coenzyme NADPH, has been employed for the selective reduction of 3,4-double bond of coumarin containing an EWG (E) at C3 (Fig. 13.46).
(d) One-pot synthesis of a number of substituted dihydrocoumarins involving Michael type addition followed by lactonization has been reported

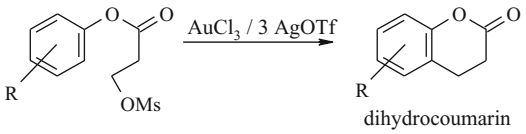


Fig. 13.44 Gold (III) catalyzed C-C bond formation leading to dihydrocoumarins

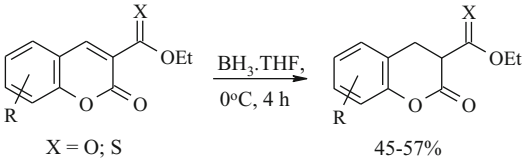


Fig. 13.45 Reduction of coumarins with EWG at C3 to dihydrocoumarin by hydroboration

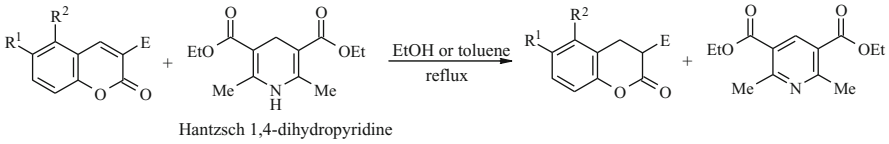


Fig. 13.46 Coumarin containing a EWG at C3 to dihydrocoumarin by reaction with Hantzsch 1,4-dihydropyridine

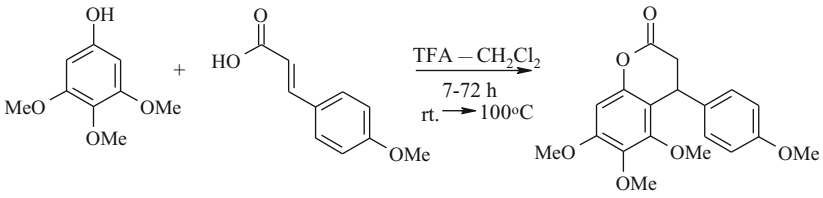


Fig. 13.47 Synthesis of 4-*p*-anisyl-5,6,7-trimethoxycoumarin

(Fig. 13.47) [40]. Nine substituted dihydrocoumarins have been synthesized, using different substituted phenols and substituted *E*-cinnamic acids (42–99 % yield).

(e) In Rapoport's synthesis of coumarins [31] at the penultimate step (Figs. 13.37 and 13.38), dihydrocoumarins are formed.

Several common chiral and achiral coumarins, e.g., *demethylsuberosin, xanthyletin, (±)-decursinol, (+)-marmesin, (–)-nodakenetin (the enantiomer oif marmesin), (±)-decursin, and (±)-prantschimgin* have been *synthesized* [41].

13.2.4 Reactions of Coumarins

Reaction with a Base A coumarin dissolves in an alcoholic KOH solution (5 %) to form a yellow colored solution from which the coumarin could be regenerated by acidification with dilute acid to give a colorless suspension. This observation involves the opening of the lactone and relactonization (Fig. 13.48) and serves as the characteristic test for most of the coumarins.

This color reaction can be carried out with a minute amount of a coumarin taken in a capillary tube and mixing it with an alcoholic KOH solution and then with dilute HCl taken in separate capillary tubes.

Reduction The coumarin double bond (at C3, C4) undergoes reduction under various conditions to give chroman-2-ones on which a review [36] has recently appeared. A few methods have been mentioned briefly in Sect. 13.2.3.9 (Figs. 13.45 and 13.46).

Addition Reactions Coumarin reacts with Br_2 in AcOH/$CHCl_3$ to give a 3,4-dibromo derivative through a bromonium ion intermediate formed by the electrophilic attack of bromine on the 3,4-double bond. It also behaves as an electrophile in its reaction with a Grignard reagent to form both 1,2 (at C=O) and 1,4 (at C4 and carbonyl like Michael reaction) addition products. A mild carbon nucleophile like $^{(-)}$CN of HCN adds on the C4 of the coumarin (like Michael reaction).

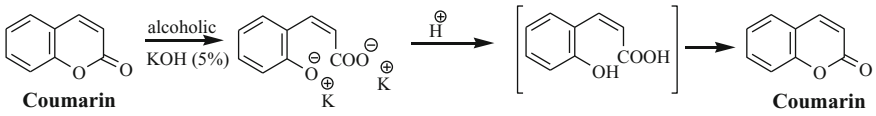


Fig. 13.48 Reaction of coumarin with KOH followed by acidification

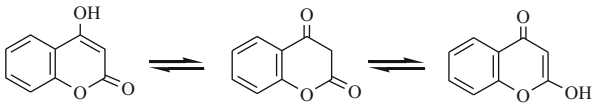


Fig. 13.49 Unstable tautomers of 4-Hydroxycoumarin

13.2.5 Reactions of 4-Hydroxycoumarin with α,β-Unsaturated Carbonyls [42]

4-Hydroxycoumarin (**1**) is endowed with both nucleophilic and electrophilic properties. A few reactions of 4-hydroxycoumarin (**1**) with active methylene compounds like α,β-unsaturated ketones, acids, and esters and the probable pathways for the formation of the products are delineated in Fig. 13.50. All products were characterized by their ^{1}H and ^{13}C NMR and mass spectral data [42]. 4-Hydroxycoumarin furnishes the dimeric coumarin derivative (**2**) in a pyridine catalyzed self-condensation process (*Reaction 1*). Pyridine catalyzed condensation of (**1**) with ethyl acetoacetate gives a tricyclic dilactone (**3**) (*Reaction 2*). On the contrary, morpholine, being a better nucleophile than pyridine, when refluxed in N_2 atmosphere with 4-hydroxycoumarin (**1**) gives an enamine derivative, 4-morpholinyl coumarin (**4**) (*Reactioin 3*), but no self-condensation dimeric product. This indicates the existence of its less stable tautomer, 2,4-chromandione (1a) (Fig. 13.49), in the equilibrium.

However, ^{13}C NMR spectrum of (**1**) does not show the presence of the tautomers (1a) or (1b), as it shows the absence of any downfield carbonyl beyond coumarin carbonyl (~δ 160–162). Perhaps the population of (1a) and (1b) in the above equilibrium is too less to be detected under the condition of the spectral measurement. Alternatively, morpholine being a good nucleophile may undergo Michael addition to C4 of (**1**) forming (**4a**), which undergoes loss of elements of water to form the enamine (**4**).

As has been shown in Fig. 13.50, the formation of the dimeric coumarin (**2**) may be rationalized by entailing the participation of the tautomer (**1a**) or alternatively by Michael addition of C_3 anion (generated by abstracting the proton of 4 OH) as nucleophile (of one molecule) to the electrophilic center C4 of the other molecule leading to the formation of (**2a**), the precursor of (**2**).

4-Hydroxycoumarin when refluxed in pyridine with other α,β-unsaturated carbonyls like acrylic acid, acetylacetone, mesityl oxide, and crotonic acid furnished the condensation products (**5**), (**6**), (**7**), and (**8**) (*Reactions 4, 5, 6 and 7*), respectively, in good yields. The probable pathways for their formation are also shown in the Fig. 13.50.

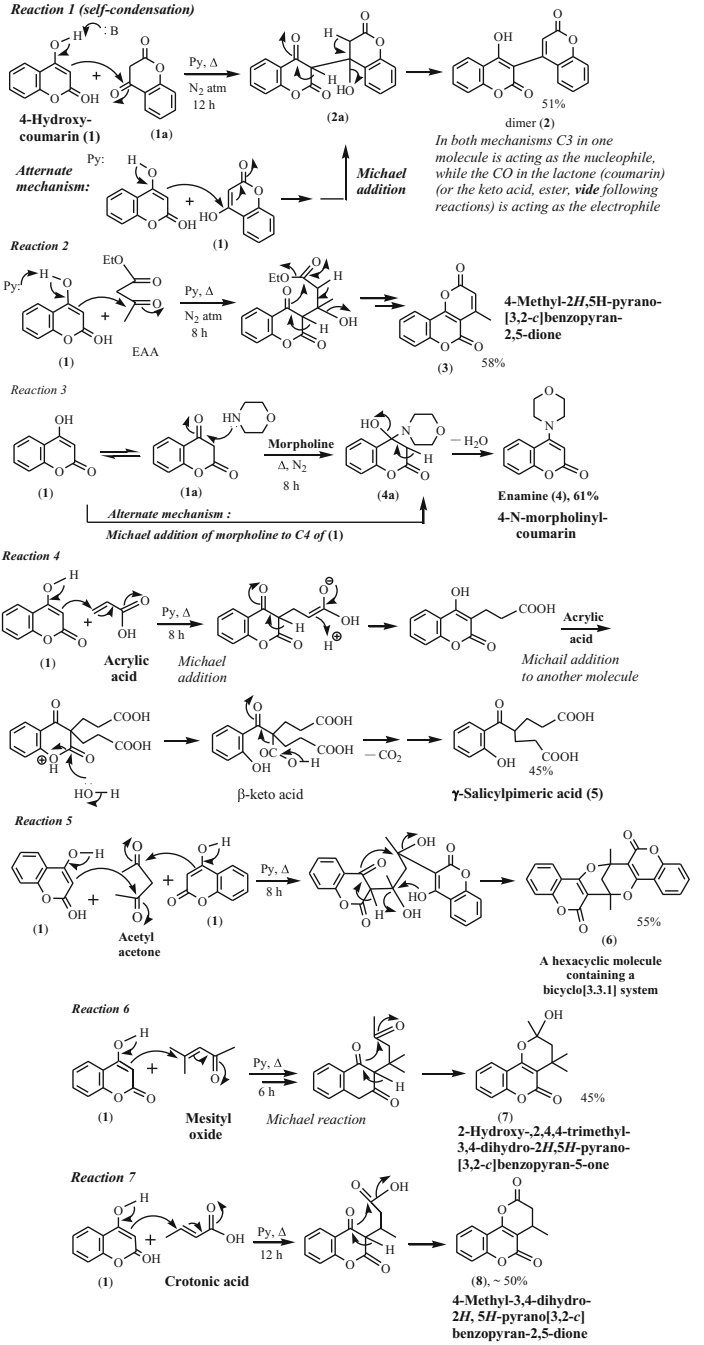


Fig. 13.50 Reactions of 4-hydroxycoumarin and their probable mechanistic pathways

13.2.6 Photochemical Reactions of Coumarins

13.2.6.1 [2 + 2]-Photodimeriztion in Solution

Photochemical reactions of coumarins in solution have been studied extensively; [2 + 2]-dimerization is the main pathway forming stereochemically and regioselectively different cyclobutane derivatives, depending much upon the experimental conditions (Fig. 13.51) *viz*., in polar and nonpolar solvents with or without sensitizer. Theoretically, such a cyclobutane may exist as an enantiomeric pair (±)-(**A**) (Pt. Gr. $\mathbf{C_1}$) and a meso (**B**) (Pt. Gr. $\mathbf{C_{1h}}$, or $\mathbf{C_S}$ having a plane of symmetry). The mass spectra of the products determine the dimeric structure. The molecular ion peaks are of low intensity as the dimers are prone to fragmentation to monomers and other entities. The relative stereochemistry and the absolute configuration at ring junctures have been determined from the ^{1}H NMR spectral data and X-ray crystallographic studies, respectively.

Activation of coumarins is caused by the energy transfer—a key step in photosensitized reactions. Hammond by mechanistic studies showed [43] that in nonpolar solvents, *e.g*., benzene, the excited singlet state coumarin interacts with ground state coumarin and gets self-quenched and forms *anti* head-to-head dimer (±)-(**A**) (Fig. 13.51). In polar solvents, *e.g*., ethanol, similar interaction leads to self-quenching and the *syn* head-to-head dimer (**B**) in low yield. In the presence of a triplet sensitizer like benzophenone, triplet coumarin is generated by energy transfer from the excited triplet sensitizer. In high dilution intersystem crossing is complete with self-quenching, and *anti* head-to-head dimer (±)-(**A**) is produced with high quantum yield. Even when most of the light is absorbed by coumarin

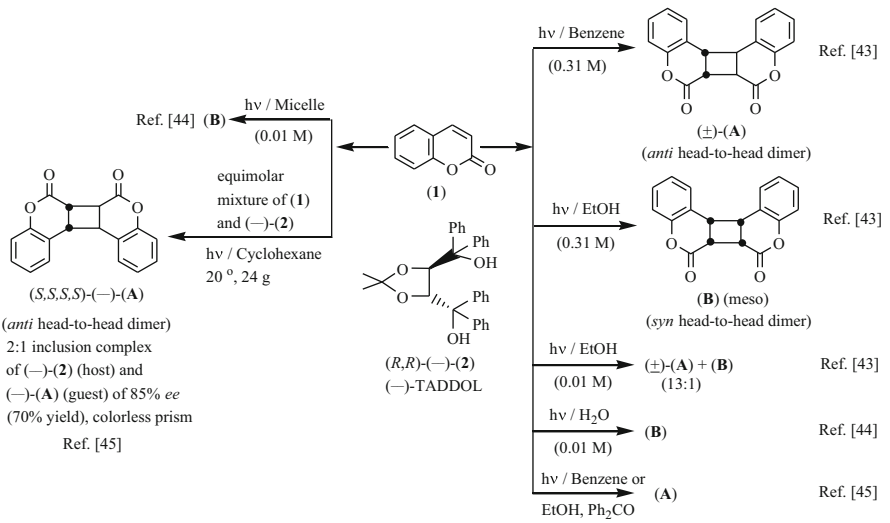


Fig. 13.51 Photodimerization of coumarin in solution (different conditions)

generating singlet coumarin, the latter transfers its energy to benzophenone to form benzophenone singlet. Upon intersystem crossing the energy is given by the sensitizer back to couamrin to generate triplet coumarin.

Coumarins dimerize more effectively in water and also in the presence of micelle with more quantum yield compared to organic solvents like benzene, methanol, etc. (Fig. 13.51). The enhanced reactivity and selectivity during dimerization might be due to the polarity of the solvents. The micelle provides a hydrophobic pocket with the bulk of water causing hydrophobic interaction and allowing the aggregation of coumarin molecules in water [44].

13.2.6.2 Photodimerization of Coumarins in the Presence of a Chiral Host [45]

Recently, it has been observed that enantioselective photodimerization of coumarin in the form of inclusion crystal with an optically active host, (*R*,*R*)-(–)-*trans*-4,5-bis (hydroxydiphenylmethyl)-2,2-dimethyl-1,3-dioxacyclopentane (**2**) also known as (–)-TADDOL (Fig. 13.51), could be achieved in a homogeneous solution when single-crystal-to-single-crystal interaction [45] takes place. Photodimerization reactions of coumarins are reversible in solvent. In the hydrocarbon solvent the complex of one enantiomer dimer with the host precipitates while the other remains in solution; it can be decomposed into the monomer and hence the reversibility of the process is arrested. The absolute stereochemistry of the product (–)-(**A**) has been determined to be (*S*,*S*,*S*,*S*) by literature comparison and X-ray crystallographic studies of the inclusion crystal.

13.2.6.3 Photodimerization in the Solid State

Photodimerization of cinnamic acid in the crystalline state is well known in the literature [46]. Thus, coumarins with potentiality for dimerization was made photoactive to dimerize in solid state by bringing the active C3=C4 double bond of the molecules of coumarin in favorable distance and disposition for interaction through controlled crystal packing geometry [47, 48]. It has been found that OMe, Me, etc. substituents are helpful in engineering the crystal packing suitable for photodimerization by displaying in-plane interstacking interactions in crystal lattice. Of several couamrins studied, only 7- and 8-methoxycoumarins and 6-chlorocoumarin when irradiated by UV light yielded corresponding dimers in 90, 50, and 40 % respectively. X-ray studies show that 7-methoxycoumarin dimerizes in *syn* head-to-tail fashion [47–49].

13.2.6.4 [2+2]-Photoaddition of Coumarin

Coumarins have been shown to undergo [2+2]-photoaddition in the presence of benzophenone with other mono-olefinic compounds [50] when no appreciable dimer was obtained (Fig. 13.52).

13.2.6.5 Light-induced Coumarin Cyclopentannelation [51]

A tricyclic compound (**3**) and a hitherto unknown tetracyclic system [(4-hetero) cyclopent[b,c]acenaphthylenes] (**5**) have been obtained from 4-cyano and 4-alk-1-ynylcoumarins when they are photochemically allowed to react with tetramethylethylene (**2**) (Fig. 13.53).

13.2.7 Thermal [2+2]Cycloaddition

Highly regio- and enantioselective thermal [2+2]-cycloaddition, *though forbidden according to Woodward Hoffmann rules*, took place with coumarin in crystalline inclusion complexes under high vacuum [52] (Fig. 13.54). In this inclusion

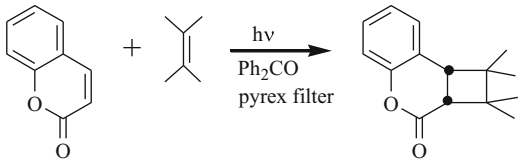


Fig. 13.52 [2+2]-Photoaddition of coumarin

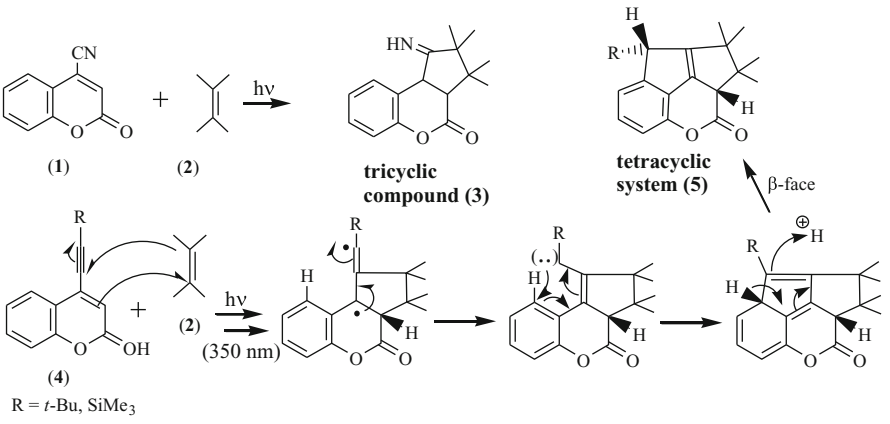


Fig. 13.53 Photochemical cyclopentannelation of some coumarins

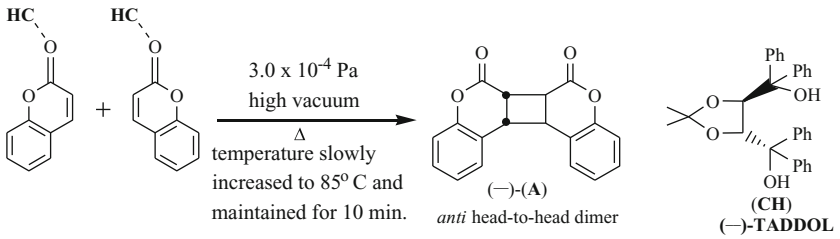


Fig. 13.54 Thermal [2+2]cycloaddition of coumarin in chiral inclusion complex at high vacuum

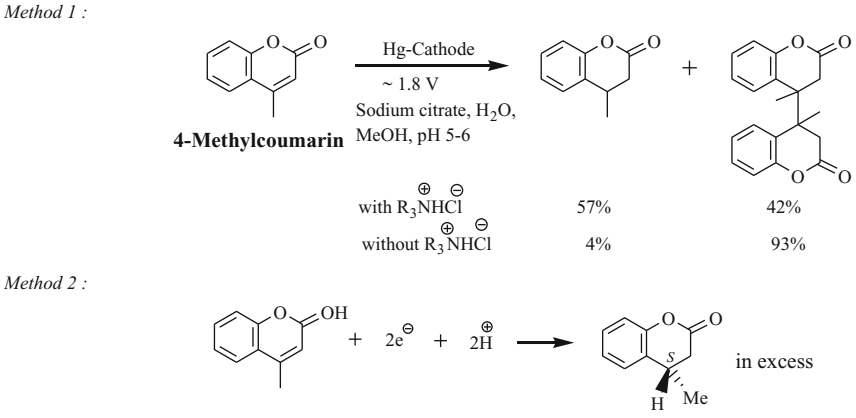


Fig. 13.55 Eletrochemical reduction and reductive dimerization of coumarin

complex having chiral environment, the double bond of the guest coumarin molecules are separated by 3.42–3.59 Å and are parallel to each other making the situation suitable for thermal dimerization. A probable stepwise radical mechanism has been offered [52]. This type of thermal dimerization under vacuum may have synthetic utility.

13.2.8 Electrochemical Reduction and Reductive Dimerization of Coumarins

Different types of dimeric compounds along with monomeric compounds are obtained, and the ratio of the products depend on the condition of the experiment (Fig. 13.55) [Methods 1 and 2] [53].

During the process, a soluble reducing agent might have been formed that could transfer hydrogen and intercept the radical before dimerization. In their absence, radicals dimerize and the dimer appears as the major product. Many amines participate in the electrochemical reductions of enones [53].

When the electrochemical reduction is carried out on a poly(*S*)-valine-coated graphite cathode, the reduction of 4-methylcoumarin on the cathode yields the (*S*)-configuration enantiomer in excess [53].

13.2.9 As a Synthon

13.2.9.1 An Efficient Synthesis of (R)-Tolterodine

Coumarins are excellent substrates for the asymmetric 1,4-addition with arylboronic acids in the presence of a rhodium catalyst (3 mol%) generated from Rh(acac) $(C_2H_4)_2$ and (*R*)-segphos. The resulting (*R*)-4-arylchroman-2-ones are obtained in high yields (75–94 % 8 examples) in over 99 % *ee*. Such an asymmetric conjugate adduct obtained from 6-methylcoumarin has been employed for the synthesis of (*R*)-tolterodine [54] (Fig. 13.56), a potent competitive muscarinic receptor antagonist, intended for the treatment of urinary incontinence.

13.2.9.2 Synthesis of 4-Heteroaryl-substituted Coumarins

Suzuki cross-coupling between 4-trifluoromethylsulfonyloxy- di- and trimethyl-coumarins and various heteroarylboronic acids yielded 4-heteroaryl-substituted methoxycoumarins in excellent yields (81–96 %) [55]. *They are potential cytotoxic and chemopreventing agents against cancer*. Thus, these compounds (Fig. 13.57) potentially constitute a new generation of neoflavonoid tubulin binding agents [55].

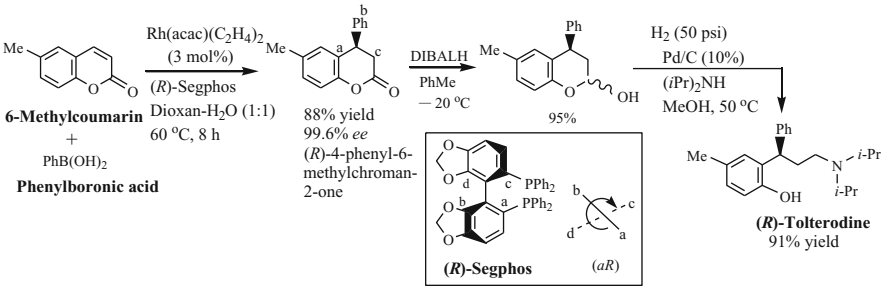


Fig. 13.56 Synthesis of (*R*)-tolterodine *via* asymmetric synthesis of 4-phenyl-6-methylchroman-2-one

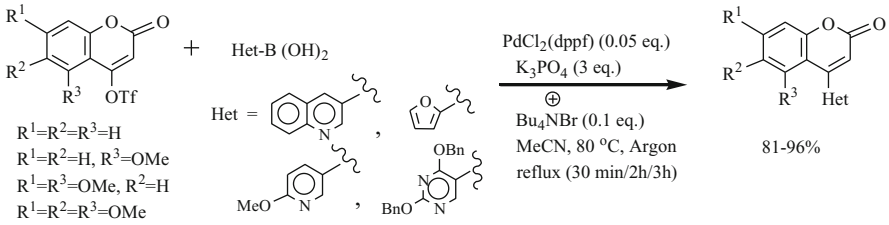


Fig. 13.57 Synthesis of 4-heteroaryl-substituted coumarins

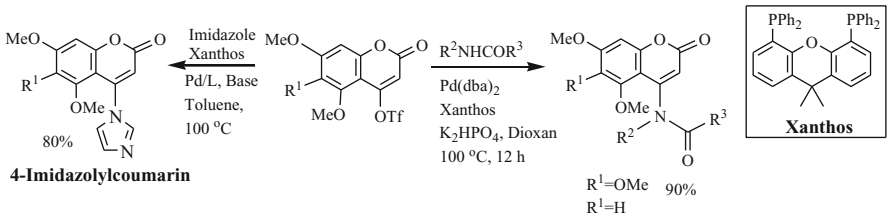


Fig. 13.58 Synthesis of 4-amido- and 4-(*N*-heteroaryl) coumarins

13.2.9.3 Synthesis of 4-Amido- and 4-(*N*-Heteroaryl)coumarins

4-Amido- and 4-(*N*-heteroaryl) coumarins constitute an important class of flavonoids, widely occurring in nature and also are components of the structures of more complex natural products; they exhibit a broad range of biological activities. Various catalytic systems for the amidation and introduction of heterocycles at C4 of coumarin-4-triflates were attempted [56]. These reactions with a few examples are shown in Fig. 13.58.

13.2.9.4 An Efficient Synthesis of the Intrinsic Fluorescent Peptide Labels

(*S*)- and (*R*)-(6,7-dimethoxy-4-coumaryl)alanines (Dmca) (via asymmetric hydrogenation) with high yield and enantioselectivity (>95 % *ee*) [57] have been achieved using 4-methyl-6,7-dimethoxycoumarin as the starting material (Fig. 13.59). During the hydrogenation the coumarin double bond is not reduced. These Dmca compounds absorb and emit at longer wavelengths, 334 nm and 440 nm, respectively, and hence reduce interference with other aromatic fluorophore moieties in amino acids. Thus, Dmca-labeled peptides, also having high quantum yield and large molar absorptivity, allow their selective determination in picomole quantities.

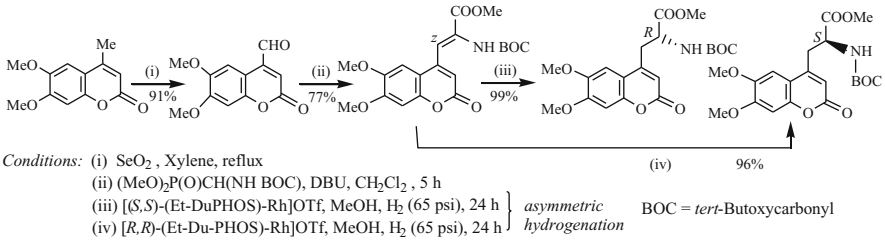


Fig. 13.59 Synthesis of the fluorescent peptide labels

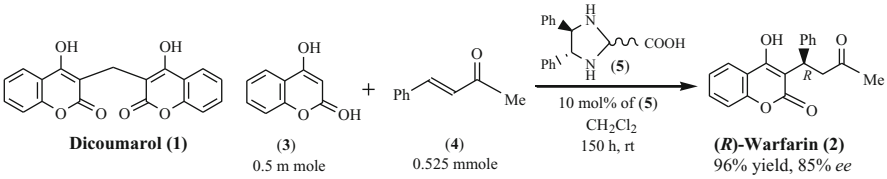


Fig. 13.60 Conversion of 4-hydroxycoumarin to (*R*)-warfarin

13.2.9.5 Synthesis of Warfarin by Catalytic Asymmetric Michael Reaction

Dicoumarol, a naturally occurring constituent of spoiled sweet clover (*Melilotus officinalis*), has been found to be the causative agent for fatal hemorrhage of cattle fed on this hay. It is thus an antagonist of vitamin K involved in the synthesis of prothrombin and related agents responsible in blood coagulation. Dicoumarol (**1**) (Fig. 13.60) being a 4-hydroxy-coumarin derivative, other 4-hydroxycoumarins have been tried. Warfarin (**2**) with a 4-hydroxycoumarin moiety has been found to be an *effective antagonist of vitamin K and is widely used as medicine given to patients of thrombosis prone to blood clotting*.

The drug has been prescribed as racemate for about 50 years. However, it has been found that the (*S*)-enantiomer possesses 5–8 times higher anticoagulant activity than that of the (*R*)-enantiomer. Further, the half-lives in the human body are 21–43 h and 37–89 h for (*S*)- and (*R*)-warfarin, respectively, indicating their different metabolic pathways. Hence, one of the major problems with racemic warfarin is the delivery and maintenance of a stable dose; too high doses might lead to internal hemorrhages in the patient. So the patient may be treated with optically pure warfarin, better with the milder (*R*)-warfarin.

A simple, effective, and highly *atom-economical synthesis* (vide Sect. 4.2) of (*R*)-warfarin (**2**) from 4-hydroxycoumarin (**3**) and benzylideneacetone (**4**) has been effected in 87 % *ee*, in the presence of the (*R*,*R*)-imidazolidine catalyst (**5**) as

delineated in Fig. 13.60 [58]. The corresponding (*S*,*S*)-catalyst produces the enantiomer (*S*)-warfarin in 80 % *ee* [58].

13.2.10 Biological Properties, Uses, and Applications

Many coumarins are bioactive and are used as antifungal and anticoagulant reagents, in various skin disorders, and other medicinal relevancies. Coumarins also find uses as flavoring agent, fluorescent brightener in textile, detergent, and paper perfluorescent and fluorescent agents, in various chemical and biochemical studies, fluorescent labels, laser dyes, light-emitting diodes, inhibitors of some metabolic paths, as substrates for the study of various reactions, and their conversion to useful compounds (Sect. 13.2.4). Some of the above uses will be illustrated.

Fluorescent Brightener in Textile Continued washing of white cotton clothes turns them yellow. Many coumarin derivatives (e.g., 4-methyl-7-dimethylaminocoumarin) are used as whitener by getting introduced into the fiber through the soap or whitening powder. It imparts blue fluorescence with UV light, which masks the yellowish color of the cotton, and the cotton appears whitish. In fact advertisement for such material sometimes reads as "Whiter than white." Medicinal uses of coumarins have been discussed in Sect. 13.2.9.5 and Chap. 33.

Uses in Fluorescence Assay [59] Protein kinases catalyze phosphate group transfer from ATP to the serine, threonine, and tyrosine residues of target proteins. Such activity of the enzymes has been measured in different ways with merits and demerits. Recently, 4-thiomethylcoumarins (*e.g.*, 4-thiomethylumbelliferone) have been employed as a fluorophore for such activity measurements based on fluorescence assay. This novel method thus developed is thought to be a better competitive one compared to the existing methodologies. Base-mediated β-elimination of the phosphate moiety and the Michael addition of a thiol-containing fluorescent molecule allow convenient and efficient detection of the enzyme activity (Fig. 13.61).

Simple coumarin, a profluorescent compound, acts as a trap for hydroxyl radical [60], generated by radiolysis in aqueous solution, and is converted to fluorescent 7-hydroxycoumarin. The fluorescence intensity is proportional to the number of 7-hydroxycoumarin molecules formed, which in turn is proportional to the locally

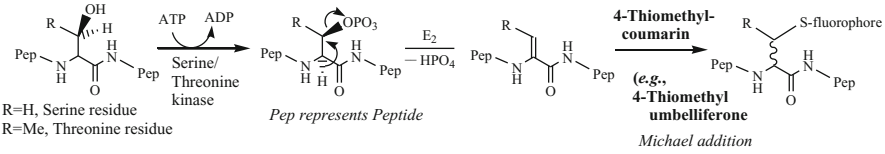


Fig. 13.61 Mechanism for the insertion of thiomethylcoumarin (fluorophore) for assay for serine/threonine kinase

available $HO^{\bullet}$ concentration produced in a variety of controlled pore glasses (CPGs) with pore sizes ranging from 8 to 300 nm leading to the better understanding of the rate of the reaction.

13.3 Marmesin

13.3.1 Occurrence, Structure

Marmesin, m.p. 189°, $[\alpha]_D$ + 26.8 ($CHCl_3$) was first isolated from the matured bark of *Aegle marmelos* Corrêa (Fam. Rutaceae) [61]. From the chemical reactions and IR spectral observations (done prior to NMR era) as outlined in Fig. 13.62, the gross structure of marmesin may be represented as linear (**a**) or angular (**b**) dihydrofuranocoumarin.

13.3.2 Synthesis and Absolute Configuration

The structure (**a**) for (+)-marmesin as well as the absolute configuration (*S*)- of its only chiral center has been established by its synthesis from a chiral synthon, (*S*)-(−)-6-benzyloxycoumaran-2-carboxylic [acid (−)-(**A**)], of known absolute configuration [62]. Thus, (−)-nodakenetin [63], the enantiomer of (+)-marmesin, obtained from *Peucedanum decursivum* Maxim as the glucoside, nodakenin, must have (*R*)-configuration.

(i) Synthesis of (S)-(−)-6-benzyloxycoumaran-2-carboxylic acid (**A**) and its absolute configuration determination is outlined in Fig. 13.63.

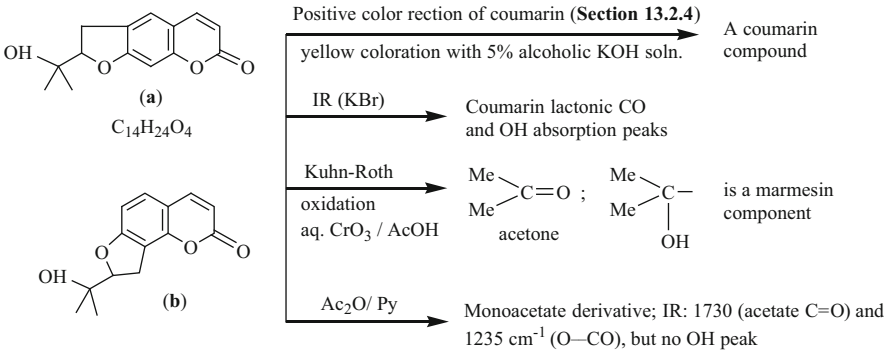


Fig. 13.62 Some basic reactions and IR spectral characteristics of marmesin

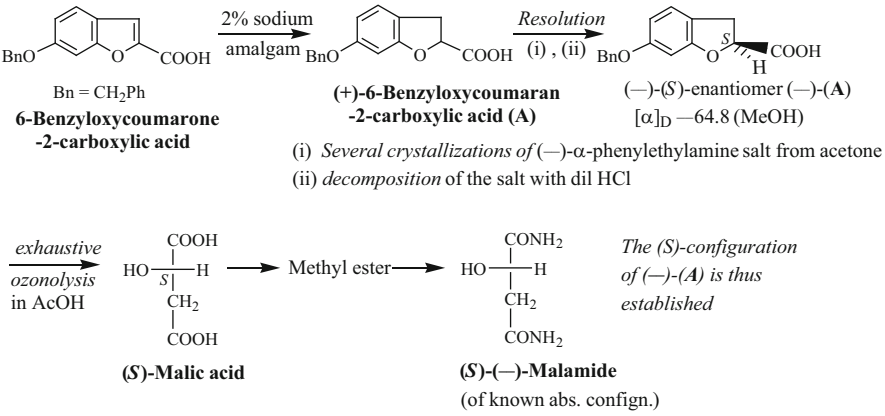
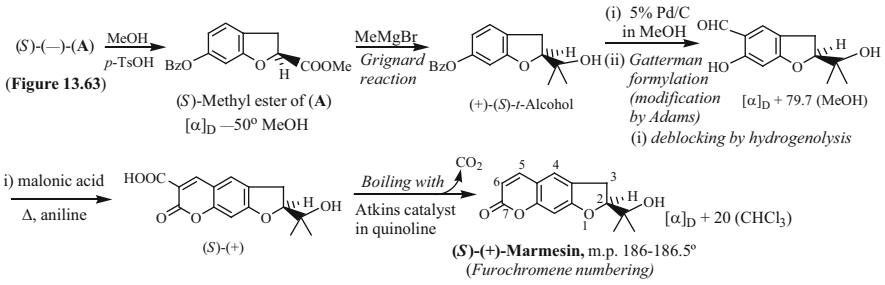


Fig. 13.63 Synthesis of (−)-6-Benzyloxycoumaran-2-carboxylic acid (−)-(**A**) and its absolute configuration



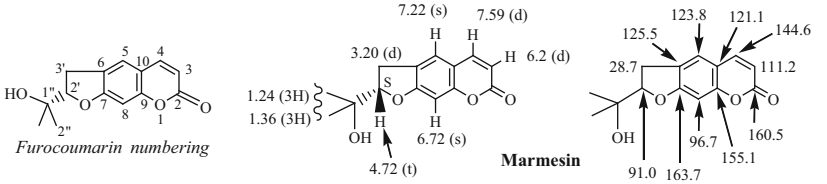
Note: (+)-*Marmesin may be systematically named (S)-(+)-2-(1-Hydroxy-1-methylethyl)-2,3-dihydro-7H-furo-[3,2g]chromen-7-one*

Fig. 13.64 Synthesis of (*S*)-(+)-marmesin starting from (−)-(**A**)

(ii) Synthesis of (*S*)-(+)-marmesin starting from (*S*)-(−)-(**A**) is outlined in Fig. 13.64.

13.3.3 NMR Spectral Data (Fig. 13.65) [64]

^{1}H NMR (δ, $CDCl_3$) [65]: 6.2 (H3, d), 7.59 (H4, d), 7.22 (H5, s), 6.72 (H8, *s*), 4.72 (H2′, t), 3.20 (H3′, d); the hydroxyisopropyl CH_3 groups (diastereotopic) appeared comparatively downfield (1.24 and 1.36, 3H each) due to their attachment to the carbon bearing OH.



Note: *The different* ^{1}*H chemical shift values of the methyl groups of the hydroxyisopropyl indicate that they are in diastereotopic environments and that the rotation about the 2′-1′′ bond is not entirely free – some conformers are preferred.*

^{13}C NMR (δ, d_6DMSO) [65]: 160.5 (C2), 111.2 (C3), 144.6 (C4), 123.8 (C5), 125.5 (C6), 163.3 (C7), 96.7 (C8), 155.1 (C9), 121.1 (C10), 91.0 (C2′), and 28.7 (C3′).

Fig. 13.65 ^{1}H and ^{13}C NMR spectral data (δ-values) of marmesin

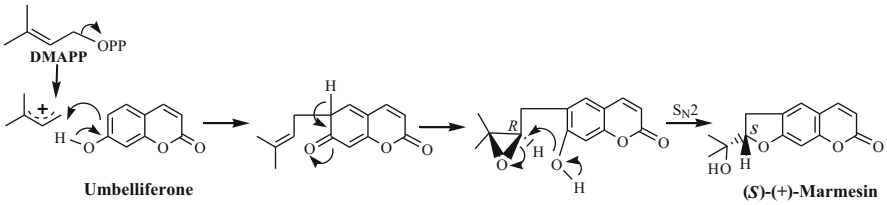


Fig. 13.66 Biosynthesis of marmesin from umbelliferone

13.3.4 Biosynthesis of Marmesin, the Obligatory Precursor of Psoralen and Other Furanocoumarins [11, 66]

Umbelliferone (for biosynthesis see Sect. 13.1.6, Fig. 13.14) undergoes C-prenylation at C6, followed by enantioselective epoxidation (2(*S*)-epoxide, DMAPP numbering) at the double bond, and intramolecular nucleophilic attack at C2 (inversion) which opens up the epoxide generating a hydroxyisopropyl group at the α-carbon atom of the newly formed dihydrofuran system of marmesin, as outlined in Fig. 13.66.

It has been shown by labeling experiments that the prenyl group being attached to umbelliferone originates from the non-mevalonate (DOXP) pathway (Chap. 5, Sect. 5.3). This has been proved by carrying out two independent biosynthetic feeding experiments in *Apium graveolens* (Apiaceae)—one provided with mevalonic acid and the other with 2-desoxy-D-xylulose, both appropriately labeled—as the two different sources of the prenyl unit (Fig. 13.67) [11]. Marmesin thus formed undergoes *cis* (or *syn*) elimination. The enzyme abstracts 3′D_{RS} creating a resonance stabilized benzyl radical (*step a*). This is followed by β-cleavage (with respect to furan O) forming acetone, water, and psoralen (*step b*). The mevalonate-independent (DOXP) pathway produces IPP/DMAPP at plastids, and umbelliferone is formed in cytosols. To interact with each other either

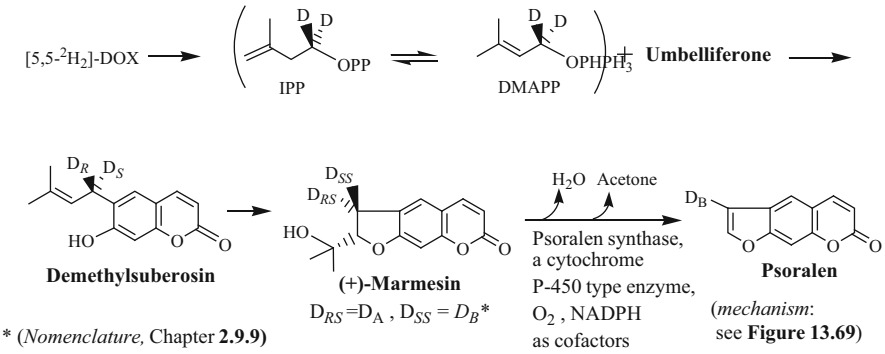


Fig. 13.67 Biosynthesis of marmesin and psoralen

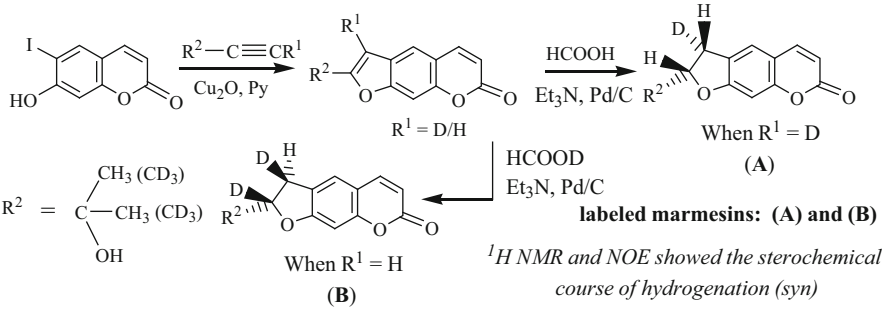


Fig. 13.68 Synthesis of labeled marmesins, (**A**) and (**B**)

IPP/DMAPP moves from plastids to cytosols or umbelliferone moves from cytosols to plastids [11].

This mechanism involving *syn* (*cis*) elimination (C3′ H_R and hydroxyisopropyl group at C2′) for the formation of psoralen has been shown by incubating labeled marmesin with a microsome preparation from a cell culture of *Amni majus*. The latter was elicited, prior to the isolation of microsome, with a cell-wall preparation from the plant pathogenic fungus *Phytophthora megasperma* to produce the desired enzyme responsible for the conversion of marmesin to psoralen. [66] The synthesis of labeled marmesin is delineated in Fig. 13.68.

Isotopomer (**A**) gave psoralen devoid of deuterium, while isotopomer (**B**) gave dideuterated psoralen (mass). Acetone and water are other products. The dealkylation of marmesin into psoralen and acetone may be rationalized by the mechanistic sequence outlined in Fig. 13.69 [66].

P-Fe(V), the active species of the enzyme, abstracts the H_R atom from C3′ of marmesin. The benzylic radical thus generated forms psoralen by α-cleavage (with respect to furano oxygen) and releases thehydroxyisopropyl radical (*step a*). The species Fe(IV)OH of the enzyme abstracts H radical from the hydroxylisopropyl group (*step b*) which self quenches to acetone and water molecule, the other two products (Fig. 13.69). These two steps may be concerted in the presence of the

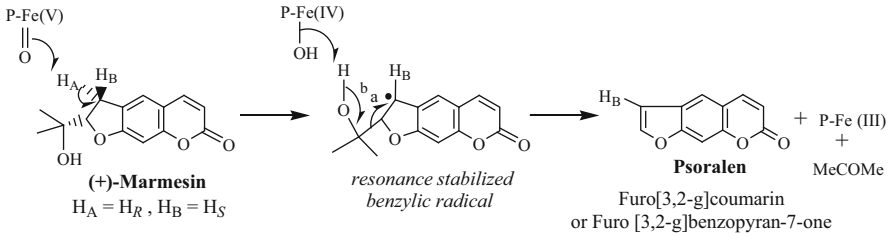


Fig. 13.69 Mechanism of biosynthesis of psoralen from (+)-marmesin

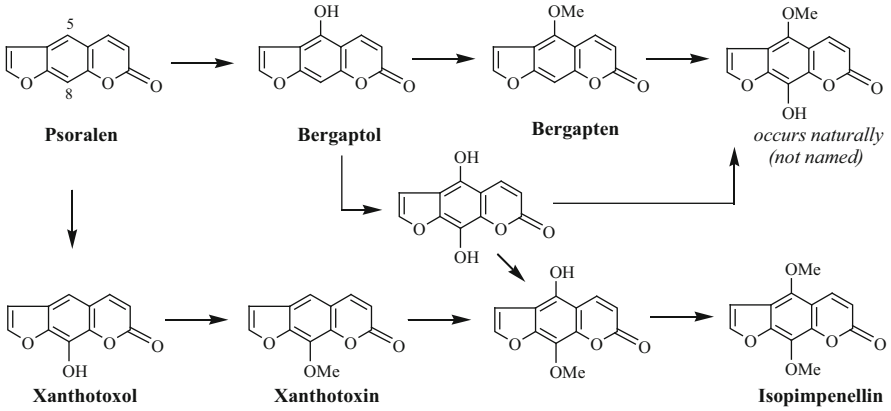


Fig. 13.70 Enzymatic transformations of psoralen into 5- and/or 8-oxygenated simple linear furanocoumarins

enzyme and cofactors. Fully labeled acetone is obtained when during synthesis of marmesin hydroxyisopropyl group is fully deuterated (Fig. 13.68). The fully deuterated acetone is derivatized as the pentafluorobenzylhydroxylamine oxime and identified mass spectrometrically.

Further enzymatic transformations of psoralen by successive regiospecific hydroxylations at C5 and/or C8 and methylations of the hydroxyls lead to other simple furanocoumarins as outlined in Fig. 13.70. However, the exact sequence of hydroxylation at C5 and C8 and methylation is not known.

13.4 Some Interesting Natural Dimeric and Trimeric Coumarins: Structure Diagrams. Nomenclature. Some Comments

Apart from monomeric diversely substituted coumarins nature elaborates bicoumarins and much less abundantly tricoumarins. The constitutional monomers of biarylbicoumarins are siderin, umbelliferone, esculatin, etc. (1.). No cross-

coupling during biological dimerization is reported. However, with two identical monomers the coupling may take place constitutionally (a) symmetrically and (b) unsymmetrically. The monomers may be connected through oxidative coupling to form the biaryl axis. Some dimers occur as biaryl ethers; some trimers contain both ether and biaryl linkages [67]. The structures of a few dimeric coumarins and a trimeric coumarin with chiral biaryl axes are depicted in Fig. 13.71.

The absolute axial configuration (*M*) (≡ *aR*) (see Sects. 2.16.5.5 and 2.19.6.2) of desertorin A (Fig. 13.71) has been established by an X-ray structure analysis of a bromo derivative. The transformation of desertorin A and desertorin B to desertorin C and their stereochemical identity by comparison of the chiroptical data of these biaryl compounds demonstrate that all these desertorins have the same (*M* ≡ *aR*) configuration. The absolute configuration of the umbelliferone trimer, triumbellitin (Fig. 13.71), at the biaryl chiral axis has been determined to be (*P* ≡ *aS*) by application of exciton chirality method (Sect. 2.14) though the two interacting groups are not identical along the biphenyl axis—one is a monomer and the other is a dimer [67].

Furanocoumarin Trimers and Dimers. Only one example of each type is given. Rivulotririn C and rivulobirin E, a trimer and a dimer, respectively, of 8-hydroxpsoralen have been isolated from the underground part of *Pleurospermum*

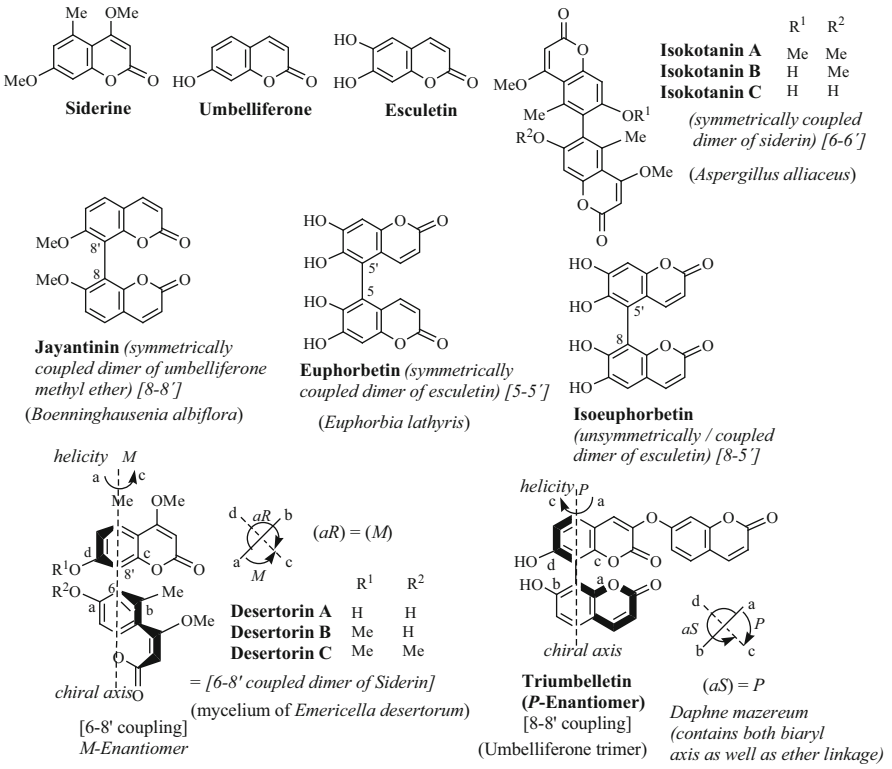
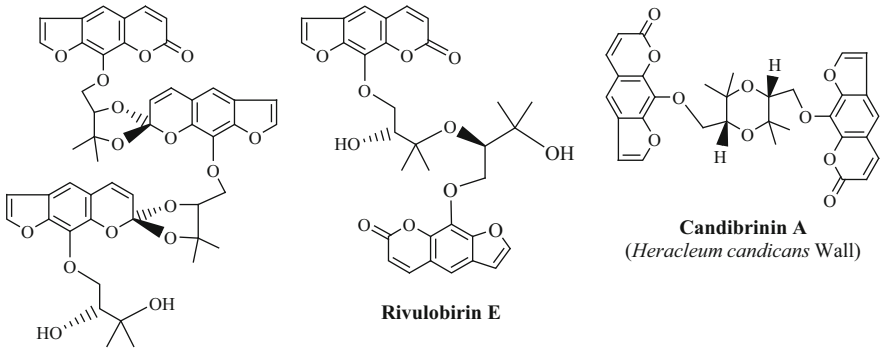


Fig. 13.71 Structures of a few monomeric and dimeric coumarins and a trimeric coumarin

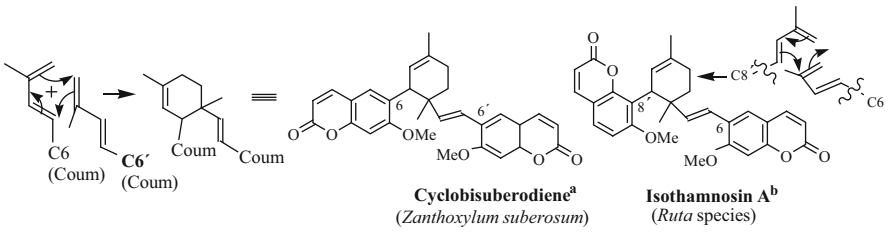
rivulorum (Umbelliferae) [68] (Fig. 13.72). Ribulotririn C is the first example of a trifuranocoumarin having two ortho ester linkages. Their structures were established by ^{1}H and ^{13}C NMR studies and HMBC and NOE correlations.

An interesting 8-Oxa-alkylated psoralen dimer, candibrinin A (Fig. 13.72), has been isolated from *Heracleum candicans* Wall (Umbelliferae) [69].



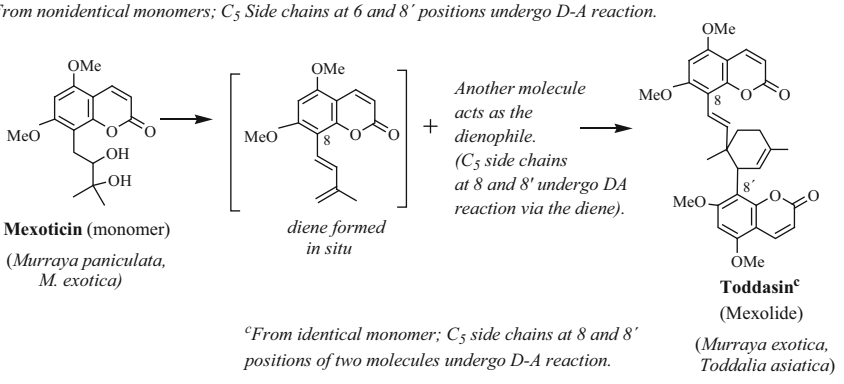
(The first example of a trifuranocoumarin having two ortho ester linkages)

Fig. 13.72 Structures of a trimeric furanocoumarin with two ortho ester linkages and two dimeric furanocoumarins



[a]*From identical monomer; C_5 side chains at 6 and 6′ positions of two molecules undergo D-A reaction.*

[b]*From nonidentical monomers; C_5 Side chains at 6 and 8′ positions undergo D-A reaction.*



[c]*From identical monomer; C_5 side chains at 8 and 8′ positions of two molecules undergo D-A reaction.*

Fig. 13.73 Some coumarin dimers formed through D-A reactions of the C_5 chains of the corresponding monomers

Sometimes dimers are possibly formed via Diels-Alder (D-A) reaction involving suitable C_5-diene side chains of the monomer units, acting as the diene and the dienophile [70] (Fig. 13.73). In some cases a non-diene C_5-side chain might undergo chemical transformation to diene prior to dimerization. The co-occurrence of such monomer with the dimer is suggestive of such speculation.

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Further Readings (of Section 13.1)

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Chapter 14
Polyketide Pathway. Biosynthesis of Diverse Classes of Aromatic Compounds

14.1 Introduction

In 1893, a young chemist, John Norman Collie at London University serendipitously discovered a set of reactions while establishing the structure of dehydroacetic acid [1]. On boiling dehydroacetic acid with $Ba(OH)_2$ and its subsequent work up with acid, Collie obtained a phenol, orcinol, whose structure he could correctly assign [1, 2]. He even offered an explanation on the formation of orcinol from dehydroacetic acid (Fig. 14.1). This concept of a triketone formation as the intermediate has been the cornerstone of the polyketide pathway [3].

In 1907 Collie proposed [3] the bioformation of polyphenolic compounds in vivo from polyketide (initially he coined the term "multiple keten") systems. However, this conjecture of Collie did not receive due attention. Since 1951, the involvement of acetyl coenzyme A in the biosynthesis of terpenoids and steroids (Chaps. 5 and 11) has been studied extensively. This study suggests the involvement of other acyl coenzyme A for the biosynthesis of aromatic compounds. In 1953 Birch hypothesized [4, 5] the polyketide pathway and in 1955 he confirmed the first polyketide from label acetic acid as the building block for 6-methyl salicylic acid [6] isolated from a fungal metabolite. The locations of ^{14}C have been arrived at by controlled chemical degradations (Fig. 14.2).

Polyketide natural products possess diverse skeletal patterns and occur in plants, fungi, and soil bacteria. They exhibit medicinally important antibiotic, anticancer, antifungal, antiparasitic, and immunosuppressive [1] activities. They are also used as food ingredients and nutraceuticals.

S.K. Talapatra and B. Talapatra, *Chemistry of Plant Natural Products*,
DOI 10.1007/978-3-642-45410-3_14, © Springer-Verlag Berlin Heidelberg 2015

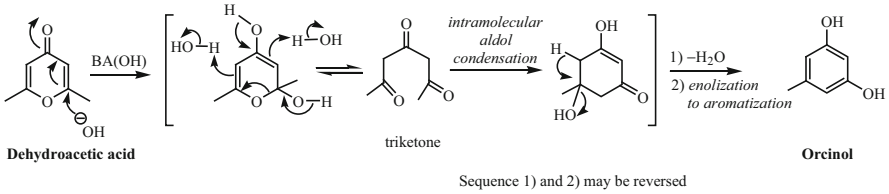
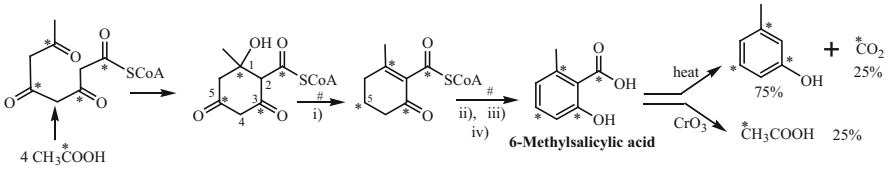


Fig. 14.1 Collie's analysis of the formation of orcinol from dehydroacetic acid



#i) *regiospecific reduction of C5-carbonyl;* ii) *enolization*; iii) *hydrolysis of thioester*, iv) *aromatization* (*dehydrogenation*): *the sequence of chemical events cannot be defined.*

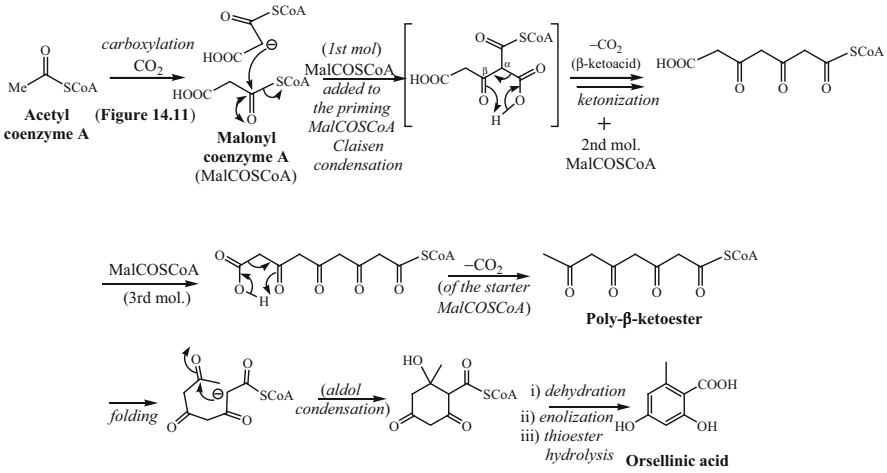
Fig. 14.2 Biosynthetic experiment for 6-methylsalicylic acid (label experiment)

14.2 Biosynthesis of Polyketide Natural Products

The initial carbon chain of a polyketide is formed by a series of intermolecular Claisen condensation of malonyl coenzyme A (elongation of the chain by C_2 unit). The latter is formed from acetyl coenzyme A by carboxylation (see Fig. 14.11). The majority of polyketide natural products are produced by three broad classes of polyketide synthases (PKSs) sharing a common mechanistic approach, the sequential decarboxylative condensation [7, 8]. The three classes of PKSs are (i) multienzyme type 1, (ii) iterative type II PKSs, and (iii) the homodimeric type III which are involved in the formation of a large range of aromatic compounds like flavonoids, chalcones, etc. [9]. Chalcone synthase (CHS) belongs to this class. The lengthening and cyclization of polyketide chain is controlled by enzymes so that unwanted mode of cyclization, especially the premature cyclization, is prevented. Biosynthesis of orsellinic acid is delineated in Fig. 14.3.

14.2.1 Biosynthesis of Phloroglucinol, the Simplest Polyketide Derived Aromatic Compound

For the Biosynthesis of phloroglucinol and dehydroacetic acid, three molecules of malonyl coenzyme are required [10]. Decarboxylation of the COOH group of the priming malonyl coenzyme would lead to the formation of 3,5-diketohexanoate (Fig. 14.4), an intermediate for triacetic acid lactone while the retention of the said



Note: *The sequence of events may be different*

Fig. 14.3 Biosynthesis of orsellinic acid

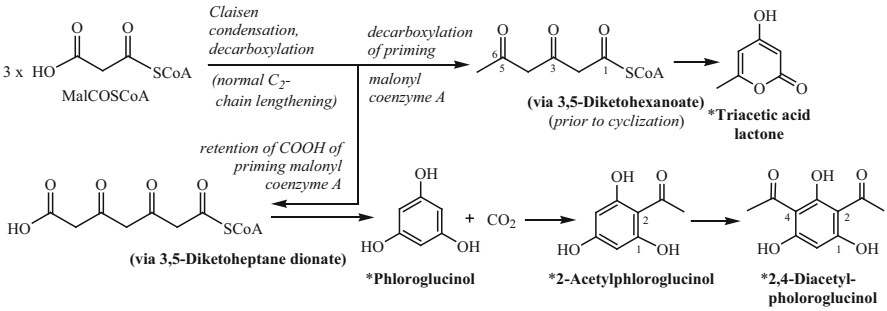


Fig. 14.4 Biosynthesis of acetylphloroglucinol via phloroglucinol [10]

COOH group would form 3,5-diketoheptane dionate, an intermediate for phloroglucinol (Fig. 14.4).

During the biosynthetic studies [10] by gene cluster *phlACBDE* encoded for acetylphloroglucinols, the synthesis of phloroglucinol in the culture was observed. By genetic manipulation the concentration of phloroglucinol could be increased in the culture. Thus, the biosynthesis of phloroglucinol as a free-standing molecule (not as a part of a molecule) is delineated for the first time [10], though phloroglucinol is found as a part in a variety of natural products.

Further, phloroglucinol is formed by microbes expressing *phlD* from malonyl coenzyme A. In the absence of malonyl coenzyme A in the gene cluster *phlACB*, no

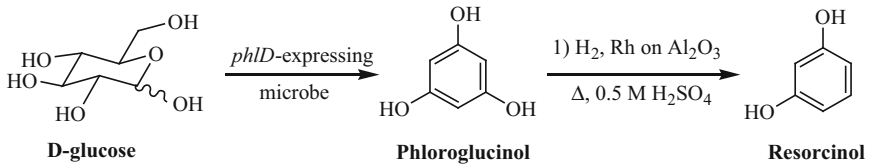


Fig. 14.5 Chemoenzymic synthesis of resorcinol via pholoroglucinol formed by *PhlD*-expressing microbe [10]

phloroglucinol could be synthesized. However, if phloroglucinol is added, it is converted into acetylphloroglucinol.

Incidentally, it may be mentioned that *PhlD* is particularly important in the new synthesis of phloroglucinol and resorcinol which are synthesized by methods either by hazardous or circuitous (chemoenzymatic) way [11, 12], and resorcinol is synthesized by alkali fusion of 1,3-benzene disulfonic acid. Heterogenous expression of *PhlD* in *Escherichia coli* converts glucose to phloroglucinol in a single microbe-catalyzed step (Fig. 14.5). Phloroglucinol may then be converted into resorcinol by hydrogenation with Rh on Al_2O_3, followed by reflux with 0.5 M H_2SO_4 [10] (Fig. 14.5).

The biosyntheses of chalcones and different flavonoids have been discussed in Sect. 14.4.

Numerous aromatic compounds are biosynthesized via polyketides. An excellent review [13] on the biomimetic synthesis of aromatic natural products via polyketides may be consulted.

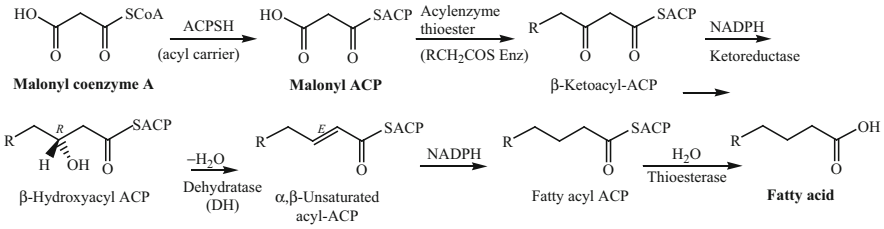
14.3 Fatty Acid Biosynthesis Using C_2 Extender [14, 15]

In fatty acid biosynthesis catalyzed by the fatty acid synthase (FAS), the further stepwise addition of $–CH_2CO$ (C_2 unit, C_2-extender) is coupled with reduction of CH_2CO-unit to $CH_2–CH_2–$ as shown in Fig. 14.6.

Most of the fatty acids are of even numbers (multiples of C_2-units). The odd numbered fatty acids may originate from different odd numbered priming acyl coenzyme A (e.g., propionyl coenzyme A) or by the loss of one carbon unit from an even-numbered acyl-ACP. For nomenclature of fatty acids see Chap. 34.

This simple Claisen type condensation involving C_2-unit addition, as observed in the polyketide chain formation and also in fatty acid synthesis, revealed another route of acetate metabolism.

In the subsequent sections some polyketide-derived plant natural products like chalcones and flavonoids, including their biosynthesis [16], have been discussed.



Notes: i) *All the reactions are catalyzed by appropriate enzymes.*
ii) The length of the fatty acid will depend on the number of C_2 units (no. of extenders) added and the specificity of the enzyme.
*iii) The chain extender malonate unit is bound to a thiol residue of a protein designated as acyl carrier protein (*ACP*)*

Fig. 14.6 Biosynthesis of fatty acids

14.4 Flavonoids Derived from Polyketide and Shikimic Acid Pathways

14.4.1 Introduction. Various Classes

Flavonoids comprise a group of one of the largest natural oxygen heterocycles having a carbon framework C_6–C_3–C_6 (a 1,3-diarylpropane).

The left-hand side aromatic ring (ring A, C_6) of flavonoids originates from the polykelide pathway, while the rest C_3–C_6 unit containing the second aromatic ring (ring B) is derived from shikimic acid pathway (Sect. 13.1). The flavonoids are thus of mixed biogenetic origin. Figure 14.7 shows various classes of flavonoids along with their skeletons. The two aromatic rings constitute ring A and ring B, respectively; the middle heterocyclic ring (ring C) is formed by Michael type nucleophilic attack of the phenolic OH of ring A on the α,β-unsaturated ketone of the chalcone, the precursor of flavonoids.

Flavonoids occur as their ethers, esters, glycosides, and also in the free state. They are also found to occur in the dimeric, trimeric, tetrameric, etc. states. More than 4,000 flavonoids have been identified and the human health protection properties of many have been assessed.

The various classes of flavonoids are responsible for the beautiful colors of the flowers, where they occur mostly in the petals, apart from various other parts of the plants. The beautiful colors of the flowers, (also caused by carotenoids) (Chap. 12), attracted the attention of our observant and curious ancestors. They naturally wanted to know what made them colored. Perhaps this curiosity is one of the causes of the humble beginning of scientific endeavors, as we find today. Special mention should be made of the flowers of *Butea frondosa* (the fire of the forest). They are soothing to the eyes and delight to the vision, and they benefit our body as an antioxidant and prevent the oxidation of endogenous biomacromolecules (Chap. 34).

Few comments on the structures of flavonoids

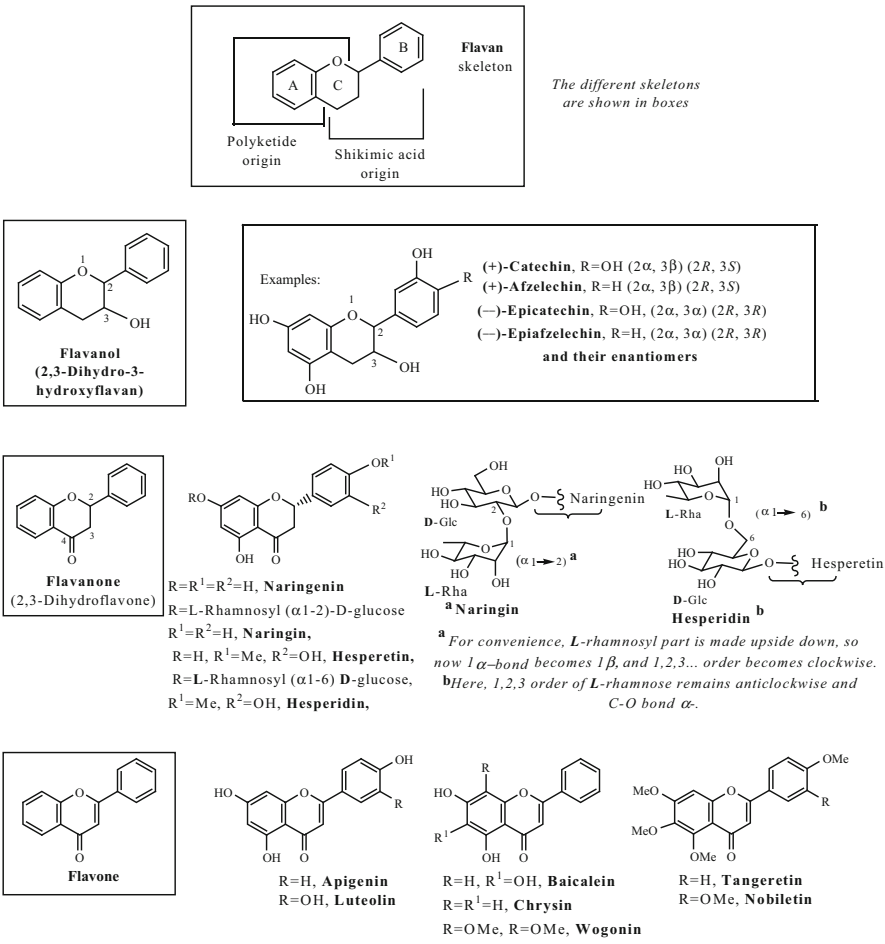
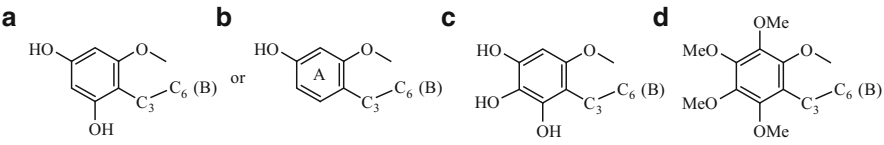
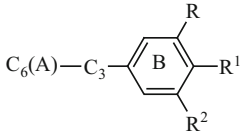


Fig. 14.7 (continued)

1. Ring A is characteristically phloroglucinol (**a**) or resorcinol (**b**) and may be further hydroxylated like (**c**) and (**d**)



Ring B is characteristically hydroxylated as shown



4	3,4	3,4,5
$R=R^2=H$ $R^1=OH$	$R=R^1=OH$ $R^2=H$	$R=R^1=R^2=OH$

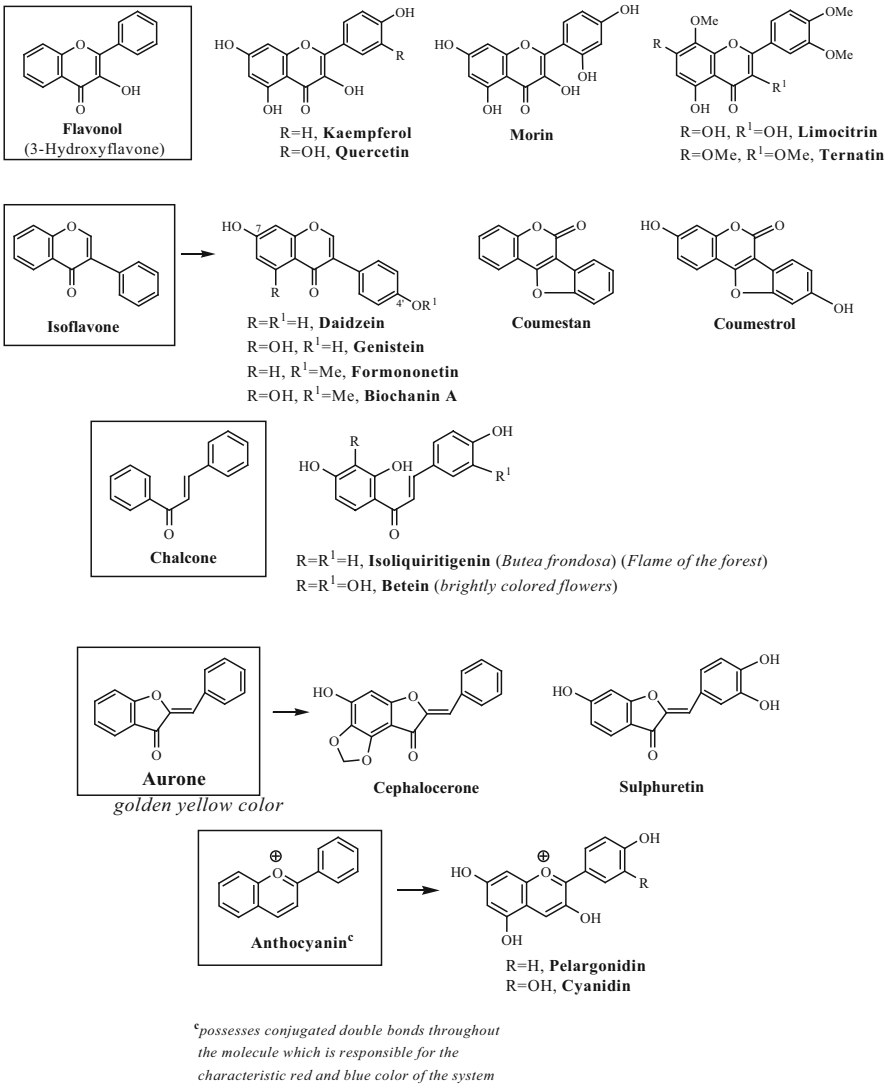
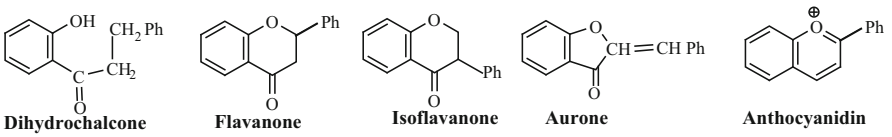


Fig. 14.7 Representative natural flavonoids and isoflavonoids and their skeletons

2. Rarely ring A or B occurs without any substituent
   The most common site of glycosylation is at C7, sometimes at C3 (in cases of flavanols and flavonols).
3. The central three-carbon fragment can basically occur in the following forms:



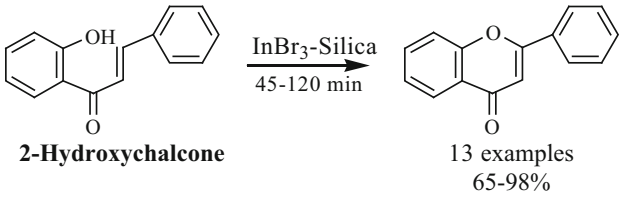


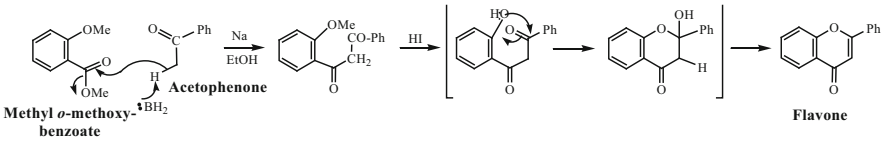
Fig. 14.8 Different syntheses of flavones

14.4.2 Synthesis of Flavonoids

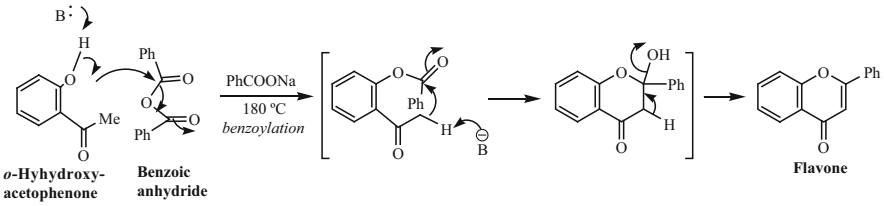
(a) **Synthesis of flavones**

Many syntheses of flavones are known. Only a few will be outlined (Fig. 14.8).

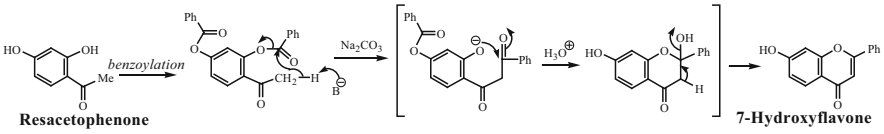
1. ***Kostanecki synthesis*** (1900)



2. ***Robinson's method*** (1924)

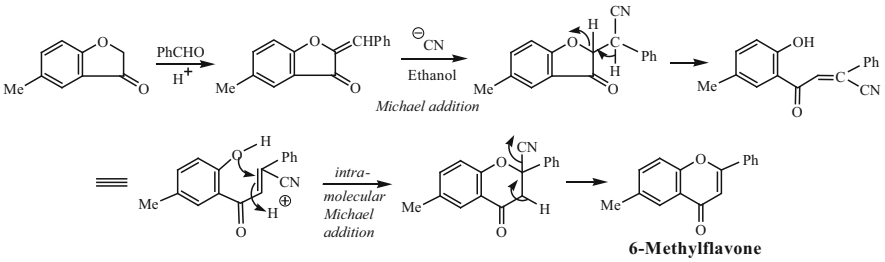


3. ***Baker–Venkataraman synthesis*** (1933)

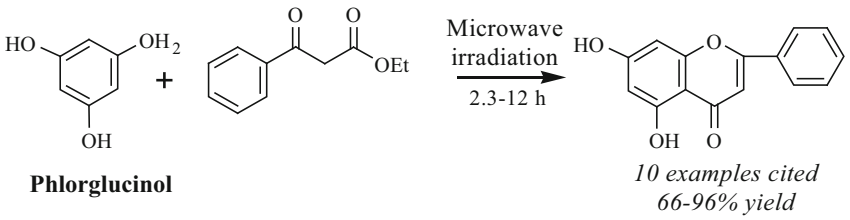


4. ***Wheeler synthesis*** (1955)

In this method ring expansion of 2-benzylidenecoumar-3-ones occurs to form flavones.



5. ***Solvent-free synthesis of functionalized flavones under microwave irradiation*** (2005) [17]



6. ***Oxidation of flavanones***

2-Hydroxy chalcones and flavanones undergo rapid and facile oxidation to flavones by catalytic functions of silica gel supported $InBr_3$ and $InCl_3$ (2005) [18].

(b) **Synthesis of flavonols (via flavanones) and of flavones via chalcones**

Flavanones formed via chalcone can be easily converted to flavonols through the reaction sequence shown in Fig. 14.9. A flavone can be formed via a chalcone in the way shown (Fig. 14.9)

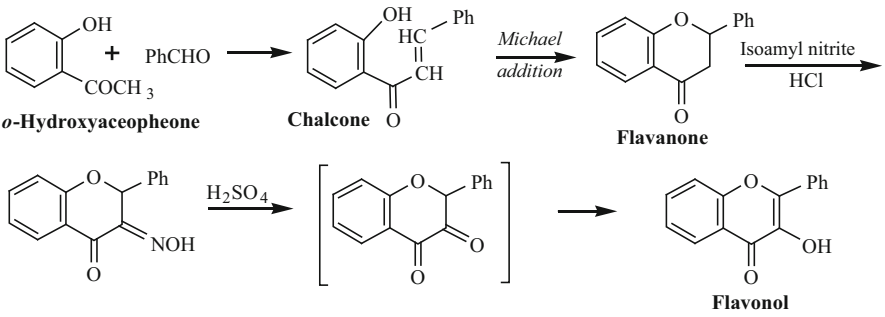
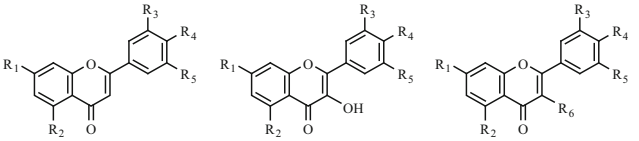


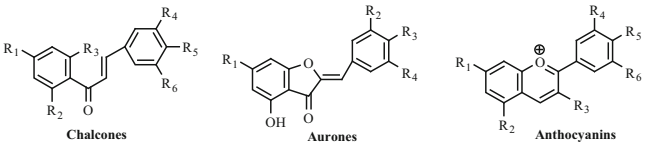
Fig. 14.9 Synthesis of flavanol via chalcone and flavanones and of a flavones via chalcone

UV and **Visible** λ_{max} (MeOH) nm: (OH or OMe is present at the positions mentioned) (two/ three /four at a time o r in some cases at all positions - 3, 5, 7, 3′, 4′, 5′), methylenedioxy group is also present.



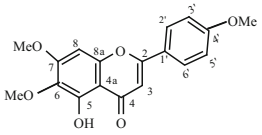
Flavones (240-285, 304-) | **Flavonols** (240-285, 352-) | **Flavones** (270-295, 300-)

R_1, R_2, R_3, R_4, or R_5 = OH, OMe, O-C_5/C_{10}, or H



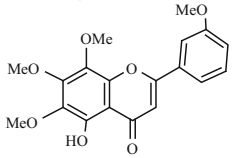
Chalcones (220-270, 340-) | **Aurones** (370-430) | **Anthocyanins** (270-280, 465-550)

IR: ν_{max} cm^{-1} 1630-1650 (1670) in general for flavonoid carbonyl, some shifts are also expected according to its environment and substitutions. *Specific examples*:



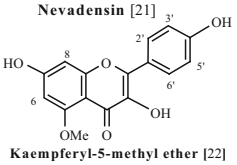
Salvigenin [20]
*Salvia triloba (*Labiatae), *Alnus japonica* (Betulaceae), *Eupatorium odoratum* (Asteraceae)

UV: λ_{max} (ErOH), 274 nm (ε 18, 700), 330 nm (ε 26,000)
IR: ν_{max} 1635 cm^{-1}
^{1}H NMR (δ $CDCl_3$, 100 MHz): 3.90, 3.94, 3.98 (3H each, 3 OMe groups), 6.54 (1H, H-3), 6.58 (1H *S*, H-8), 7.84 (2H, d, H-2′ and H-6′), 7.02 (2H, d, H-3′ and H-5′), 12.76 (1H, chelated O*H* at C5)



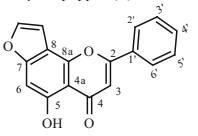
Nevadensin [21]

UV: λ_{max} (EtOH), 285 nm (log ε 4.36), 332 nm (4.23)
IR: ν_{max} 1655 cm^{-1}
^{1}H NMR: (δ, $CDCl_3$, 80 MHz), 3.82, 3.89, 3.95 (3H, each 3 OMe groups), 6.44 (1H, s, H-3), 6.59 (OH, D_2O exchangeable), 7.12 (2H, d, J = 8 Hz, H-3′, H-5′), 8.28 (2H, d, J = 8 Hz, H-2′, H-6′), 11.59 (1H, s, exchangeable with D_2O, chelated O*H* at C5)



Kaempferyl-5-methyl ether [22]
(*Eupatorium erythropappam*) (Asteraceae)

IR: ν_{max} 1660 cm^{-1} (>=O)
^{1}H NMR: (δ, DMSO-d_6), 3.82 (3H, OMe), 6.30 (1H, d, J = 2 Hz, H-6), 6.65 (1H, d, J = 2 Hz, H-8), 6.90 (2H, d, J = 8 Hz, H-3′, H-5′), 8.05 (2H, d, J = 8 Hz, H-2′ and H-6′)



Pongaglabol [23]
Pongamia globra (Leguminosae)

UV: EtOH λ_{max} (log ε) 221 (4.48), 257 (4.29), 281 (4.47)
$\lambda_{max}^{EtOH-AlCl_3}$: 223.5 (4.43), 265 (4.47), 306 (4.46)
IR: ν_{max}^{KBr} cm^{-1} 1650 (conjugated >=O)
^{1}H NMR: (80 MHz, $CDCl_3$), δ 6.8 (1H, *s*, H-3), 6.95 (1H, d, $J_{3'',6}$ 0.9 Hz, H-6), 7.04 (1H, dd, $J_{3',2'}$ 2.1, $J_{6,3'}$ 0.9 Hz, H-3′′), 7.53-7.61 (4H, m, H-2′, H-3′, H-6′ and H-5′), 7.90-8.01 (2H, m, H-2′ and H-6′), 12.73 (1H, D_2O exchangeable, chelated OH at C5)

Fig. 14.10 Spectral data of flavonoids having different skeletal patterns and substitutions

14.4.3 Spectral Properties [19]

Expected UV absorption peaks are given in a general way for the different skeletal patterns of flavonoids (Fig. 14.10). Appearance of specific peaks for individual compounds is mostly governed by the substituents. Bathochromic and hypsochromic shifts of some peaks in alkali and $AlCl_3$, respectively, are observed in compounds with suitably disposed phenolic OH groups playing resonance with the carbonyl group [19].

14.4.4 Biosynthesis of Flavonoids [24, 25]

The gross C_6–C_3–C_6 carbon content of flavonoids is derived from (a) 4-coumaroyl coenzyme A, a product of shikimic acid pathway (see Fig. 13.15), which provides the ring B and the C_3 part of the middle heterocyclic ring (ring C), and (b) three molecules of malonyl coenzyme A (precursor of ring A), a biosynthetic entity in polyketide formation (Fig. 14.11). In the biosynthetic pathway of flavonoids, the first C_6–C_3–C_6 compound is a chalcone (catalyzed by chalcone synthase) from which the middle six-membered heterocyclic ring is generated by intramolecular Michael addition. The classification of flavonoids is based on the oxidation level of the central ring (Fig. 14.7). The biosynthetic pathway of different flavonoids are delineated in Figs. 14.11 and 14.12.

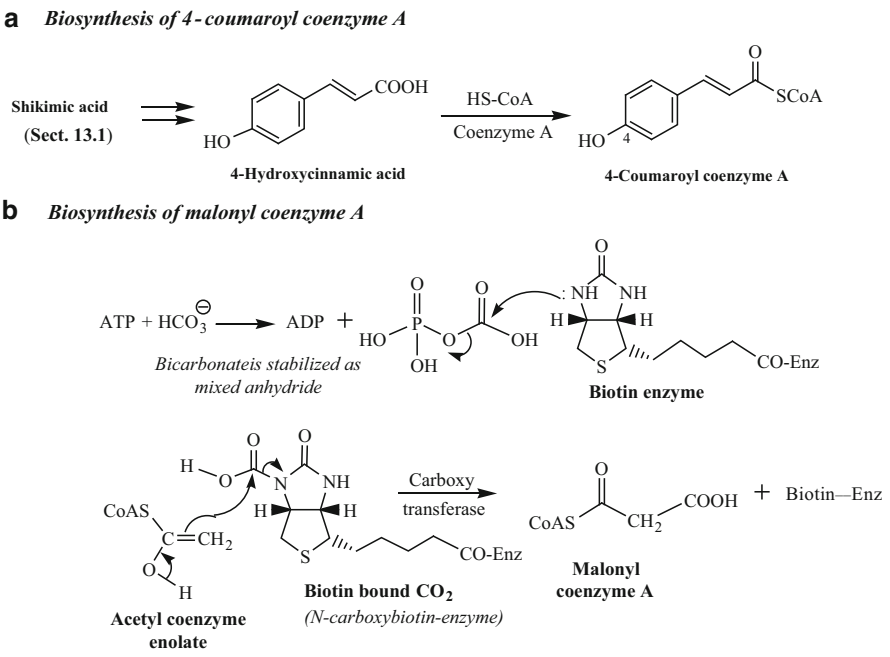


Fig. 14.11 Bioformation of 4-coumaroyl coenzyme A and malonyl coenzyme

14.4.4.1 Biosynthesis of the Precursors of Chalcones and Flavones (Fig. 14.11)

14.4.4.2 Biosynthesis of Chalcones and Flavones

Since in malonyl coenzyme A the methylene group is flanked by two electron withdrawing groups, it becomes a better anionic center, and its methylene protons are more acidic compared to the methylene protons of acetyl coenzyme A. The enzymes responsible for polyketide chain extension accept malonyl coenzyme A unlike the mevalonic acid pathway, where the enzyme accepts acetyl coenzyme A and not malonyl coenzyme A (Chap. 5). In the subsequent chain elongation concerted enzymatic decarboxylation is facilitated for being β-keto acid system in each step and the elimination of three molecules of CO_2 and four molecules of HSCoA makes the chalcone synthesis an irreversible process (Fig. 14.12).

Absolute Configuration It may be mentioned at this stage that (–)-flavanones possess (2*S*)-configuration. It has not been established by individual experiments for the flavanones, but comparison of their ORD curves suggests the above generalization.

The absolute configuration of (–)-hesperetin has been correlated with L-(–)-malamide serving as the reference compound (Fig. 14.13).

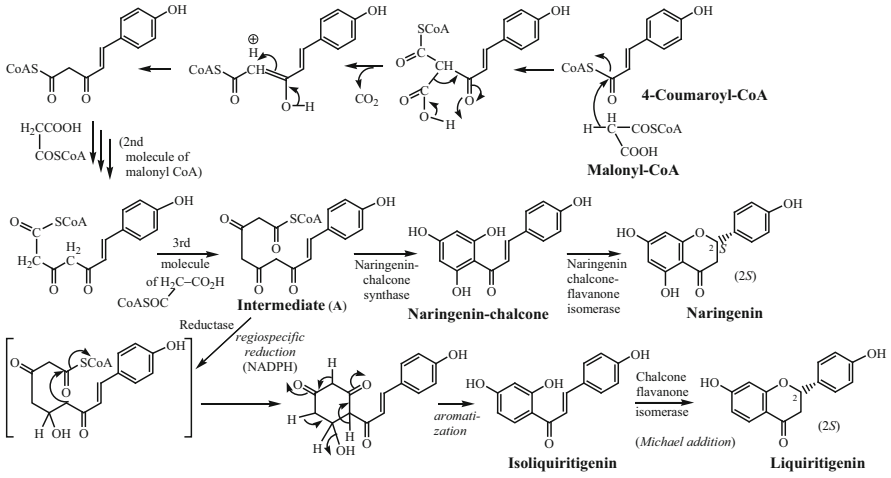


Fig. 14.12 Biosynthetic route of chalcones and flavanones

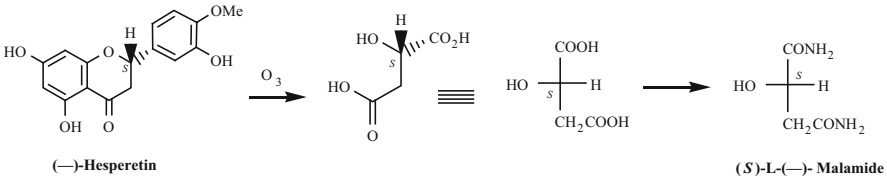


Fig. 14.13 Determination of the absolute configuration of (–)-hesperetin

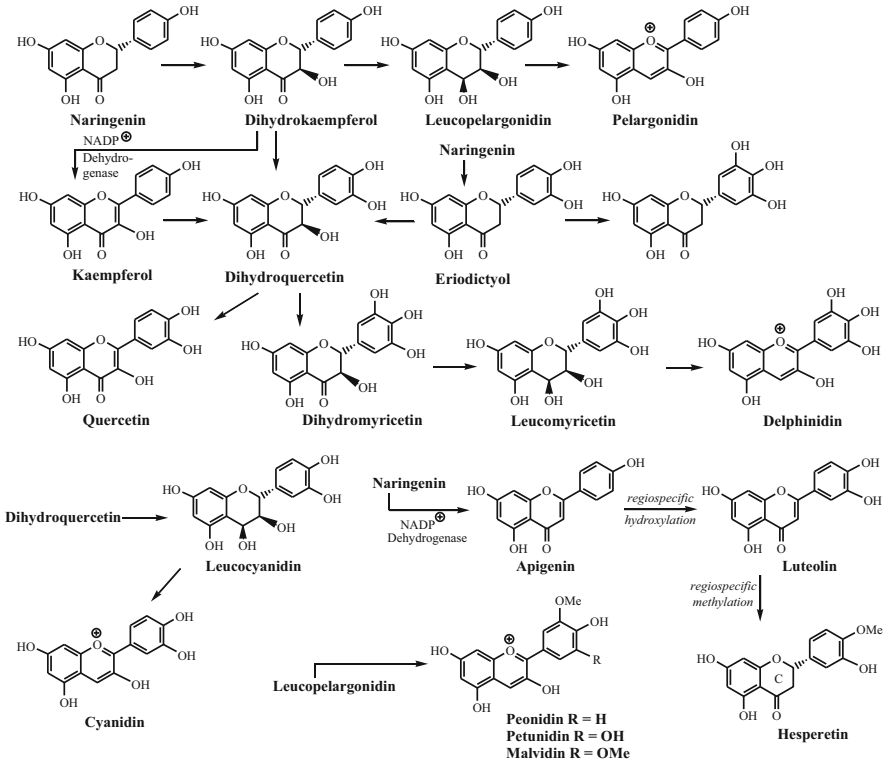


Fig. 14.14 Probable biogenetic relationship of natural flavanones, hydroxylated flavanones, flavonoids, anthocyanidins, etc.

14.4.4.3 The Probable Biogenetic Relationships

Flavanones, hydroxylated flavanones, flavonoids anthocyanidins are biogenetically related as shown in Fig. 14.14. Regiospecific hydroxylase, reductase, dehydrogenase, etc. are used in vivo for the necessary conversions.

14.4.4.4 Biogenesis of Isoflavones from Flavanones Through Radical Formation (Fig. 14.15)

Regiospecific methylation and hydroxylation by appropriate enzymes leads to several substitutions around the isoflavone nucleus (Fig. 14.15).

14.4.4.5 Biosynthesis of Isoflavones from Chalcone Precursors

This is conceived to take place by 1,2-aryl migration. A chemical analogy to this conjecture has been shown by Ollis et al. [26] by converting a chalcone to an isoflavone using thallic acetate (Fig. 14.16).

This conversion provides a new general route for the synthesis of isoflavones from the corresponding chalcones. Thallic acid oxidation and reorganization of a chalcone to the corresponding isoflavone is a remote model for the biosynthesis of isoflavone, but the oxidative processes could correspond.

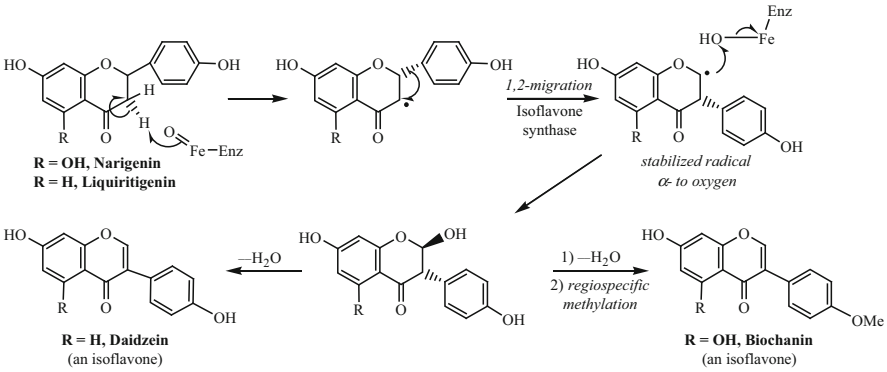


Fig. 14.15 Biogenesis of isoflavones from flavanones through radical formation

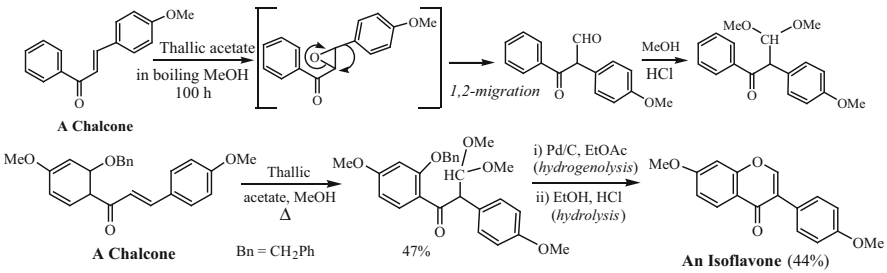


Fig. 14.16 Chemical analogy for isoflavone biosynthesis

14.4.4.6 Biosynthesis of Aurones

Probable bioformation of aurones and an isoflavanoid variant medicarpin are outlined in Fig. 14.17.

14.4.4.7 Bioformation of Isoflavonoid Variants

The ring juncture between dihydrofuran and tetrahydropyran rings may be enantiomeric, as observed in cases of (–)-(6*aR*, 11*aR*)-maackiain and (+)-(6*aS*, 11*aS*)-maackiain, probable bioformation of which from the corresponding dihydroisoflavone, (–)-sophorol, is shown in Fig. 14.18.

The stereochemical features at the ring juncture is not obvious for the (+)-variety. However, label incorporation of (–)-(3*R*)-sophorol into both (+)- and (–)-maackiain has been observed; perhaps the stereochemistry at C3 has been changed in a later stage, after the dihydrofuran ring formation takes place by nucleophilic

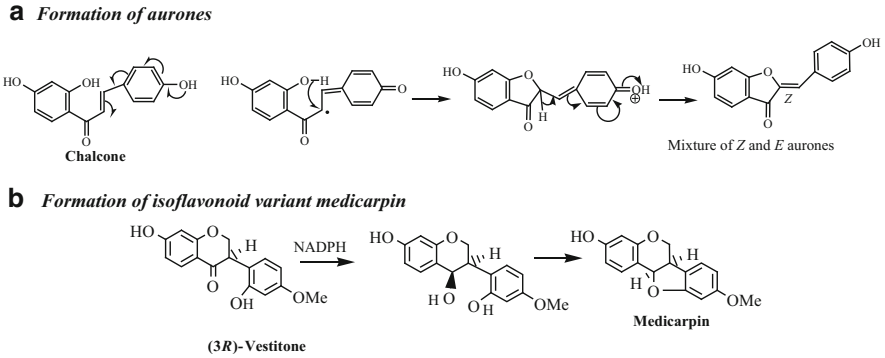


Fig. 14.17 Bioformation of aurones and an isoflavonoid variant medicarpin

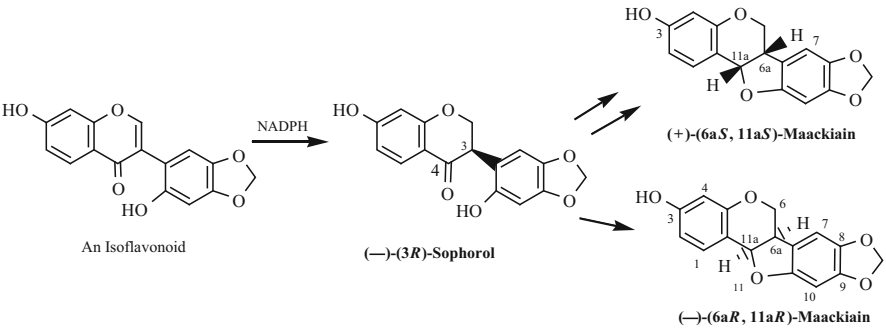


Fig. 14.18 Bioformation of isoflavonoid variants

attack by OH at CO (at ~109°) (see Sect. 2.13.4.3) carbon of sophorol from both Re (β-) and Si (α-)faces, made possible by rotation around C3-aryl bond.

14.4.4.8 Biosynthesis of Resveratrol and Veniferin

In some plants like pine, grape vines, and peanuts, the presence of stilbene synthase uses the same substrate (**A**) (Fig. 14.12) and converts it to a stilbene derivative resveratrol through intramolecular Claisen condensation. The elimination of four molecules of CO_2 and four molecules of HSCoA in the stilbene formation process during the formation of the intermediate (**A**) (Fig. 14.12) make the process of resveratrol biosynthesis an irreversible one (Fig. 14.19).

Stilbenes being potential fungicides, a gene from grapevine has been expressed in tobacco by genetic engineering. The expressed gene elaborates the enzyme in transgenic tobacco needed for resveratrol synthesis and other potent plant

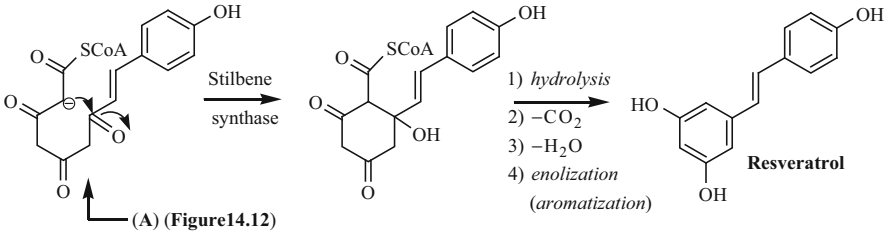


Fig. 14.19 Biosynthesis of resveratrol

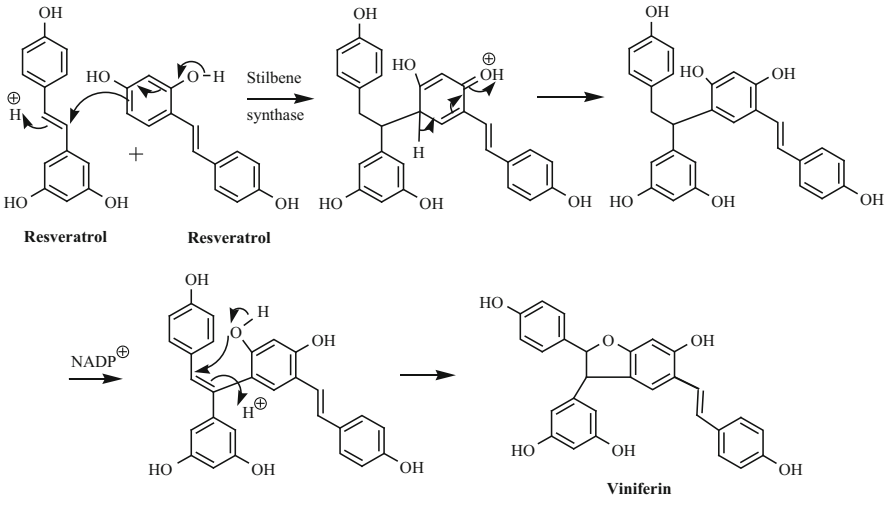


Fig. 14.20 Biosynthesis of veniferin via dimerization of resveratrol

fungicides including **veniferin** (Fig. 14.20). The transgenic tobacco plants thus become resistant to the fungus *Botrytis cinerea*. Veniferin is biosynthesized via dimerization of resveratrol (Fig. 14.20).

14.4.4.9 Flavonoids Containing C_5 or C_{10} Side Chain or C_5 Derived Ring

Flavonoids also contain substituents other than methoxyl, hydroxyl, and methylenedioxy groups, viz, C_5 or C_{10} side chain and C_5 derived rings. Some examples are given in Fig. 14.21.

A large number of flavonoids also occur as their glycosides and as acylated (sinapic acid, ferulic acid, gallic acid, etc.) derivatives.

14.5 Catechins

14.5.1 Introduction. Various Classes

F. F. Runge (the discoverer of aniline) first described catechins as early as 1821. Catechin was first isolated from *catechu*, the extract of Indian *Acacia catechu* (Fam.

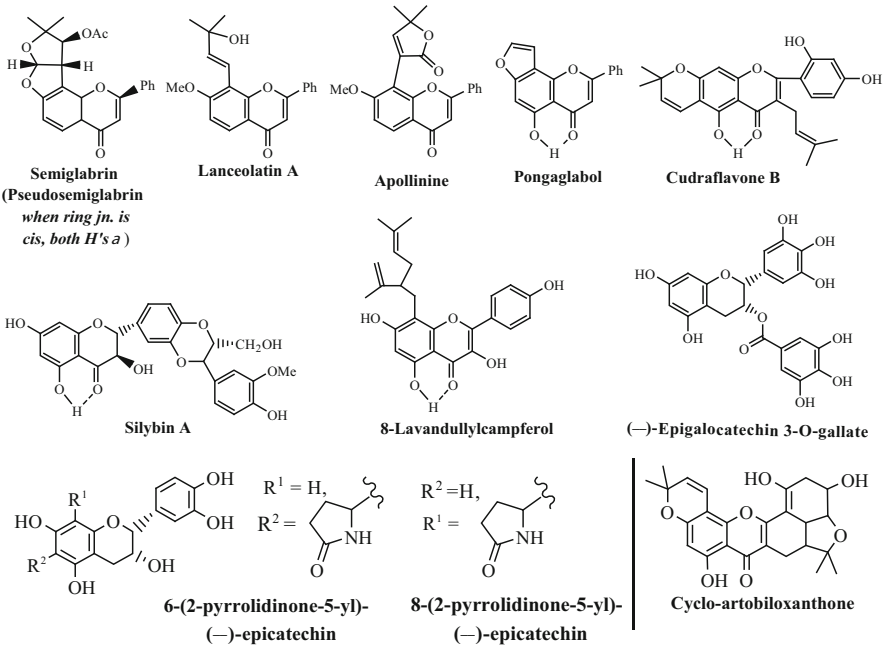


Fig. 14.21 Some flavonoids with C_5 or C_{10} side chain or C_5 derived rings

Leguminosae) along with some of its rearranged products. Another source of catechin is *gambir catechu*, the chief constituent of which is (+)-catchin. Gambir catechu is available as the commercial source of catechin.

14.5.2 Structure Elucidation

From the degradation products (Fig. 14.22), Perkin suggested that catechin is most probably a reduction product of quercetin and thereafter related it to flavonoids. He put forward two structures of which structure **1** turned out to be the correct one. Freudenberg confirmed it in 1920 from the identification of the reduction product of catechin followed by complete methylation (α,γ-diarylpropane derivative) and its synthesis as shown in Fig. 14.23. The dehydration product of catechin has been found to be optically inactive, hence (**a**) represents catechins.

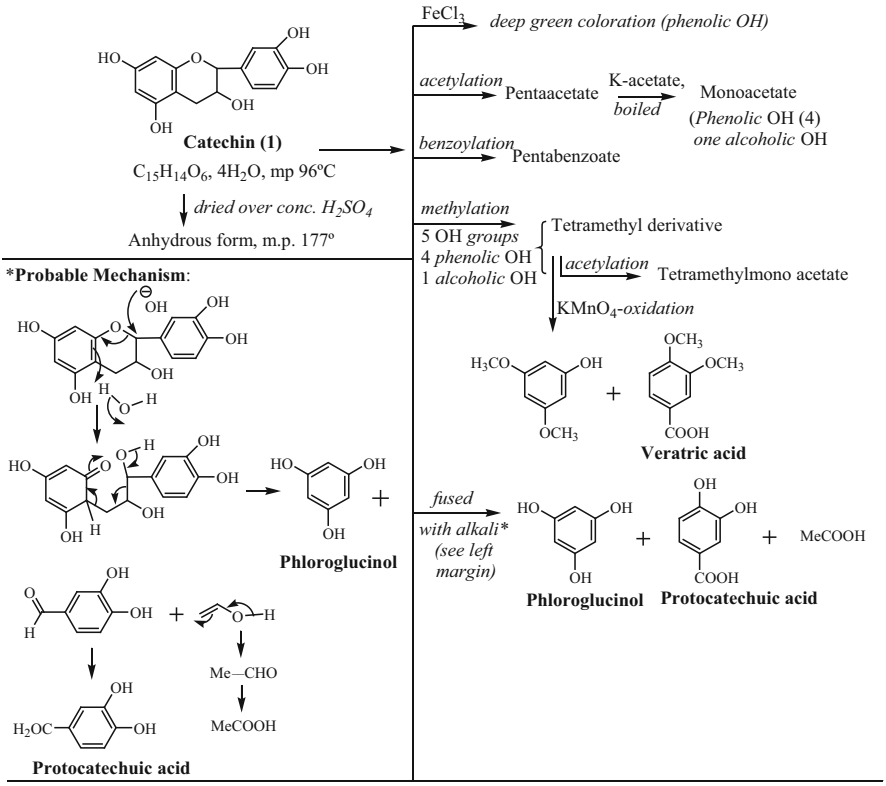


Fig. 14.22 Degradation products of catechin

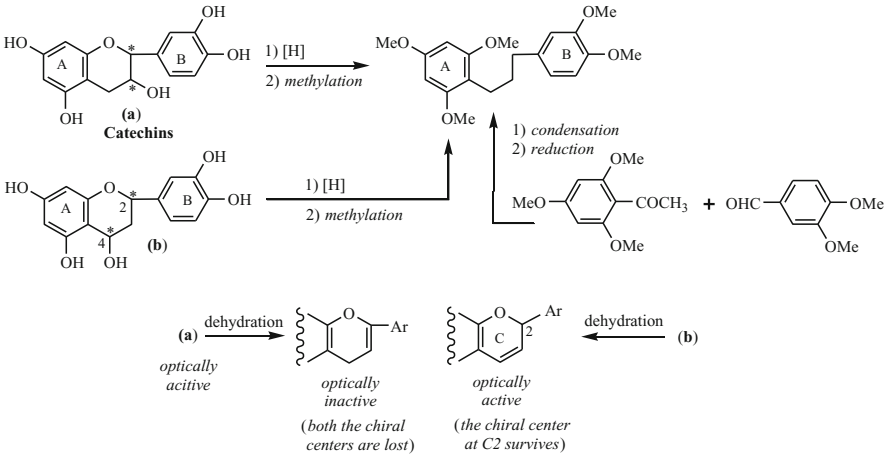


Fig. 14.23 Confirmation of the gross structure of catechins (Freudenberg)

14.5.3 Relative and Absolute Configurations of Catechins

14.5.3.1 Relative Configuration

Catechins contain two adjacent asymmetric centers (C2 and C3). Consequently, they are present as two diastereomeric (±)-pairs and hence as four stereoisomers. One set of diastereomeric pair eliminates the elements of water easily via the corresponding tosylate and hence holds 3-OH and 2-H in *trans* relationship. However, the other diastereomeric pair does it, but not so smoothly, indicating the *cis* disposition of C3-OH and C2-H. The former set is referred to as epicatechin enantiomers and the other set catechin enantiomers. The diastereomeric relationship of epicatechins and catechins has been arrived at by comparing the rotations of the products from (+)-catechin tetramethyl ether and (−)-epicatechin tetramethyl ether by reductive ring opening, followed by methylation (Fig. 14.24).

Rotation measurements indicated compounds (**A**) and (**A′**) to be enantiomers. (+)-Catechin and (−)-epicatechin, being diastereoisomers, will have identical absolute configuration at C2, the chirality of which is lost in both (**A**) and (**A′**) during their formation, while the configurations at C3, being preserved, are opposite in (+)-catechin and (−)-epicatechin. Further, as already mentioned, C2-H and C3-OH are *trans* oriented in epicatechin and *cis* oriented in catechin. Thus, the establishment of the absolute stereochemistry at one chiral center of any optically active diastereomer will establish the absolute configurations of both chiral centers of all the catechin stereoisomers from their relative configurational relationship.

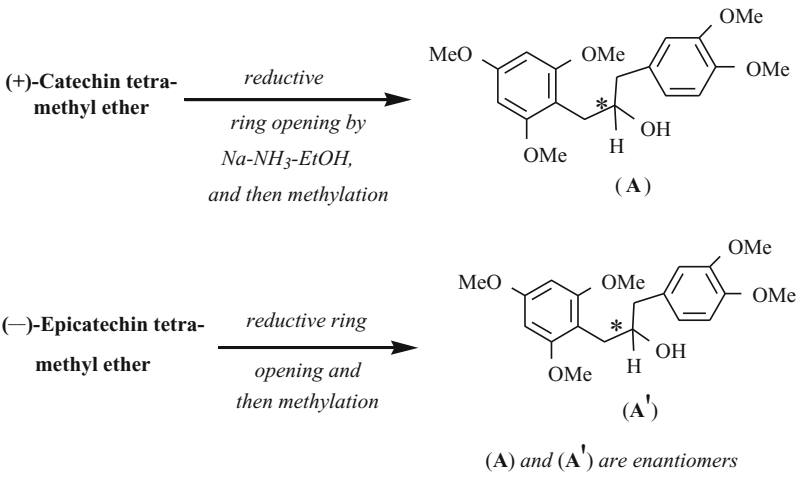


Fig. 14.24 Reductive ring openings of diastereomers to form enantiomers

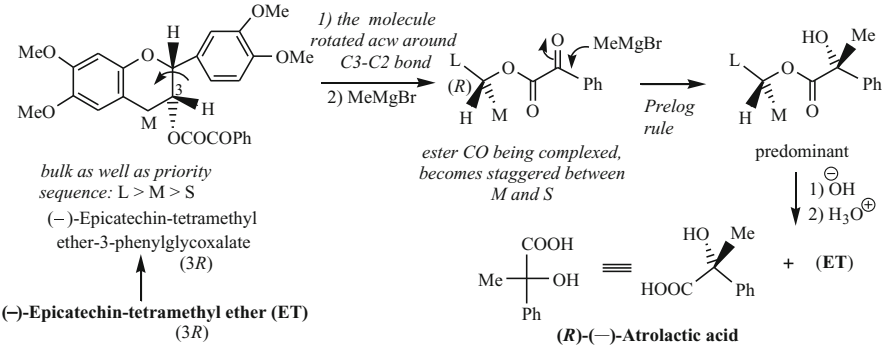
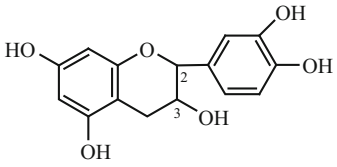


Fig. 14.25 Absolute configuration of (−)-epicatechin by application of Prelog rule



14.5.3.2 Absolute Configuration by Application of Prelog Rule

(−)-Epicatechin-tetramethyl ether in the atrolactic acid synthesis as per Prelog rule (see Chap. 2) furnished preferentially (*R*)-(–)-atrolactic acid indicating the (*R*) absolute configuration of the former at C3 [27] (Fig. 14.25). However, (3*S*)-(+)-

catechin-tetramethyl ether upon subjecting to Prelog procedure unexpectedly gave predominantly (*R*)-(+)-atrolactic acid instead of (*S*)-(–)-atrolactic acid. These two diastereomers, being epimeric at C3, should give enantiomeric atrolactic acids. The reverse and weak stereoselectivity of the reaction of (+)-catechin-tetramethyl ether having C3 (*S*) configuration and the presence of the oxygen atom close to the induction center along with its conformational mobility (see Fig. 14.29) imply that the correct environment for the application of the Prelog rule is not fulfilled in this case. Thus, this result should be considered as an anomaly rather than an exception to the rule [27]. The absolute configurations of catechins that follow are shown in Fig. 14.26.

14.5.3.3 Absolute Configuration by Chemical Correlation [14]

The absolute configuration of (+)-catechin (hence for its three other stereoisomers from their relative configurational relationship) has been settled by chemical correlation of one of its degradation products with 2-deoxy-D-ribose [14] (Fig. 14.27).

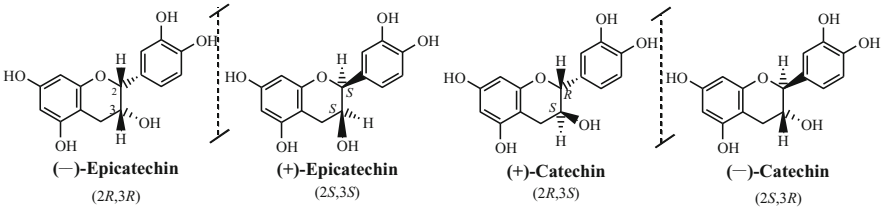


Fig. 14.26 Absolute configurations of catechins

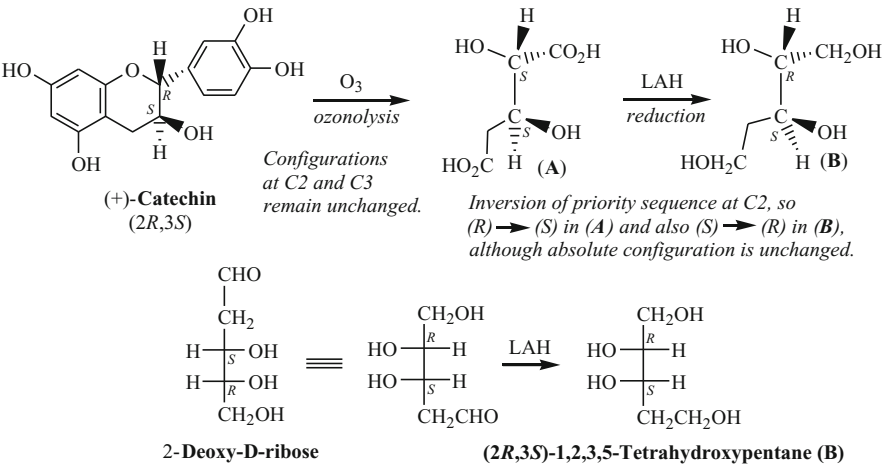


Fig. 14.27 Chemical correlation of (+)-catechin with 2-deoxy-D-ribose

Likewise, following the sequence of reactions for (+)-catechin (Fig. 14.27) (−)-epicatechin could be correlated to 2-deoxy-D-xylose (Fig. 14.28).

14.5.4 Conformations of Catechin and Epicatechin

In (+)-catechin and (−)-epicatechin and their enantiomers, the cyclohexene ring C will exist in a half chair conformation (Fig. 14.29). (+)-Catechin tetramethyl ether (**2**) as well as (−)-epicatechin tetramethyl ether (**4**) absorbs in their IR spectra at 3,594 and 3,587 cm^{-1}, respectively, which has been explained in terms of intramolecular hydrogen bonding [28, 29]. These absorptions are assignable to H-bonded OH as evident from their *aa* and *ea* conformers respectively

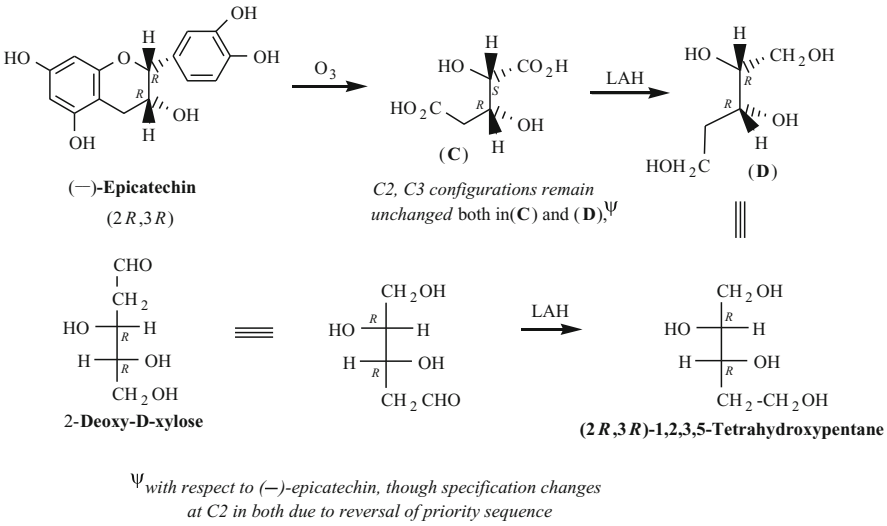


Fig. 14.28 Chemical correlation of (−)-epicatechin with 2-deoxy-D-xylose

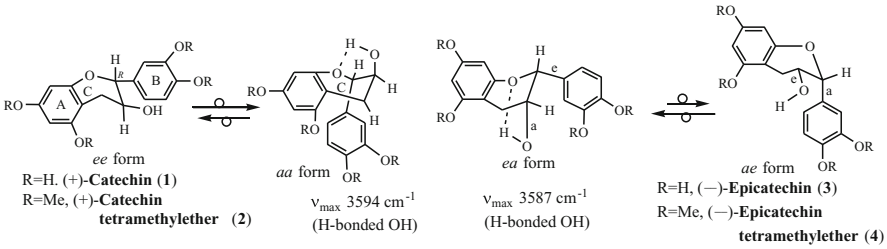


Fig. 14.29 Preferred conformations of (+)-catechin and (−)-epicatechin and their tetramethylethers

(Fig. 14.29). Compounds (**2**) and (**4**) are reported to lack any free OH absorption near 3,630 cm^{-1}. Thus, the compound (**2**) [and hence compound (**1**)] having *trans* stereochemistry of the aryl and OH groups will have preferred H-bonded *aa* conformation, whereas the compound (**4**) [and hence compound (**3**)] having *cis* stereochemistry of the aryl and OH groups will have preferred H-bonded *ea* form. In case of (**1**) and (**2**) in the *aa* conformer stabilization due to H-bonding is more than outweighing the destabilization due to nonbonded interaction between the aryl group at C2 and a *syn*-axial H at C4. The slightly higher frequency of (**2**) than that of (**4**) represents slightly weaker H-bonding in (**2**), presumably due to the axial aryl group in its *aa* conformer.

14.5.5 Synthesis of Catechins

Flavan-3-ols display various biological properties, e.g., antioxidant and antiaging properties in human, activity against neurodegenerative diseases, and cancer. They are important starting materials for the synthesis of dimers, e.g., procyanidins and other biologically important coupling products. They are synthesized generally by intramolecular epoxide ring opening of 1,3-diarylpropene epoxide or propene dihydroxylation, followed by cyclization (Fig. 14.30).

Ferreira [30, 31] obtained flavanols in good yield and high enantiomeric excess by subjecting 1,3-diarylpropene to asymmetric hydroxylation followed by acid catalyzed cyclization. Thus, 1,3-diarylpropene is the key precursor (or synthon) in the synthesis of flavan-3-ols. Gession et al. [32] designed a general and efficient alternative entry into 1,3-diarylpropenes based on cross metathesis of protected 2-allylphenols with styrene in presence of Grubbs catalyst. $Cl_2(PCy_3)_2Ru{=}CHPh$ (Fig. 14.31). The products were identified by spectral analysis.

Grubbs [33] used olefin cross metathesis as a method for the synthesis of styrenyl-olefins. Working with several styrenes and a variety of substituted olefins substituted styrenes were synthesized with excellent control of olefin geometry. The substituted styrenes could be easily converted to flavan-3-ols. One such example is shown in Fig. 14.32.

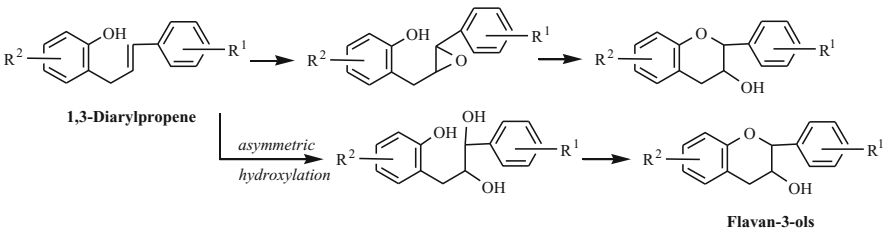


Fig. 14.30 Alternate routes for the synthesis of flavan-3-ols

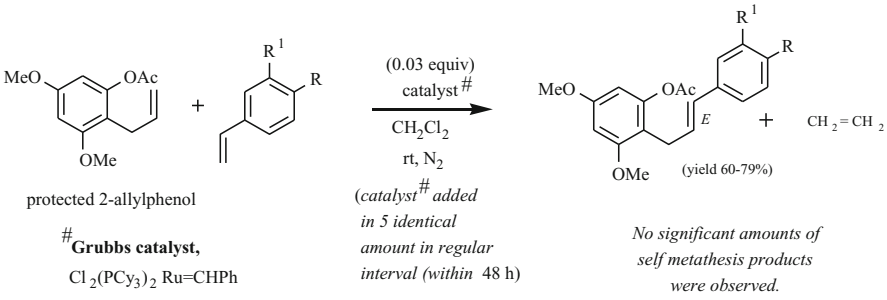


Fig. 14.31 Synthesis (2001) of a chalcone by cross metathesis by Grubbs catalyst

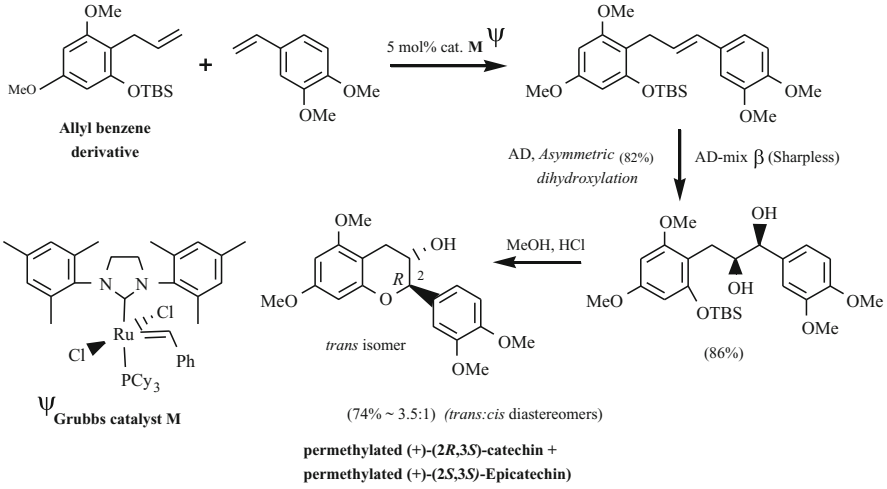


Fig. 14.32 Cross metathesis route for the synthesis of permethylated catechins

For the synthesis of flavan-3-ols, Karsten [34] used Mitsunobu cyclization of the AD product of β-benzyl styrene derivatives in which the phenolic OH group *ortho* to benzyl carbon remains free. Unlike other methods, protection and deprotection are not needed—hence two steps are eliminated.

14.5.6 A Rearrangement Reaction of Catechin

Catechin tetramethyl ether undergoes a rearrangement involving 1,2-shift which is mechanistically displayed in Fig. 14.33.

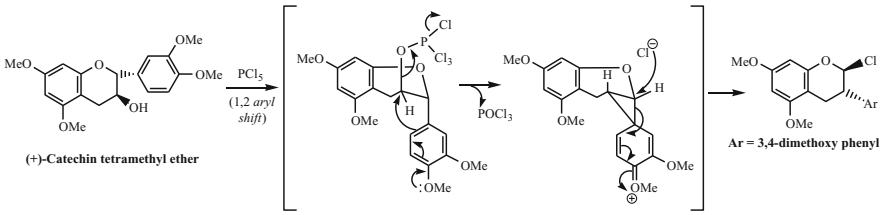


Fig. 14.33 A rearrangement reaction of (+)-catechintetramethyl ether

14.5.7 (+)-Catechin, A Potential Synthon for Representative PPAP (Polyprenylated Acylphloroglucinol) Natural Products (e.g., Garsubellin A, Hyperforin, Clusianone, etc.)

(+)-Catechin has been reported to undergo a remarkable highly stereoselective rearrangement into a bicyclo[3.3.1] nonane-1,3,5-trione derivative *catechinic acid* [=enol of 6-(3,4-dihydroxyphenyl)-7-hydroxy-2.4.9-bicyclo[3.3.1]nonatrione (X-ray)] under alkaline condition (Fig. 14.34) [35].

The genesis of this study has an interesting background. Flavan-3-ols and/or flavan-3,4-diols get polymerized into polyphenolic polymers, which are present in all the studied coniferous bark. They also occur in the heartwood and bark of a significant number of deciduous plants [34]. The conventional way of solvent extraction failed to isolate them from their sources, but they could be isolated using dilute alkali or alkaline bisulphate. The isolate contained COOH group. To find out whether the COOH is an original component of polymer or generated during the process of isolation, catechin being a common monomeric component of many such polyphenolic polymers has been chosen as a substrate to verify the fact. Hence, the study.

Catechinic acid could serve as the promising enantiopure entry into the bicyclo [3.3.1] nonanetrione core of PPAP (polyprenylated acylphloroglucinol) natural products (Fig. 14.34) [36]. In an attempt to utilize catechinic acid for the synthesis of PPAP natural products, the desired substituents and the replacement of the existing substituents by the desired ones have been planned and tried. In this endeavor selective substitution at the C5 bridge head, C3 sp^2 carbon and oxidative cleavage of the catechol part of catechinic acid have been done.

The conversion of catechin to catechinic acid under alkaline condition may proceed into its retrobiosynthetic fashion. It opens up into dihydrochalcone intermediate followed by C-alkylation (instead of o-alkolation as in catechin) of the phloroglucinol part by the methide part of the intermediate as shown in Fig. 14.35.

Attempts have been made for the syntheses of the natural PPAP from catechinic acid by appending substituents to the core nucleus derived from catechinic acid. While furan ring could be introduced as in garsubellin A (Fig. 14.34), the oxidative

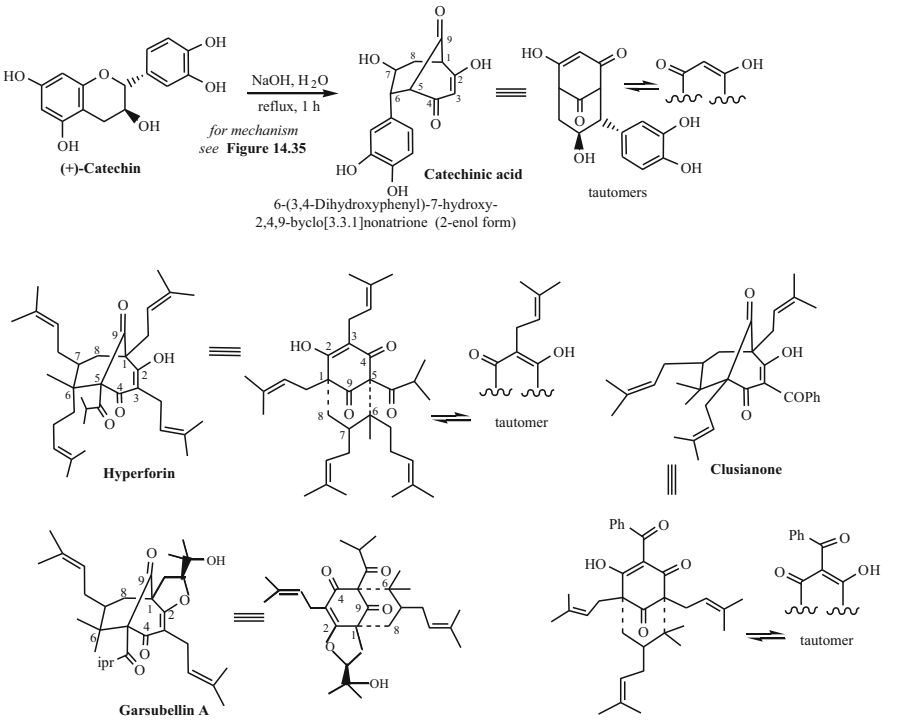


Fig. 14.34 Catechin to catechinic acid and some examples of PPAP natural products

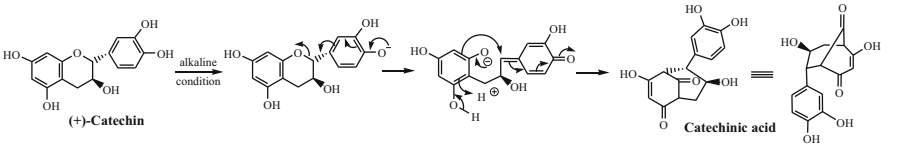


Fig. 14.35 Plausible mechanistic explanation for the formation of catechinic acid from catechin

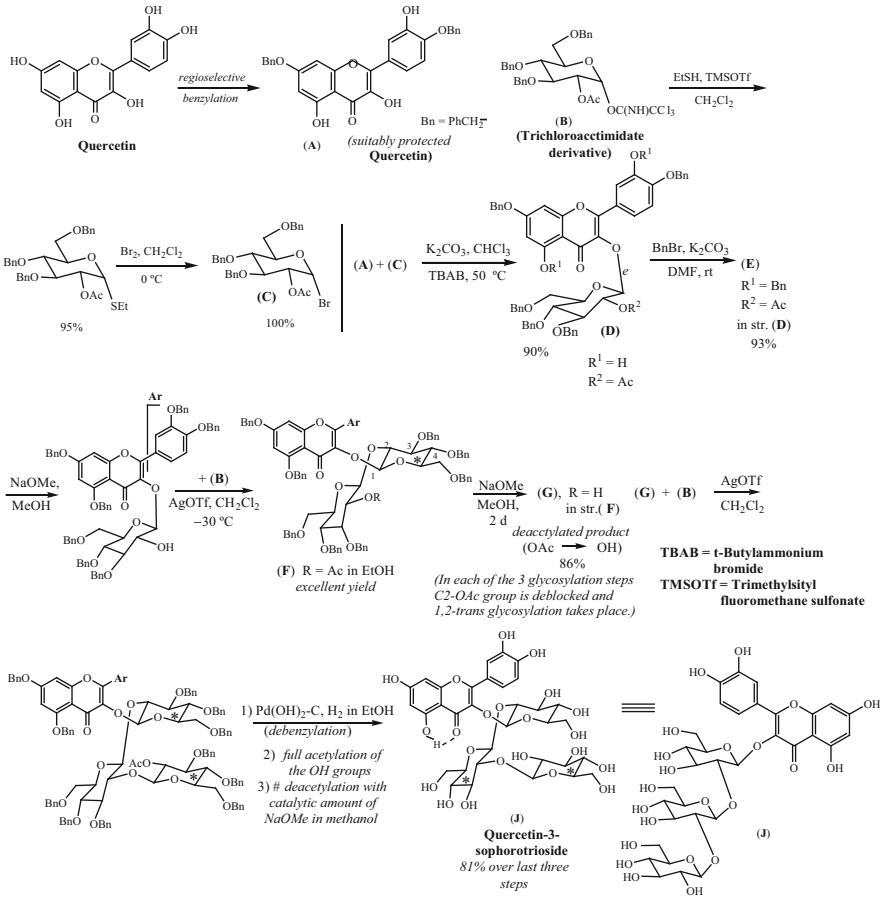
degradation of the aromatic ring to COOH was not successful. However, the products obtained from catechinic acid have been tested for biological properties.

14.5.8 Preparation of Some Useful Derivatives

14.5.8.1 O- and C-Glycosylation of Flavonoids

Flavonoid glycosides are potential drugs and health protective phytonutrients. They are widely distributed in fruits, vegetables, and many medicinal plants. Their

antimicrobial, anticancer, and antioxidant properties made them potent drug candidates. They are better absorbed in the system compared to aglycones. The regiospecific glycosylation, however, remains a difficult task. Various blocking reagents and their deblocking conditions are involved. Glycosylation of a few flavonoids will be discussed briefly. A total synthesis of quercetin-3-sophorotrioside (**J**) (isolated from the young seed pods of *Pisum sativum*, Fam. Leguminosae) (Fig. 14.36) has been reported [37]. Soforose (Sophorose) is the trivial name of the disaccharide, 2-D-β-glucopyranosyl-D-glucose [38]. β-Sophorotrioside means 3 glucose units in the form of a trisaccharide through glycosidic bond as in



Notes: (i) * *All tetrahydropyranose units in this* ***Figure*** *are D-glucose derivatives; those with an asterisk were drawn enantiomeric L - glucose derivatives by the authors in their paper [37] by oversight. Here, in each ring the O atom to the anomeric C, and hence carbons 1,2…5 are arranged clockwise, keeping the α,β orientation of the substituents unchanged, so that the oxymethyl group is β (up position) ; thus each ring represents a D-glucose unit.*

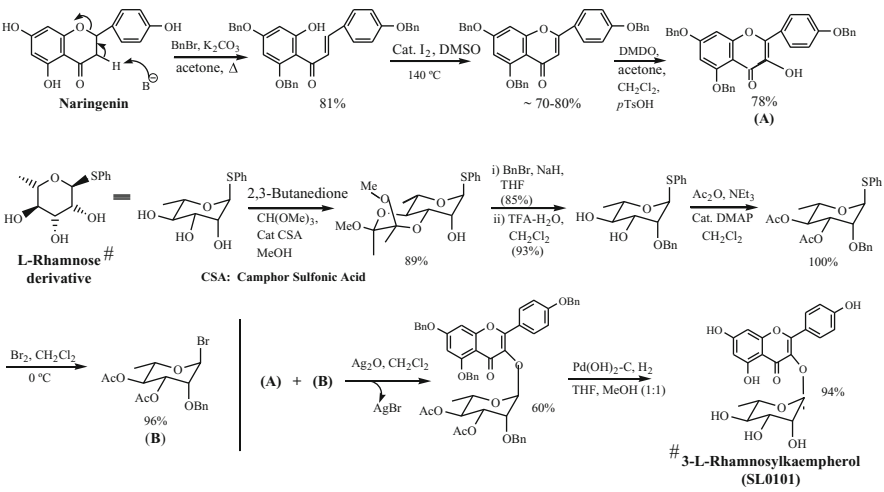
(ii) # *Deacetylation at this stage is extremely difficult.*

Fig. 14.36 Glycosylation of quercetin to quercetin-3-sophorotrioside

3-*O*-β-D-glucopyranosyl(1-2)-β-D-glucopyranosyl-(1-2)-β-D-glucopyranoside, 3 being the site of attachment of quercetin (aglycone). After several unsuccessful attempts, the following sequence (Fig. 14.36) of simple linear eight-step synthesis of quercetin-3-sophorotrioside has been achieved [37]. The method using a combination of AgOTf (silver triflate) and phase transfer catalyst-promoted carbohydrate chain elongation during quercetin-3-glycosylation with both sugar bromide and trichloroacetimidate donors. Suitably protected quercetin has been prepared according to Jurd's method [39].

Synthesis of a Naturally Occurring Kaempherol Glycoside: A Potential Anticancer Agent [40]

Naringenin (4′,5,7-trihydroxyflavanone) when treated with benzyl bromide and excess K_2CO_3, the hydroxyl groups are protected and a facile β-elimination takes place to yield the corresponding protected chalcone. The latter is converted into the flavone by treatment with catalytic amount of I_2 in DMSO at 140 °C. The 3-OH group has been introduced using dimethyldioxane (DMDO) followed by opening of the resulting epoxide with *p*-toluenesulfonic acid to yield the flavone (**A**) (Fig. 14.37). The latter [compound (**A**)] is condensed with the **L**-rhamnose derivative (**B**) to produce after deprotection of the naturally occurring 3-**L**-rhamnosylkaempherol (**SL0101**) (Fig. 14.37). **SL0101** exhibits selective and potent p90 Rsk inhibitory activity at nanomolar concentrations without inhibiting the function of upstream kinases [40].



#*Starting from the oxygen the anomeric carbon is in the clockwise direction and the methyl at C 5 is α (down position), hence the sugar is* **L**.

Fig. 14.37 Synthesis of SL0101, a potent selective inhibitor of p90 Rsk

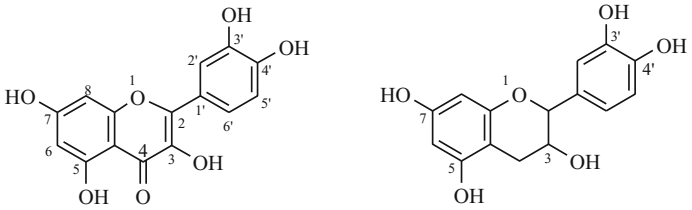


Fig. 14.38 Structures (with numbering) of quercetin and catechins

Glycosylation and Malonylation of Quercetin, Epicatechin, and Catechin by Cultured Plant Cells [41]

Supported by experiment it has been shown that cultured plant cells of *Nicotiana tabacum* are able to convert quercetin in the early stage of incubation into its 3-*O*-β-glucoside and its subsequent malonylation regiospecifally at C6 position of the glucose moiety. It has been postulated that C2–C3 double bond is necessary for the regiospecific bioglycosylation at C3 of quercetin as the major products in 51 % yield and its 6-malonyl (sugar C6) derivative in 10 % yield along with other glycosides in low yields formed after nearly 10 h incubation. This is the first report on the glycosylation and malonylation of exogenously added quercetin by cultured plant cells. The percentages of yields of the products have been calculated on the basis of HPLC peak areas.

In a similar experiment after 5 days of incubation, epicatechin-3′-*O*-β-glucoside (38 %), 5-*O*-β-D-glucoside (97 %), 7-*O*-β-D-glucoside (15 %), catechin-3′-*O*-β-D-glucoside (46 %) (see Fig. 14.37), 5-*O*-β-D-glucoside (10 %), and 7-*O*-β-D-glucoside (17 %) could be obtained. This is the first report on the glycosylation of epicatechin by cultured plant cells. (The numberings of quercetin and catechins are shown in Fig. 14.38.)

14.5.8.2 C-Glycosylation

A variety of C-glycosylflavonoids are known to occur in plants. The C-glycosidic bonds are located at C6 and C8 of ring A of flavonoid moiety. These glycosides are well known as antioxidants and feeding stimulants for insects and are also responsible for color development of flowers. The synthesis of *flavocommelin* (7-*O*-methylapigenin-6C-, 4′-di-β-D-glucose) (Fig. 14.39), a component of the blue supramolecular pigment *commelinin* (a metalloanothocyanin, structure done by X-ray), isolated from *Commelina communis*, Fam. Commelinaceae) first appeared in 2004 [42]. In this synthesis a direct C6-glycosylation has been achieved. The synthesis of a flavone bearing both O- and C-glycosides has not been reported earlier. The sequence of steps in the synthesis of flavocommelin is delineated in

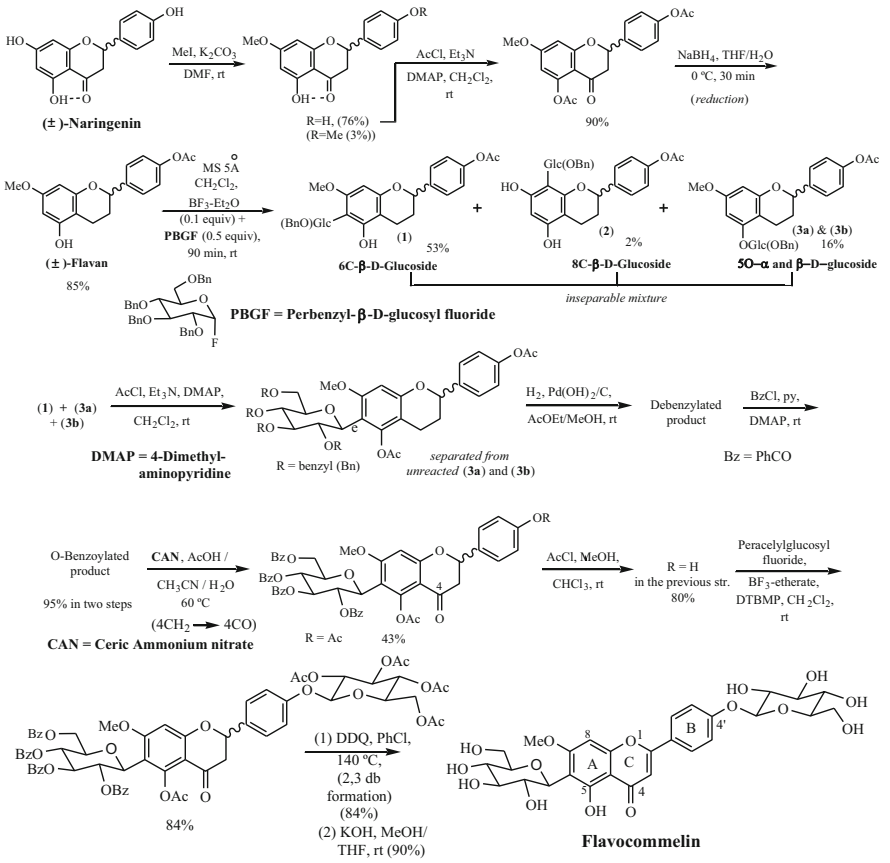


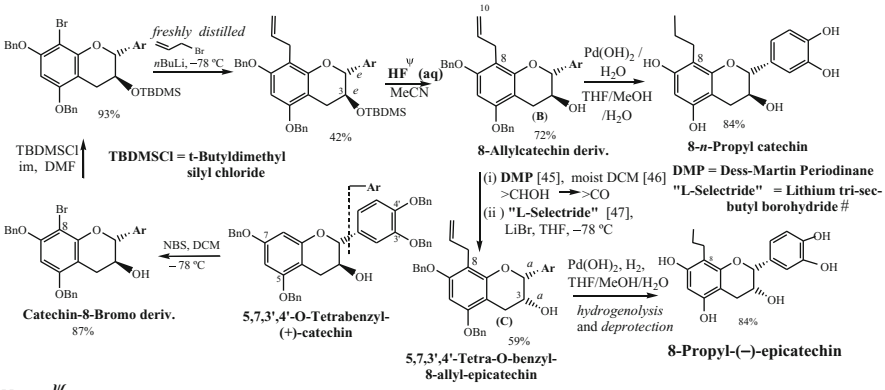
Fig. 14.39 Synthesis of flavocommelin (via direct C-glycosylation)

Fig. 14.39. It may be mentioned that commelinin is the most stable of the metalloanthocyanins and is a stoichiometric self-assembled supramolecule composed of six molecules of malonylawobanin (M), six molecules of flavocommelin (F), and two magnesium ions ($M_6F_6Mg_2^{+2}$). These components are specially self-assembled by strict chiral molecular recognition [42].

14.5.8.3 Preparation of Some Potential Anticancer Derivatives from (+)-Catechin and (−)-Epicatechin via Functionalization at C4 and C8 [43]

Protection of Phenolic OH Groups in Catechins

The positions C4 and C8 of catechins are the linking positions of the flavonoids in natural procyanidins. Prior to the stereoselective functionalization at C4 and C8, the



Notes: Ψ *HF being corrosive plastic reaction vessel and plastic syringe were used.*

Lithium tri-sec-butylborohydride appears to be the most stereoselective reagent known for reduction of ketones, aldehydes, and esters. The highly hindered borohydride reagent stereoselectively attacks the CO group from the equatorial side to form the axial alcohol with>93% stereo selectivity [47]..

Fig. 14.40 Benzylation of (+)-catechin and structure of a procyanidin

phenolic groups of the catechins are blocked as benzyl derivatives (Fig. 14.40). It was not needed to protect the 3-OH, which could be esterified with the galloyl group at a later stage.

Stereoselective Functionalization at C4

Protected (+)-catechin is treated with DDQ in dichloromethane (DCM) in the presence of allyl alcohol. The DDQ oxidation is followed by the trapping of the quinonemethide with allyl alcohol acting as an external nucleophile. From the formed allyl ether, a quinonemethide could be regenerated by BF_3-etherate and trapped by a wide range of external nucleophiles [43] (Fig. 14.41).

On NBS treatment, the protected catechin-8-bromo derivative is formed. The latter could be alkylated at C8. Some of the nucleophilic substitutions of the protected (+)-catechin at C8 are presented in Fig. 14.41. Deprotections of the phenolic OH groups have been carried out at the final step.

Synthesis of C8 Substituted Catechin and Epicatechin Derivatives

Since commercially available epi-catechin is considerably more expensive than catechin, the authors [43] decided to use the C3-alcohol inversion methodology developed earlier [44].

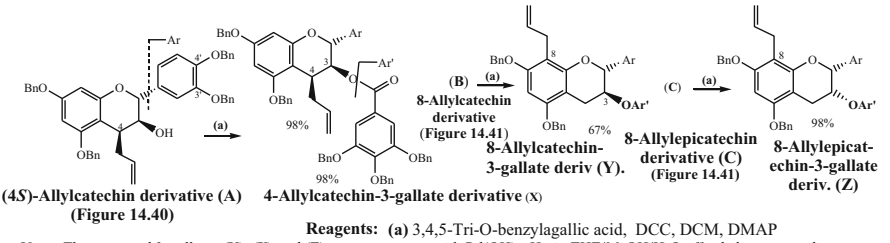


Fig. 14.41 Stereoselective nucleophilic substitution at C4 of catechin [43]

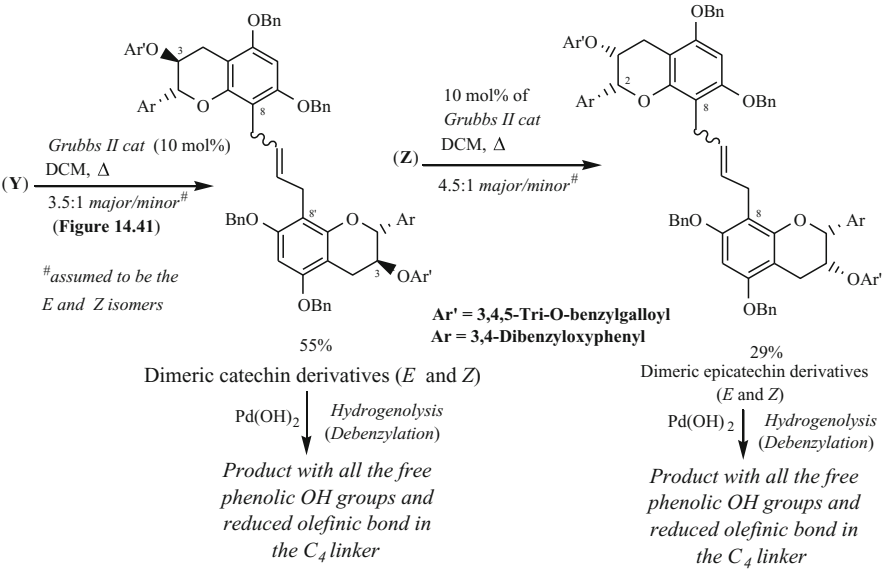


Fig. 14.42 Synthesis of C8 substituted catechin and epicatechin analogues

Galloylation

Galloylation at the available C3-OH of (**A**) (Fig. 14.41), (**B**), and (**C**) (Fig. 14.42) gave the corresponding galloyl esters of (**X**), (**Y**), and (**Z**) in 98, 98, and 67 % yields, respectively (Fig. 14.43). The compounds (**X**), (**Y**), and (**Z**) were treated with Pd $(OH)_2$, H_2, in THF/MeOH/H_2O to give the corresponding totally debenzylated 8-dihydroallyl (8-*n*-propyl) derivatives in 76, 80, and 78 % yields, respectively. These gallate ester derivatives of the novel C4 and C8-substituted catechins were screened for potential anticancer activity in a range of human cell lines. It was found from preliminary cytoxicity assay that *C8 propyl-catechin gallate was more active than catechin gallate or epicatechin gallate*. Differential sensitivity in pancreas, bladder, stomach, liver, and fibroblasts cell lines was also observed [43].

Metathesis Dimerization

Dimerization of catechin derivatives by C_4-linker between C8–C8 and C4–C4 using Grubbs catalyst has also been achieved. The protected 3-galloyl esters (**Y**) and (**Z**) with C4 and C8 allyl side chains were allowed to undergo cross-metathesis using 10 mol% of Grubbs second generation catalyst to yield "pseudo" flavonoid homodimers as shown in Figs. 14.43 and 14.44 [44]. Both dimers were isolated as a mixture of major and minor products assumed to be the *E* and *Z* isomers. They possess a C8 → C8 unsaturated linker between the monomer units.

The acetyl derivative of compound (**A**) was subjected to identical metathesis condition to form the C4 → C4 homodimers (mixture of *E* and *Z* isomers) in 73 %

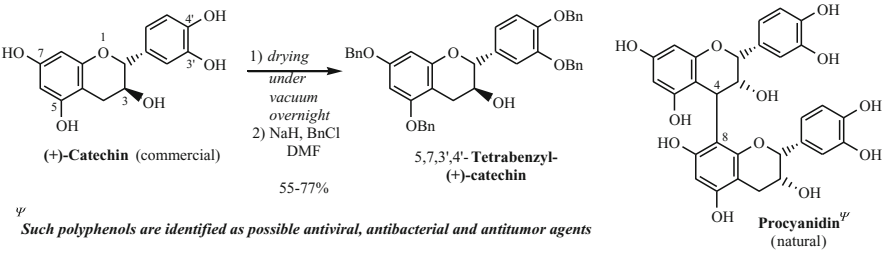


Fig. 14.43 Synthesis of C4 and C8 substituted 3-gallate derivatives of catechin and epicatechin

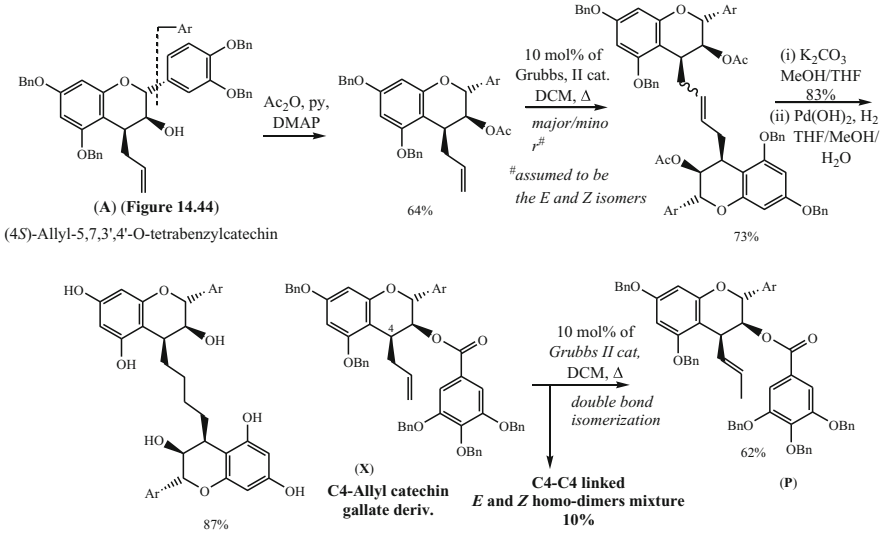


Fig. 14.44 Synthesis of dimeric catechin and epicatechin-3-gallates with a C4-linker between C8–C8

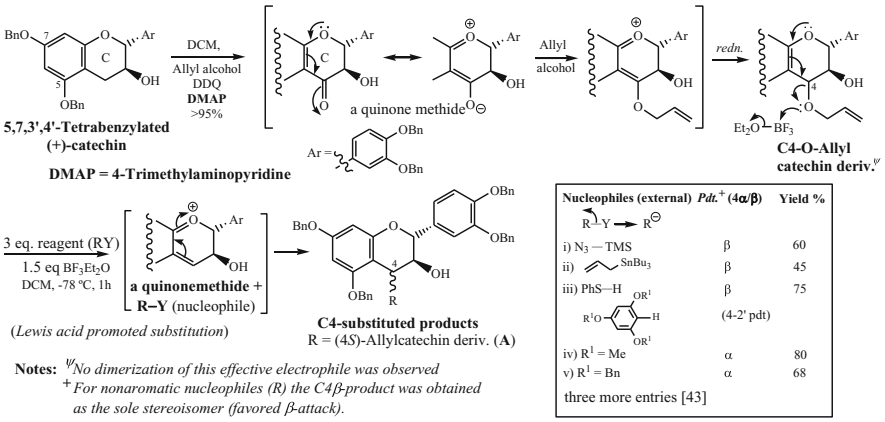


Fig. 14.45 Synthesis of C4–C4 linked catechin dimer and Grubbs catalyst-promoted double bond isomerization [43]

yield (Fig. 14.45). But when C4 allyl catechin gallate derivative (**X**) was subjected to identical condition, only 10 % of the desired C4 → C4 linked homodimer mixture was formed, and the isomeric disubstituted alkene (**P**) was isolated as the major product (62 %). Perhaps steric hindrance was retarding the cross-metathesis reaction.

Interestingly, attempted hetero-dimerization between 8-allyl derivative (**Y**) and 4-allyl derivative (**X**) failed to form natural (Fig. 14.40) type C4 → C8 linked hetero-dimers because of the competing homo-dimerizations.

A few dimeric catechin and epicatechin gallates with C_4 linker between C8–C8′ and C4–C4′ have been subjected to cell proliferation assays and were found to have slightly lower activity than the corresponding monomeric parent compounds [44].

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Chapter 15
Alkaloids. General Introduction

Alkaloids constitute a major class of natural products. The compulsory presence of nitrogen/s in their elemental contents confers basic character in these compounds save a few. Pelletier (Appendix A-21) collaborated with Dumas and showed the presence of nitrogen in alkaloids. The basic or alkali like property of alkaloids was first observed by Sertürner while working with opium constituents and he used the term "*vegetable alkali*." The term alkaloid [(derived from "alkali" and Greek *oeides* (like)] was coined by W. Meissner, an apothecary of Halle, in 1819. Later it became a generally accepted term.

The alkaloids always enjoy an important status among the natural products because of their important biological properties guided by their stereochemistry and conformation, whenever applicable. These properties are manifested remarkably in some cases, *e.g.*, *quinine, reserpine, morphine, vinblastine, vincristine, camptothecine, taxol* (a diterpene alkaloid), *atropine, nicotine*, etc. (Chaps. 18–29). They are superbly remarkable drug molecules (Chap. 33). Further, their structural deductions and synthesis have always been challenging. The first total synthesis of quinine by Woodward in 1945 [1, 2] (Chap. 25) remains a masterpiece in the synthetic repository of organic chemistry. Yet, its one stereoselective total synthesis has been achieved [3] in 2001 after 171 years of its discovery and after 56 years of its first synthesis. This is followed by another report on catalytic asymmetric total synthesis of quinine [4] in 2004 and a stereocontrolled formal synthesis of quinine and 7-hydroxyquinine by Webber et al. [5] in 2008.

In almost all cases of alkaloids excepting a few, nitrogen is supplied by L-amino acids which get incorporated into the alkaloidal scaffold, decarboxylation taking place at some stage of their biosynthesis. Rarely, nitrogen is supplied by transamination to a carbonyl precursor (nicotine, Chap. 18). The nitrogen/s mostly remain/s as a part of one or more ring/s. Sometimes nitrogen falls outside the ring and remains as a part of a side chain as amino or substituted amino group as we find in mescaline (a potent hallucinogen), choline (a component of vitamin B complex), betaines (trialkyl derivatives of amino acids, e.g. glycine betaine), histamine, serotonin, etc. In a betaine nitrogen can be a part of a ring, e.g., proline betaine. They are sometimes called *protoalkaloids* or *biological amines* (Fig. 15.1).

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There are also examples of heterocyclic nitrogenous bases, which are biosynthetically not related to amino acids. They are known as *pseudoalkaloids*, *e.g.*, caffeine, xanthine, etc. (Fig. 15.2).

These are conservative classifications. However, all these compounds could be generally classified as alkaloids. Though colchicine (Chap. 24) is neither basic in character nor a nitrogenous heterocycle, it has been included in the family of alkaloids as its nitrogen is supplied by an L-amino acid, L-tyrosine, and it possesses profound biological activity. Taxol (isolated from *Taxus brevifolia)* has the gross skeleton of a diterpene (taxadiene) and a nonbasic aromatic amide side chain; it has, therefore, been included in the Sect. 8.4 of diterpenoids. However, since its side chain is derived from rearranged L-phenylalanine, and it has exceptional biological activity, it is also included in the ambit of alkaloids by many. In fact, M. Hesse's book on "Alkaloids" [6] carries the structure of taxol on the cover, and Wall and Wani have written an article on camptothecine and taxol in a book dedicated to alkaloids [7].

Alkaloids are mostly solids and are known to occur in higher plants. They are prevalent in the plants belonging to the following botanical families: Apocynaceae, Annonaceae, Amaryllidaceae, Berberidaceae, Boraginaceae, Gnetaceae, Liliaceae, Leguminoceae, Lauraceae, Loganiaceae, Magnoliaceae, Menispermaceae, Papaveraceae, Piperaceae, Rutaceae, Rubiaceae, Ranunculaceae, Solanaceae, etc. Isolated examples of families elaborating alkaloids are also known. Some familiar alkaloids along with their plant sources and families [8] are given in Table 15.1.

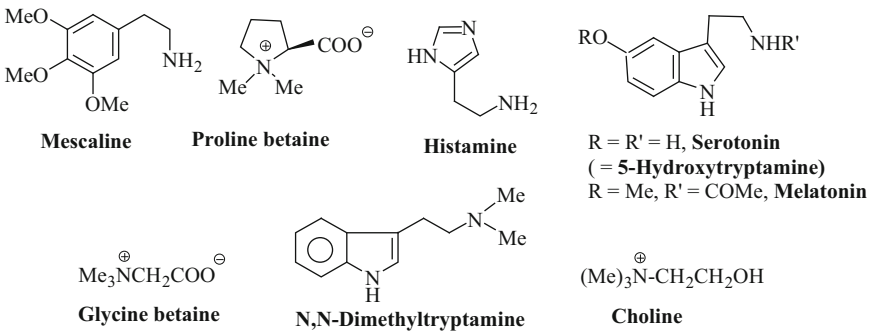


Fig. 15.1 Biological amines or protoalkaloids

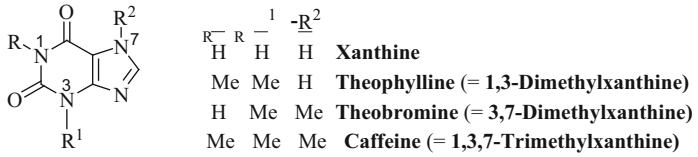


Fig. 15.2 Pseudoalkaloids

Table 15.1 Some alkaloids, their plant sources, and families

Family	Plant source	Alkaloids
Apocynaceae	*Vinca rosea*	Vincristine, Vinblastine
	Rauwolfia serpentina	Reserpine, Serpentine, Sarpagine
Amaryllidaceae	*Amaryllis belladonna*	Lycorine
Annonaceae	*Annona reticulata*	Reticuline, Anonaine
Berberidaceae	*Berberis asiatica*	Berberine
Boraginaceae	*Echium plantagineum*	Echimidine
Ephedraceae (Gnetaceae)	*Ephedra sinica*	Ephedrine, Pseudoephedrine
Lauraceae	*Alseodaphne semecarpifolia*	Srilankine
Leguminoceae	*Calycotome spinosa*	D and DL-Calycotomine
Liliaceae	*Colchicium agrippinum*	Colchicine
Loganiaceae	*Strychnos nux-vomica*	Strychnine
Magnoliaceae	*Michelia lanuginosa*	Lanuginosine
Menispermaceae	*Cocculus laurifolius*	Coclaurine, Laurifoline, Trilobine
Papaveraceae	*Papaver somniferum*	Morphine, Papaverine
Piperaceae	*Piper nigrum*	Piperine
Ranunculaceae	*Thalictrum foliolosum*	Thalictrine
Rubiaceae	*Cinchona officinalis*	Quinine, Quinidine, Cinchonidine, Cinchonine
Rutaceae	*Aegle marmelos*	Aegelenine
Solanaceae	*Atropa belladonna, Nicotiana tabacum*	Atropine, Nicotine
Umbelliferae	*Conium maculatum*	Coniine, Conhydrine

15.1 Classification Based on Precursor Amino Acids and Heterocyclic Rings

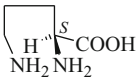
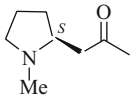
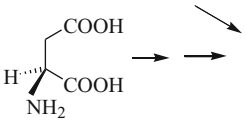
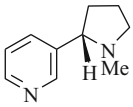
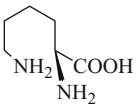
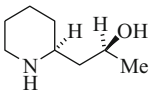
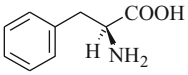
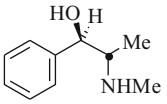
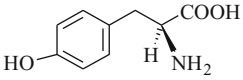
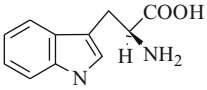
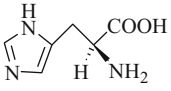
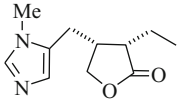
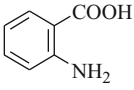
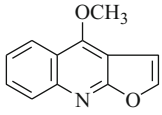
On the biogenetic ground, the alkaloids can be classified on the basis of the amino acids from which they are derived. Some examples are given in Table 15.2.

Sometimes alkaloids are also classified according to the heterocyclic ring/s they contain, *e.g.,* indole alkaloids, isoquinoline alkaloids, furanoquinoline (furoquinoline) alkaloids, piperidine alkaloids (Table 15.2). Alkaloids may also be classified according to the plant genera from which they are derived, *e.g.*, *Cinchona* alkaloids, *Rauwolfia* alkaloids, *Ephedra* alkaloids, etc.

The presence of an alkaloid is generally detected by Dragendorff test. The alkaloid/crude extract is treated with dil. HCl in a small watch glass and to it a very small amount (crystal) of the Dragendorff reagent (potassium bismuth iodide crystals) is added when the appearance of a yellow-orange precipitate suggests the presence of an alkaloid [9]. An alkaloid should be basic in character for this test, so that it can form a salt with HCl and can dissolve in water.

In conclusion, we can summarizedly define alkaloids as *the biologically active basic nitrogenous heterocycles, the nitrogen of which comes directly or indirectly from* L*-amino acids* (exception, coniine). They are mostly of higher plant origin and

Table 15.2 Classification of alkaloids based on precursor amino acids

Precursor amino acid	Example	Heterocyclic nucleus
 L-Ornithine	 (−)-Hygrine (Chap. 16)	Pyrrolidine
 L-Aspartic Acid	 (−)-Nicotine (Chap. 18)	Pyridine–Pyrrolidine
 L-Lysine	 (+)-Sedridine	Piperidine
 L-Phenylalanine	 (−)-Ephedrine (Chap. 20)	β-Phenylethanolamine (nonheterocyclic)
 L-Tyrosine	Various Indole Alkaloids Various Isoquinoline Alkaloids	Indole Isoquinoline
 L-Tryptophan	Various Indole Alkaloids Quinine (Chap. 25)	Indole Quinoline
 L-Histidine	 (+)-Pilocarpine (Chap. 21)	Imidazole
 Anthranilic Acid	 Dictamnine	Furoquinoline

solid in nature (sometimes liquid, e.g., hygrine, coniine, nicotine). Some other liquid alkaloids are also there.

Several alkaloids, as mentioned earlier, are superbly remarkable drug molecules. They may also serve as the lead molecules for cost-effective synthetic and semi-synthetic drugs (Chap. 33).

15.2 Metabolic Engineering (Combination of Microbial and Plant Genes) [10]

Metabolic engineering is a potential technique for a large scale preparation of alkaloids. Of several classes of biologically active alkaloids, benzylisoquinoline alkaloids play a prominent role, *e.g.*, *morphine* and *codeine* (age-old analgesics), *berberine*, *palmatine*, and *magnoflorine* (antibacterial agents). Further, magnoflorine has been reported to protect HDL from oxidant stress and from human lymphoblastoid cell-killing caused by HIV [10]. Berberine has recently been shown to possess cholesterol lowering properties. Save a few, the drugs and potential pharmaceuticals cannot be obtained from their plant sources as such for commercial use because of their low yields, lengthy isolation processes, and consumption of large quantities of plant materials causing a threat to the existence of the species. Neither they could be synthetically delivered, because their complex structures (in most cases) require multistep syntheses; thus, cost-effective synthesis is not possible. Efforts are now given to combine microbial and plant enzymes to produce the enzymes in which optimized expressions of the level of the synthetic genes for the desired alkaloid synthesis could be generated.

(*S*)-(+)-Reticuline, an important branch-point intermediate in the biosynthesis of several benzyltetrahydro-isoquinolines, aporphines, and protoberberines, has been synthesized using this metabolic engineering technique [10] in as good as 14.4 % yield in 1 h. Transgenic *Escherichia coli* (*E. coli*) expressing the biosynthetic genes [MAO (monoamine oxidase), NCS (norcoclaurine synthase), 6-OMT (nor-coclaurine 6-O-methyltransferase), CNMT (coclaurine-*N*-methyltransferase), 4′-OMT (3′-hydroxy-*N*-methylcoclaurine-4′-O-methyltransferase)] necessary for (*S*)-reticuline synthesis from dopamine were cultured with dopamine. Microbial MAO synthesizes 3,4-DHPA (3,4-dihydroxyphenylacetaldehyde) from DOPA. In the culture an increased amount of dopamine increases the yield of (*S*)-reticuline. Because of substrate specificity they work in succession. Microbial production of plant benzylisoquinoline alkaloids [10] is outlined in Fig. 15.3.

Two excellent reviews on plant biotechnology [11] and molecular genetics of plant alkaloid biosynthesis [12] focused on their prospects and uses for the change in alkaloid profiles in the plant cells and the formation of the desired alkaloids with desired yields have appeared in the literature. This is a very important area since the pharmaceutical concerns need bioactive alkaloids in large scale for their products.

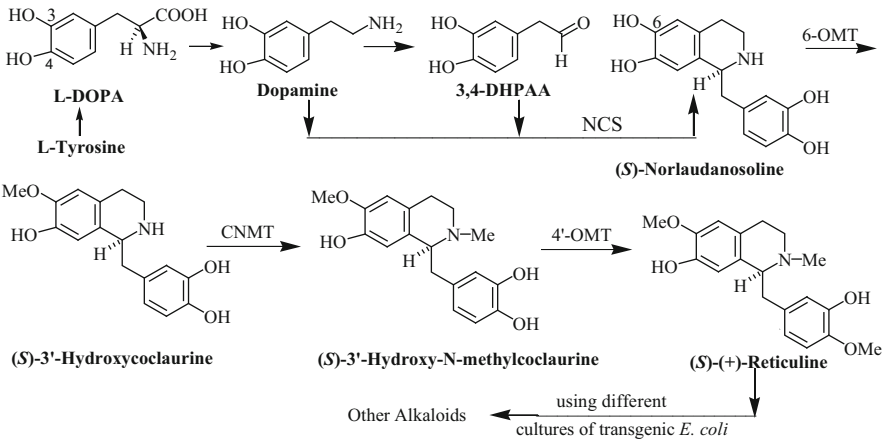


Fig. 15.3 Microbial production of plant benzylisoquinoline alkaloids

15.3 Alkaloids as Chiral Auxiliaries (See Chap. 32)

Many alkaloids as such or with their chemically manipulated structural scaffolds serve as the chiral auxiliaries in asymmetric synthesis—one of the most importantly needed area of research related to pharmaceutical products for biological systems.

Cinchona alkaloids play a leading role in the area of asymmetric organic catalysis (Chap. 32).

15.4 Historical Data Concerning Some Well-known Alkaloids

For thousands of years man has used plant preparations as remedial measures (Chap. 33). Many such preparations contain biologically active alkaloids. Attempts for the evaluation of such drugs in early nineteenth century led to the isolation of a number of alkaloids in the pure state. In the absence of modern sophisticated spectral and X-ray instruments (Sect. 4.2), many years would pass between the isolation of an alkaloid and the determination of its structure and absolute configuration (Table 15.3). In cases of strychnine and morphine, 120 and 129 years passed between their isolation and structure determination, and further 30 and 9 years, respectively, to determine their absolute configurations (Table 15.3). With the advent and availability of modern sophisticated instruments from 1960 onwards, it has been possible to determine the structure and absolute configuration of even a moderately complex compound within a year of its isolation. Since 1960 or so the minor new compounds are also being isolated from even well-studied plants as well as chemically virgin plants with the help of modern sophisticated separation and

Table 15.3 Historical data concerning some well-known alkaloids isolated in the nineteenth century [13]

Alkaloid	First isolation in the pure state	Correct structure determination	First synthesis (±)/inactive	Absolute configuration
Morphine	1805	1925	1952	1955
Xanthine	1817	1882	1882	–
Emetine	1817	1948	1950	1959
Strychnine	1818	1947	1954	1956
Piperine	1819	1874	1882	–
Atropine	1819	1883	1902	1959
Quinine (Cinchonine)	1820	1908	1944	1944
Caffeine	1820	1882	1895	–
Colchicine	1820	1955	1961	1955
Aconitine	1821	1963	1969	1971
Solanine	1822	1954	1964	1955
Chelidonine	1824	1931	1971	1979
Coniine	1827	1885	1886	1932
Nicotine	1828	1893	1904	1925 [14]
Sparteine	1851	1931	1960	1961
Cocaine	1860	1898	1898	1955
Ephedrine	1887	1889	1928	1950

Adopted from the Table 10.1, p. 316, in "*Alkaloids: Nature's Curse or Blessing?*" by Manfred Hesse, Wiley-VCH, New York, 2002, with the permission of the Publisher

isolation techniques (Sect. 4.1). The important historical data showing the years of first isolation, of correct structure determination, of synthesis of the (±)/active compound, and of the absolute configuration determination of some well-known alkaloids isolated in the 19th century are given in Table 15.3.

15.5 The Chaps. 16–29

These chapters that follow concern the important aspects in detail of the chemistry including stereochemistry, synthesis, and biosynthesis of some well-known alkaloids of different skeletal patterns. Because of the size limitation many other important alkaloids could not be included. In Chap. 30 the structures of some other alkaloids of miscellaneous types with leading references have been recorded.

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Chapter 16
Hygrine, Hygroline, and Cuscohygrine (*Ornithine-Derived Alkaloids*)

16.1 Occurrence

An alkaloid of *a simple structure*, hygrine, $C_8H_{15}NO$, light yellow oil, b.p. 193–195 °C, $[\alpha]_D$ –1.3, occurs in coca leaves (*Erythroxylum coca*, Fam. Erythroxylaceae), *Convolvulus hanadae* (Fam. Convolvulaceae) and some other plants.

16.2 Structure

Hygrine is a simple molecule and its structure has been decided by its degradations and reactions (Fig. 16.1). Formation of (**a**) and (**b**) suggests it to be an α-substituted N-methylpyrrolidine derivative; the structure of hygrinic acid (**a**) was established by its synthesis in the (±)-form by Willstätter [1, 2] (Fig. 16.2).

Formation of (**c**) shows the presence of a carbonyl group. Formation of (**a**) further suggests that the CO group is located in the side chain and not in the ring.

16.3 Absolute Configuration

The absolute configuration of (–)-hygrine at the only chiral center has been shown to be L by Karrer in 1948 by correlating (–)-hygrinic acid, obtained as one of the degradation products of (–)-hygrine (Fig. 16.1), with L-(+)-glutamic acid and L-(–)-proline, as outlined in Fig. 16.3. During oxidation of (–)-hygrine to (–)-hygrinic acid, the chiral center remains unaffected.

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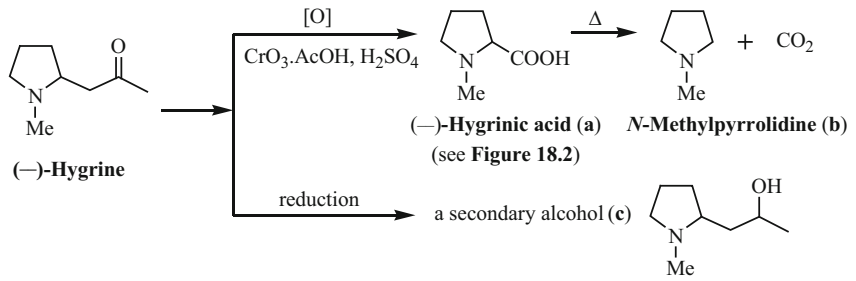


Fig. 16.1 Degradations and reactions of hygrine

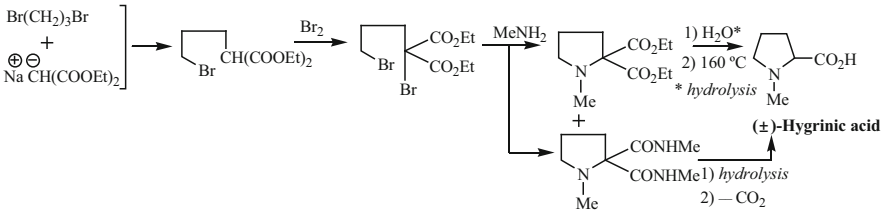


Fig. 16.2 Willstätter synthesis of (±)-hygrinic acid [1, 2]

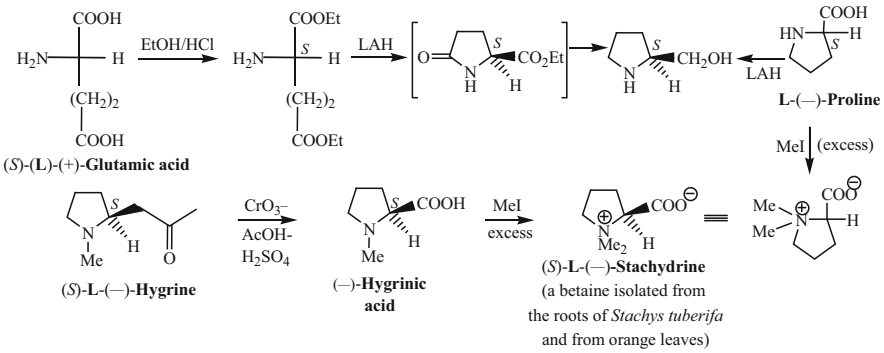


Fig. 16.3 Configuration of (–)-hygrine by chemical correlation with L-(+)-glutamic acid and L(–)-proline

16.4 Synthesis of (±)-Hygrine

1. ***Sorm's two-step synthesis*** [3], starting from *N*-methylpyrrole is shown in Fig. 16.4.
2. Anet et al. [4] synthesized (±)-hygrine by condensation of γ-methylaminobutyraldehyde with excess acetone dicarboxylic acid in a buffer

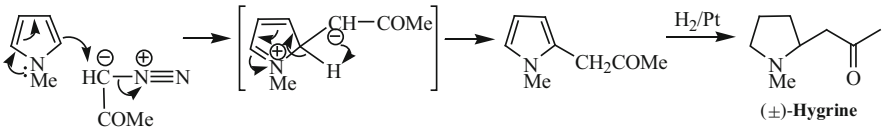


Fig. 16.4 Sorm's synthesis of (±)-hygrine [3]

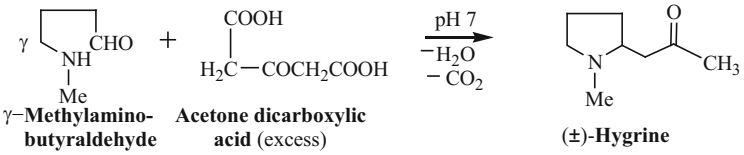


Fig. 16.5 Anet's synthesis of (±)-hygrine

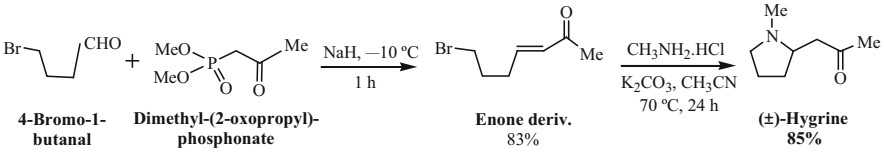


Fig. 16.6 Synthesis of (±)-hygrine [5]

at pH 7 (Fig. 16.5), followed by decarboxylation. Ethyl acetoacetate (excess) may also be used instead of acetone dicarboxylic acid; the condensation product upon hydrolysis and decarboxylation yields (±)-hygrine.

3. In 2011 Muthusubramanian has reported a synthesis of (±)-hygrine in good yield [5], using the strategy of Duenren Hou of the tandem S_N2 *intramolecular aza-Michael reaction* (Fig. 16.6).

For a number of other routes reported for the synthesis of hygrine during 1981, 1983, 1989, 2006, 2008, 2009, and 2010; see reference 6 of the publication [5].

16.5 Enantioselective Synthesis of (+)-Hygrine [6]

Hygrine suffers facile racemization in neutral and basic media; hence, its enantioselective synthesis leading to optically pure form is challenging. In 2006 the first enantioselective synthesis of (*R*)-(+)-hygrine has been accomplished in 12 steps with 29 % overall yield and 91 % ee via asymmetric phase-transfer catalytic alkylation and ring-closing metathesis as key steps [6]. This synthesis directly confirms the *R*-configurarion for (+)-hygrine. The synthesis is delineated stepwise in Fig. 16.7.

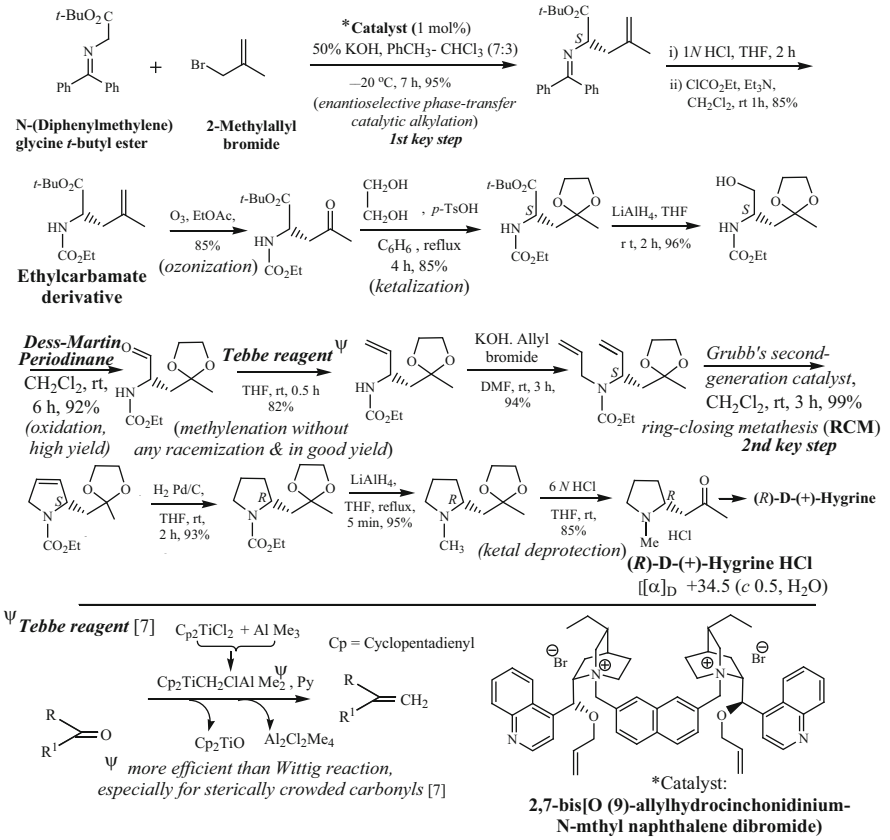
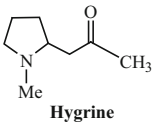


Fig. 16.7 First enantioselective synthesis of (+)-hygrine via asymmetric phase-transfer catalytic alkylation (2006) [6]



IR ν_{max} (neat): 1731 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.69-1.80 (m, 4H), 2.16 (s, 3H), 2.31 (s, 3H), 2.40-2.45(dd, 1H), 2.53-2.56 (m, 1H), 2.78-2.83 (dd, 1H), 3.03-3.07 (*m*, 1H)

^{13}C NMR (100 MHz, $CDCl_3$, δ): 22.2, 31.2, 31.4, 40.5, 48.5, 56.9, 61.9, 208.2

Mass [5, 8]: M^+ at *m/z* 141.1, base peak at *m/z* 84 (α-cleavage, $M^+ - CH_2COMe$)

Fig. 16.8 Spectral data of hygrine

16.6 Spectral Data [5] of Hygrine

The spectral data of hygrine are given in Fig. 16.8.

16.7 Biosynthesis of (–)-Hygrine

Biosynthetic sequence leading to (–)-hygrine starting from L-ornithine and involving stepwise addition of two acetyl-CoA units to *N*-methyl-Δ′-pyrrolinium ion is delineated in Fig. 16.9.

16.8 Hygroline and Pseudohygroline

(+)-Hygroline, $C_8H_{17}NO$, m.p. 29 °C, $[\alpha]_D$ +50 (EtOH, *c* 0.77), has been isolated from the leaves of *Carallia brachiata* (Fam. Rhizophoraceae). The alkaloid is absent in the bark. Hygroline has been shown to be 2(2′-hydroxypropyl)-*N*-methylpyrroline [9]. It has been synthesized [10] from anodically prepared α-methoxylated carbamates (**A**) as the key intermediates and their subsequent conversion to hygroline and pseudohygrolines (Fig. 16.10). All salient details for following the synthetic steps are given in the figure.

Biogenetically, hygroline and pseudohygroline may be formed by face-discriminating carbonyl reductions of (–)-hygrine and (+)-hygrine.

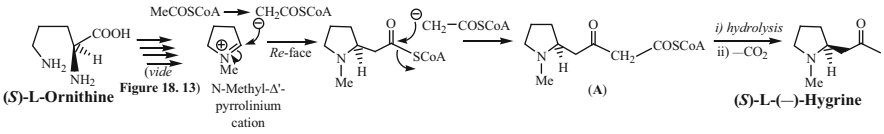
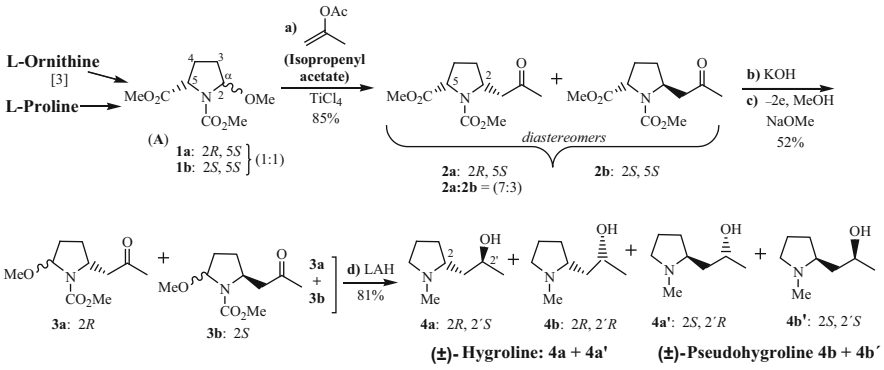


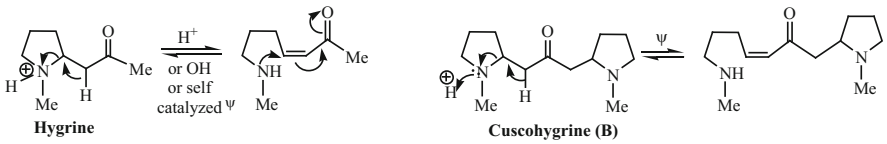
Fig. 16.9 Biosynthesis of (–)-hygrine



Notes

*Step **a**): C-C bond formation giving a similar mixture of **2a**:**2b** (7:3) from both (**1a**) and (**1b**) suggests involvement of the same active intermediate and hence a mechanism similar to S_N1.*
*Step **b**): hydrolysis. Step **c**): methoxylation by anodic oxidation (the **key step**).*
*Step **d**): Reduction gives a mixture of the diastereomers (**4a**) and (**4b**) [along with their enantiomers (**4a′**) and (**4b′**)] in the ratio of 2:3, separated by GLC. Their optical rotations are $[\alpha]_D$ +21.3 [pure (+)-hygroline has $[\alpha]_D$ +50 (EtOH), hence the optical purity is 42.6%] and 45.62 respectively.*

Fig. 16.10 The synthetic route to optically active (+)-hygroline and (+)-pseudohygroline [10]



Ψ *Intramolecular retro-aza Michael reaction (see* ***Figure 16.6****). This type of facile racemization may be responsible for the inactivity of cuscohygrine and for the low rotation of hygrine.*
N.B. *Both heterocyclic moieties of cuscohygrine can be reopened and recyclized.*

Fig. 16.11 Probable self-catalyzed racemization of hygrine and cuscohygrine

16.9 Cuscohygrine

Cuscohygrine, $C_{13}H_{24}ON_2$, b.p. 169–170 °C, a congener alkaloid of hygrine, has been shown to possess structure (**B**) (Fig. 16.11). It occurs in "cusco" leaves and also in *Convolvulus hamadae* (Fam. Convolvulaceae).

16.9.1 Stereochemistry of Cuscohygrine

It is optically inactive though it contains two chiral centers. Its stereochemistry is not definitely assigned. Its optical inactivity may be due to internal compensation because of its symmetrical (*S*, *R*) configuration of the two like chiral centers (AA type) (cf. *meso*-tartaric acid, see Sect. 2.7.2). The optical inactivity may also be due to rapid self-catalyzed racemization in solution, as may be happening in case of hygroline also. Probable self-catalyzed racemization of hygroline and cuscohygrine is delineated in Fig. 16.11.

Reduction of cuscohygrine with sodium and ethanol produces a mixture of *two* epimeric meso alcohols (CO → CHOH), α- and β-dihydrocuscohygrine (ABA type, see Sect. 2.7.4), indicating that natural cuscohygrine has the (*S*, *R*)-*meso*-configuration, since the racemate (*R*, *R* and *S*, *S*)-cuscohygrine would have produced only *one* racemic alcohol (see Chap. 2).

16.9.2 Synthesis of Cuscohygrine

Cuscohygrine has been synthesized by Rapoport and Jorgensen [11] and Anet et al. [4], as outlined in Fig. 16.12.

Condensation of acetone dicarboxylic acid with two molecular proportions of γ-methylamino- butyraldehyde at pH 7 takes place smoothly to yield excellent yield of cuscohygrine [4].

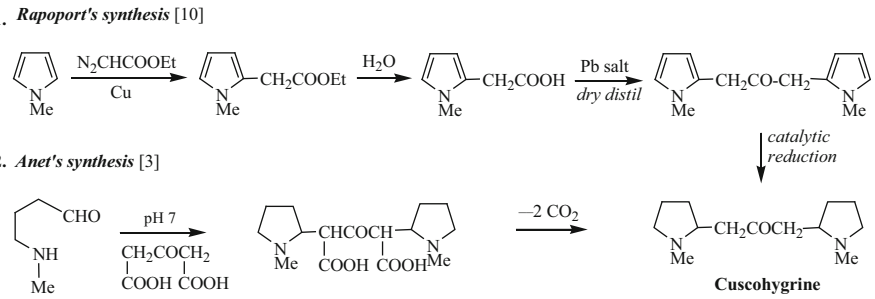


Fig. 16.12 Synthesis of cuscohygrine by Rapoport and Jorgensen [11] and Anet et al. [5]

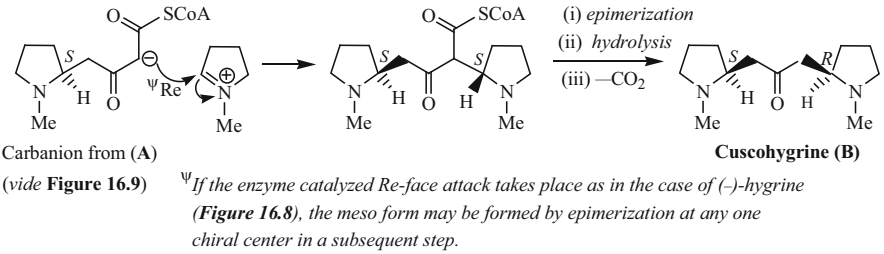


Fig. 16.13 Biosynthesis of cuscohygrine

16.9.3 Biosynthesis of Cuscohygrine

Cuscohygrine is biogenetically formed by the addition of the anion of (**A**) (Fig. 16.9) to another molecule of *N*-methyl-Δ′-pyrrolinium cation (Fig. 16.13).

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Chapter 17
Coniine, Conhydrine, and Pseudoconhydrine (*The C-Skeleton Derived from a C_8-Fatty Acid and N from Transamination*)

17.1 Introduction. *Conium* Alkaloids

In ancient Greece, the extracts of hemlock (*Conium maculatum* L., Fam. Umbelliferae) were used for the execution of criminals. Plato (427–347 BC) described the death of Socrates (about 470–399 BC): Socrates was made to drink a cup of hemlock extract as he was thought to be a Godless man who corrupted the young. This incident is perhaps one of the worst man-made tragedies in the history of civilization. Incidentally, it may be mentioned that Lavoisier was arrested and guillotined for no fault of his own, when the French Revolutionary terror was at its height. The death of Lavoisier is considered by some historians, the worst single casualty of the Revolution; a great French mathematician and astronomer Joseph Louis Compte Lagrange (1736–1813) said, "You have in a moment cut off a head whose like may not be seen again for a century."

All parts of the plant *C. maculatum* contain the alkaloids coniine, conhydrine, pseudoconhydrine (also written as ψ-conhydrine), γ-coniceine, and *N*-methylconiine [1] (Fig. 17.1). South African *Conium* species in addition to the above alkaloids also elaborate *N*-methyl ψ-conhydrine. The latter has been isolated as the major alkaloid from the leaves and stems of some high altitude species [2]. (+)-Coniine, $C_8H_{17}N$, a colourless oily liquid with a pungent smell, is the principal toxic alkaloid present in the oil of hemlock, from which it was first isolated in the pure state in 1827.

17.2 Coniine

17.2.1 Structure and Absolute Stereochemistry

The reactions and degradations of coniine outlined in Figs. 17.2 and 17.3 lead to the structure and stereochemistry of (+)-coniine and its enantiomer (−)-coniine

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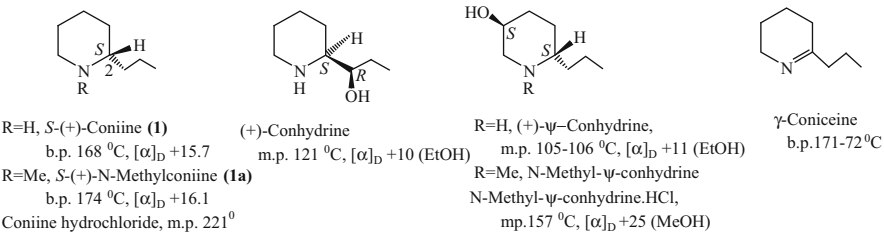


Fig. 17.1 *Conium* alkaloids

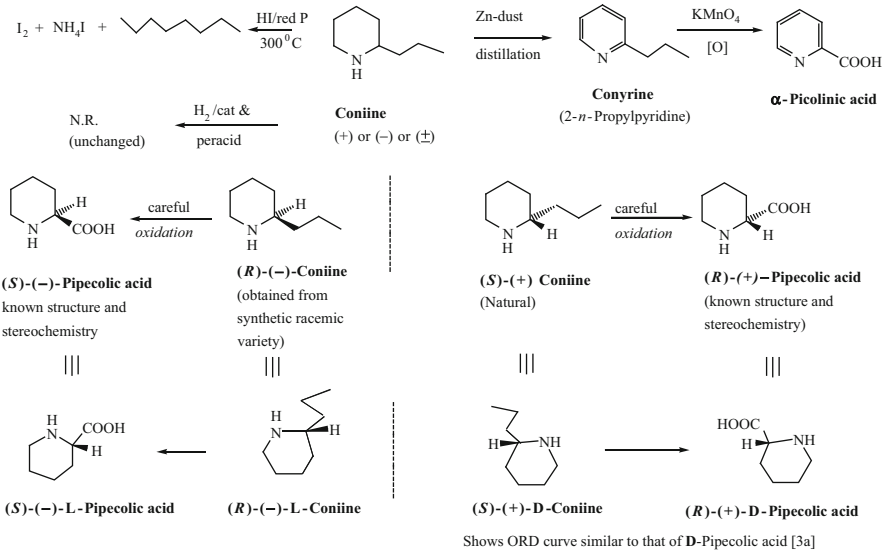


Fig. 17.2 Elucidation of structure and stereochemistry of (+)-coniine and (−)-coniine

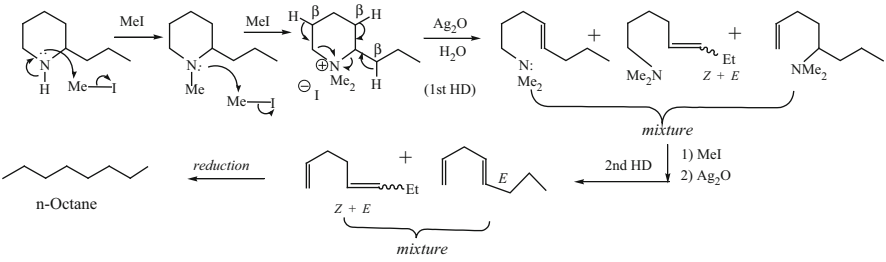
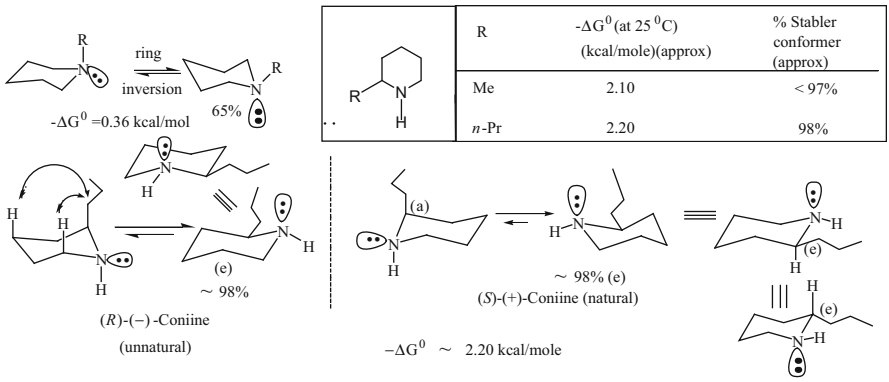


Fig. 17.3 Hofmann degradations (or eliminations) of coniine, followed by reduction to give *n*-octane



R	-ΔG⁰ (at 25 °C) (kcal/mole)(approx)	% Stabler conformer (approx)
Me	2.10	< 97%
n-Pr	2.20	98%

Fig. 17.4 Conformations of (−)-coniine and (+)-coniine

(unnatural). They have D- and L-configurations, respectively, as shown (Fig. 17.2). The conversion of coniine to *n*-octane by two successive Hofmann degradations (HD), followed by reduction, may be rationalized on the basis of its structure as shown in Fig. 17.3.

17.2.2 Conformation

It is known [4] that piperidine itself has in its conformational equilibrium approximately 65 % of the stabler conformer with N–H in the equatorial orientation, the conformational free energy difference ($-\Delta G^\circ$) at 25° being 0.36 kcal/mole (calculated from the equation $\Delta G^\circ = -RT \ln K$) (Fig. 17.4).

So, $-\Delta G^\circ_{\text{2-Methylpiperidine}} = -(\Delta G^\circ_{\text{Methylcyclohexane}} + \Delta G^\circ_{\text{Piperidine}}) = (1.74 + 0.36) = 2.10$ kcal/mole, and $-\Delta G^\circ_{\text{2-Propylpiperidine}} = -(\Delta G^\circ_{\text{Propylcyclohexane}} + \Delta G^\circ_{\text{Piperidine}}) = (1.84_{\text{expected}} + 0.36) = 2.20$ kcal/mole

Thus, the presence of *n*-propyl at C2 in coniine makes $-\Delta G^\circ \sim 2.2$ kcal/mole, making the equatorial conformer ~98 % in the conformational equilibrium (Fig. 17.4). Hence, although conformational equilibrium is possible, coniine has an anancomeric conformation, the side chain in almost all molecules being in the equatorial conformation; thus, the corresponding axial conformer in equilibrium is negligible.

17.2.3 Synthesis

1. The first synthesis of coniine was achieved by Albert Ladenburg in 1886. ***This constitutes the first synthesis of an alkaloid*** and thus it bears significance in the history of organic synthesis. The natural alkaloid (+)-coniine was later obtained

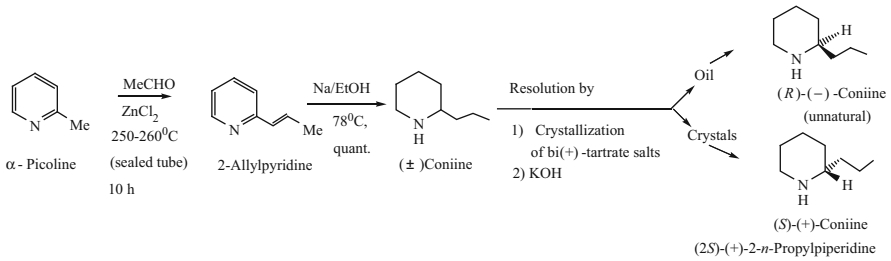


Fig. 17.5 Ladenburg's synthesis of (±) coniine (1886) and resolution to natural (+)-coniine

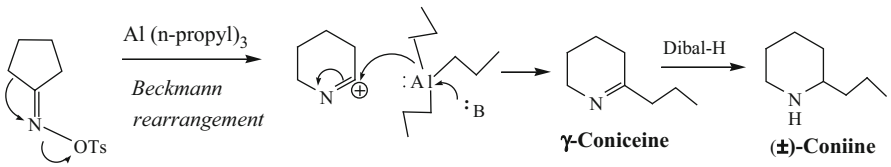


Fig. 17.6 Synthesis of (±)-coniine (1983) [5]

by crystallization of bi(+)-tartrate, followed by alkali treatment as outlined in Fig. 17.5.

2. In 1983, a synthesis of (±)-coniine was reported using organoaluminium promoted Beckmann rearrangement of cyclopentanone oxime sulfonate [5]. The organoaluminium compound might be employed as an amphoteric reagent which induces the Beckmann rearrangement of the oxime sulphonate as well as captures the iminocarbocation (Fig. 17.6).
3. (±)-Coniine has also been synthesized from carbamate [6] through the sequence of reactions outlined in Fig. 17.7. A [3+3]-type cyclization of α,α'-dimethoxylated amide and allylthrimethylsilane takes place.
4. The first enantioselective synthesis of (+)- and (−)-coniine has been achieved [7] through chiral synthons, 2-cyano-6-oxazolopiperidine derivatives, prepared through Robinson–Schopf type condensations of glutaraldehyde with a chiral components (chiral auxiliary) (−)-norephedrine followed by the addition of KCN in each case. The different steps of the total synthesis of (+)-coniine and (−)-coniine are delineated in Fig. 17.8.

The high stereoselectivity observed in the reaction of (**B**) with hydride ion implied a mechanism involving a prior formation of the iminium ion (**C**) by elimination of the cyano group and subsequent approach of H^- under complete stereoelectronic control from the axial direction (upper face) to the iminium conformer (**C**) generating the 2(*S*) absolute configuration. This step is followed by reductive opening of the oxazolidine ring to form (**D**). Likewise the oxazolidine ring in (**E**) undergoes reductive opening (by $NaBH_4$) to form (**F**).

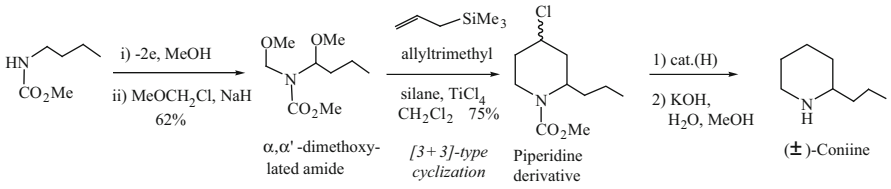


Fig. 17.7 Synthesis of (±)-coniine (1985) [6]

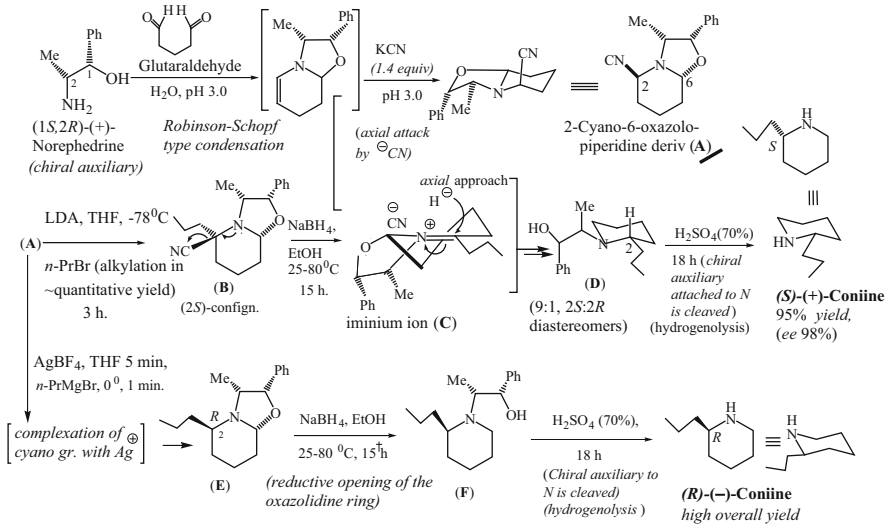


Fig. 17.8 Enantiospecific synthesis of (+)-coniine and (−)-coniine (1983) [7]

5. *Synthesis of (R)-(−)-Coniine starting from a known (R)-allene* [8]

In the synthesis of (R)-(−)-coniine, the key step is the silver-(1)-catalyzed cyclization of (R)-(-)-N-benzylocta-5,6-dienylamine (**B**) upon treatment with silver tetrafluoroborate (0.5 equiv.) (Fig. 17.9). In this step the axially dissymmetric simple allenic amine (**B**) is converted to a centrodissymmetric compound (**D**) through an intermediate silver complex (**C**), which undergoes an internal nucleophilic attack by the amine in a stereospecific fashion. The synthesis of (**B**) [containing 10 % of the (*S*)-(+)-isomer] has been accomplished in six steps in overall 47 % yield, starting from a known (*R*)-(-)-bromomethylallenenic derivative (**A**) as delineated in Fig. 17.9. The compound (**D**) is reduced in two steps by treatment with *p*-toluenesulfonylhydrazine-sodium hydroxide, followed by catalytic hydrogenation to yield (*R*)-(-)-coniine (76 %) (Fig. 17.9), isolated as the hydrochloride salt.

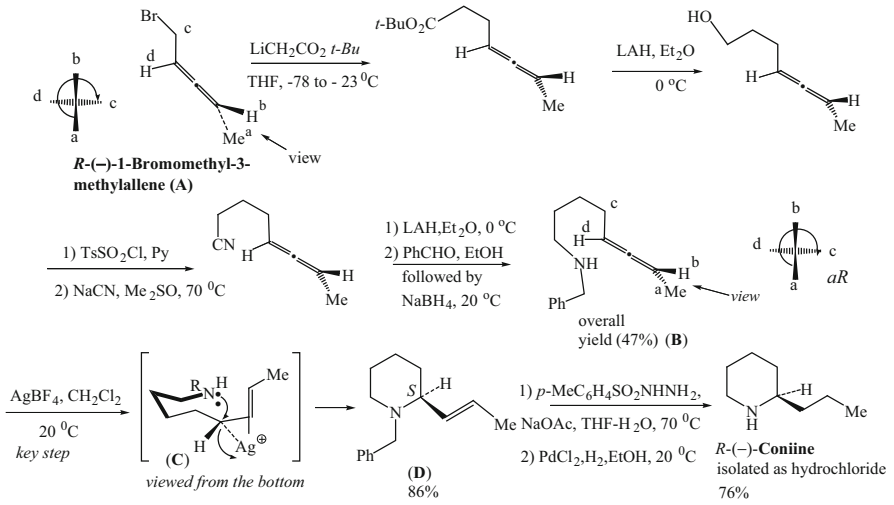


Fig. 17.9 Synthesis of (*R*)-(−)-Coniine from a known (*R*)-allene derivative (A) (1986) [8]

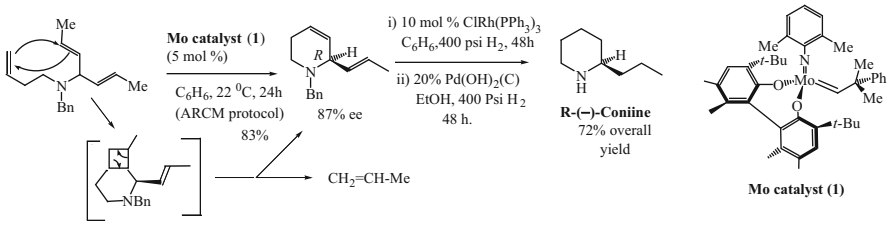


Fig. 17.10 Schrock's synthesis of *R*-(−)-coniine (2005) [9]

6. In a paper [9] Schrock (NL 2005) et al. showed that by the Mo-catalyzed asymmetric ring-closing metathesis (ARCM) protocol, a suitable benzylamine could be successfully converted into optically enriched cyclic amine in 87 % *ee* (83 % yield), from which *R*-(−)-coniine is obtained in 72 % overall yield in two simple reductive stages: olefin hydrogenation in the presence of Wilkinson's catalyst [$ClRh(PPh_3)_3$] followed by Pd-catalyzed debenzylation (Fig. 17.10). Olefin hydrogenation is done prior to debenzylation since Pd-catalyzed hydrogenation causes some C–N bond fission.
7. In a one-pot three component reaction (trimethylsilylacetylene, butanal, and dibenzylmine in the presence of a catalyst *R*-Quinap), the chiral propylpropargylamine derivative (**A**) is synthesized. The latter is alkylated with ethylene oxide. The hydroxyl group thus generated is protected by TiPSCl followed by hydrogenation of the triple bond and hydrogenolysis to achieve debenzylation. The free base (**B**) on Mitsunobu reaction [10] furnished (*S*)-(+)-coniine in 60 % yield and 90 % *ee* [11] (Fig. 17.11).

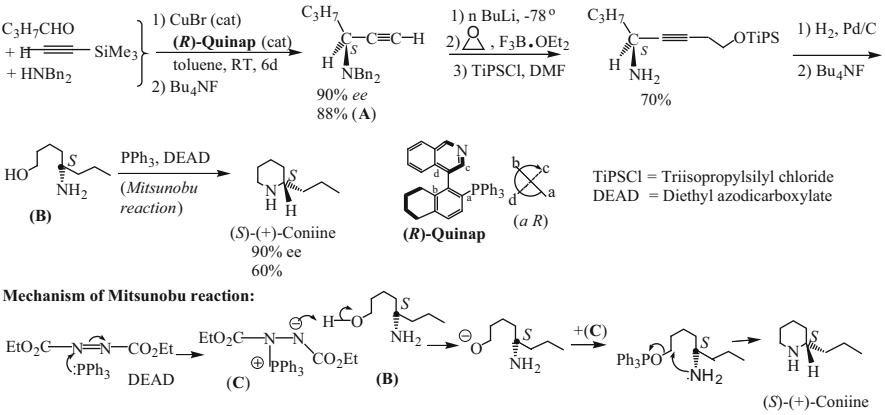


Fig. 17.11 Synthesis of (*S*)-(+)-coniine (2004) [11]

8. An enantioselective synthesis of (+)-*N*-methylconiine by Shono et al., starting from L-Lysine and using anodic oxidation as key steps, has been outlined in Sect. 17.4.3 (Fig. 17.24).

Several more asymmetric syntheses of (S)-(+)-coniine and (R)-(−)-coniine have appeared in the literature from time to time [12–16].

Natural coniine has been shown to have (*S)* absolute configuration at its only chiral centre at C2 with (+) rotation. However, absolute configuration (*R)* with (+) rotation and absolute configuration *S* with (−) rotation have been mentioned for natural (+)-coniine and its unnatural enantiomer, respectively, by oversight [17]. Change in rotation (both sense and magnitude) is observable only in some cases with the change of solvent and concentration (see Sect. 2.2.8.4). One such chiral molecule is (*R*)-ar-tumachalene which is dextrorotatory in hexane, but levorotatory in chloroform [18]; but the change in absolute configuration, which needs the breaking of a bond to the chiral center, cannot happen here.

17.2.4 Chemoenzymatic Resolution

In addition to the conventional method of resolution, the chemoenzymatic method has been found to be useful in the preparation of optically active amines from their racemates via oxalamic esters [19]. The racemate oxalamic esters when treated with a protease enzyme, the *R*-esters, get stereoselectively hydrolyzed, while the *S*-esters remain unaffected, and thereby the latter could be separated from the hydrolyzed one. By this method (+)- and (−)-coniine could be obtained [19] in the pure state. The process is delineated in Fig. 17.12.

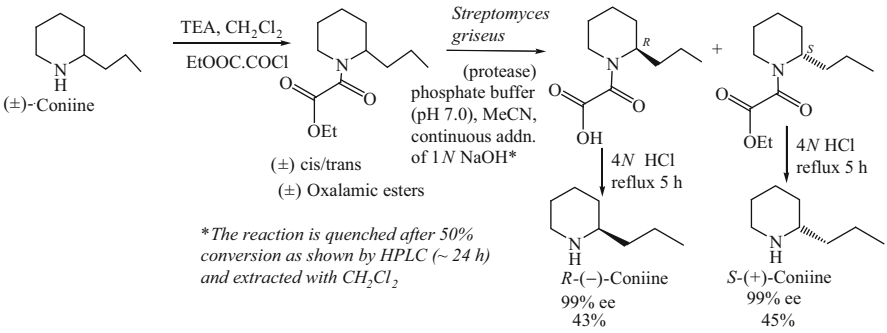
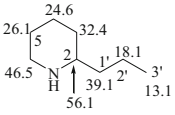


Fig. 17.12 Chemoenzymatic resolution of (±)-coniine (2005) [19]

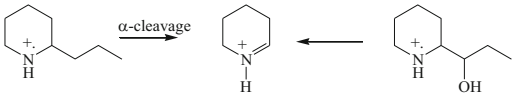
IR (liquid film) 3275 (w), 2915 (s), 2850 (s), 2800 (w), 1440 (m), 1380 (w), 1327 (m), 1314 (w), 1121 (m), 1055 (w), 750 cm^{-1} (w) [5].

^{1}H NMR ($CDCl_3$) : δ 2.87-3.31 (1H, m, N-C*H*), 2.22-2.87 (2H, m, NCH_2), 1.67 (1H, s, NH), 0.90 (3H, br.t CH_3) [5].

^{13}C NMR (Dioxan):



MS: Coniine as well as conhydrine being 2-alkyl piperidine showed characteristic α-cleavage



·Coniine
(M^+, *m/z* 127)

m/z 84

Conhydrine
(M^+, *m/z* 143)

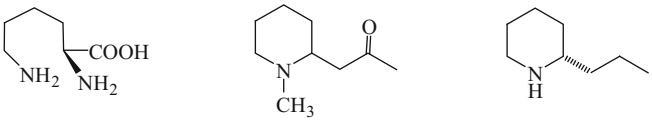
Fig. 17.13 Spectral data of coniine

17.2.5 Spectral Data

Spectral data [5, 20] of coniine are given in Fig. 17.13.

17.2.6 Biosynthesis of Coniine

Piperidine alkaloids (*e.g.*, N-methylpelletierine), excepting coniine (and probably other *Conium* alkaloids) have been shown to be biosynthesized from L-lysine and acetate. However, coniine that resembles pelletierine so closely in structure does not utilize lysine to form its piperidine ring.



(*S*)-**L**-Lysine N-Methylpelletierine Coniine

Labeling experiment with [1-^{14}C]-acetate showed the alternate incorporation in coniine which indicates the formation of a C_8-polyketide or its equivalent on the way to the formation of coniine [21]. 5-Oxooctanoic acid is likely to be an intermediate. When octanoic acid (capric acid) is tested, incorporation is observed which suggests that coniine may be derived oxidatively from a C_8-fatty acid and not reductively from a polyketide [22]. *If this conjecture is correct, this alkaloid would serve as a unique example of a secondary metabolite formed from a fatty acid.* Biosynthesis of (+) coniine is outlined in Fig. 17.14.

No acetate-derived metabolite is known to be formed from fatty acids other than polyacetylenes. Transamination and imine formation lead to the product γ-coniceine which co-occurs with coniine. An enzyme has been isolated from young hemlock leaves which catalyzes the formation of γ-coniceine from 5-ketooctaldehyde in the presence of L-alanine which donates the amino group, and hence (+)-coniine is formed (Fig. 17.14).

A laboratory analogy of such conversion of γ-coniceine to coniine has been reported [23] in which γ-coniceine is reduced by lithium butyl(hydro)dipinan-3α-yl-borate to coniine. *S*-(+)-Coniine, the natural enantiomer, is formed in predominance when the borate is prepared from (−)-pinene, while R-(−)coniine is formed in predominance when (+)-pinene is used to prepare the borate [23] (Fig. 17.15) The authors interpreted the asymmetric reduction of the 2-alkyl-tetrahydropyridine ring with the borate in terms of a suggested transition state in a preferred conformation having two pinanyl residues mutually at right angles, resulting in the product of correct stereochemistry.

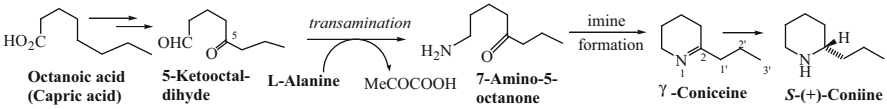


Fig. 17.14 Biosynthesis of Coniine [21, 22] (Leete)

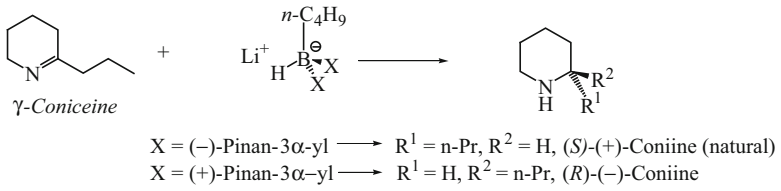


Fig. 17.15 Asymmetric reduction of γ-coniceine to (*S*)-(+)-coniine or (*R*)-(−)-coniine

17.3 Conhydrine

(+)-Conhydrine, $C_8H_{17}NO_2$, a strongly basic colorless solid, mp. 121 °C, $[\alpha]_D$ +10 (EtOH), sublimes readily (for its stereostructure see Figs. 17.18 and 17.19).

17.3.1 Stereostructure

Conhydrine contains a secondary hydroxyl group which is present in the side chain since it gives (−)-coniine on reduction and (*S*)-(−)-pipecolic acid on careful oxidation (see Fig. 17.19). Of the two possible locations (β and γ) for the hydroxyl group β- has been its site. That it holds e*rythro* relationship around C–N bond has been shown by sequence of reactions outlined in Fig. 17.16. Both e*rythro*-octane-3,4-diol (**A**) and *threo*-octane-3,4-diol (**B**) have been synthesized (Fig. 17.17). The diol obtained from conhydrine (Fig. 17.16) has been found to be identical with the synthetic *erythro* diol [24].

The erythro relationship in conhydrine can be well recognized when the reactions shown in Fig. 17.17 are represented with Newman projection formula.

Hofmann degradation (elimination) products of (+)- conhydrine are depicted in Fig. 17.18. The epoxide formed by the 1st Hofmann degradation upon 2nd Hofmann degradation forms an olefine epoxide, which upon acid hydrolysis produces the racemate of the *erythro* glycol shown.

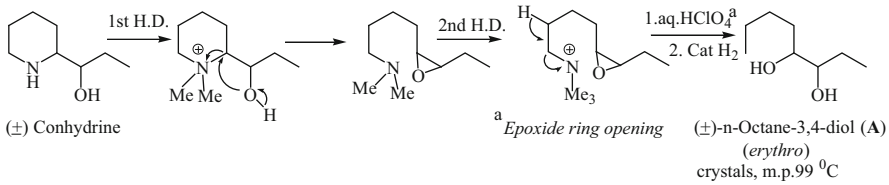


Fig. 17.16 Conversion of (±)-conhydrine to *erythro*-(±)-*n-o*ctane-3,4-diol

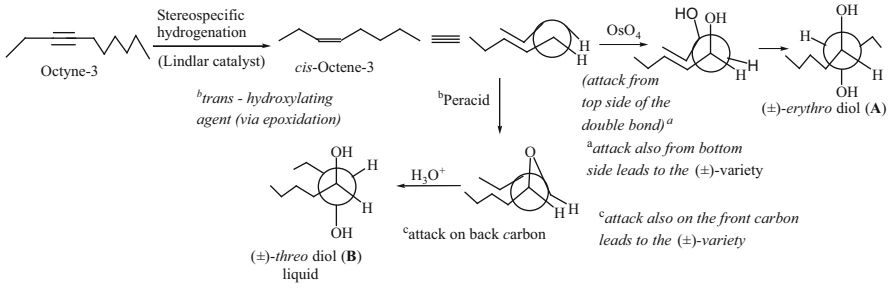


Fig. 17.17 Synthesis of *erythro*-diol (**A**) and *threon*diol (**B**)

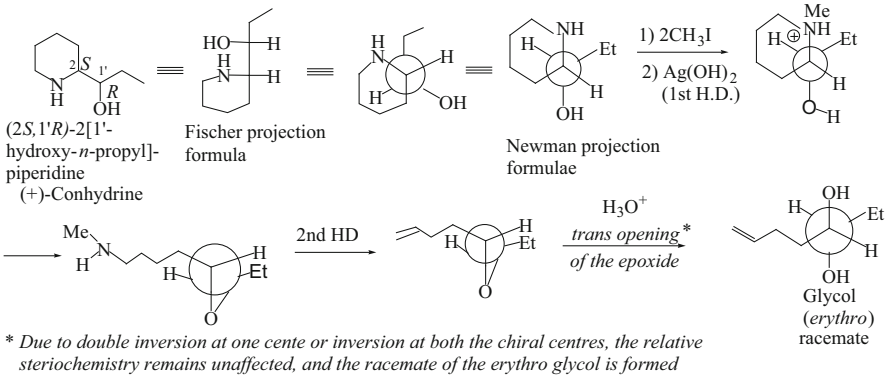


Fig. 17.18 Hofmann degradation products of conhydrine

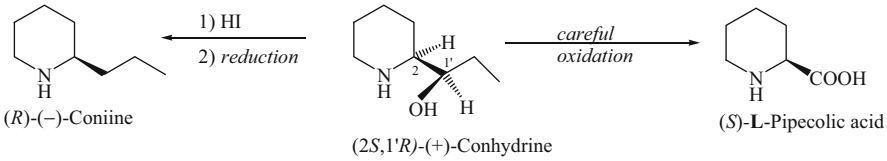


Fig. 17.19 Conversion of (+)-conhydrine into (*R*)-(−)-coniine and (*S*)-pipecolic acid

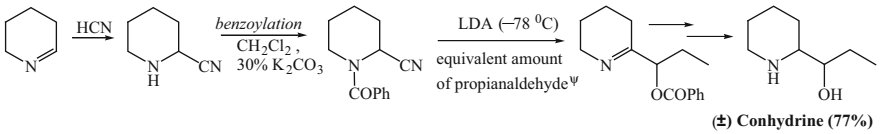
The relative stereochemistry of conhydrine is thus erythro. The absolute configuration at C2 has been established as (*S*) by correlating it with (*R*)-(−)-coniine and L-pipecolic acid. This correlation study also confirms *S*-configuration at C1′ holding erythro relationship with C2 (Fig. 17.19).

17.3.2 Synthesis of (±)-Conhydrine

(±)-Conhydrine has been synthesized [25] by substitution at the suitable latent α-aminocarbanion (Fig. 17.20).

17.4 Pseudoconhydrine

Pseudoconhydrine**,** $C_8H_{17}NO$, m.p. 105–106 °C, $[\alpha]_D$ + 11(EtOH) has been shown to have the 5-hydroxy coniine structure.



ψ The probable sequence of events in this transformation is shown below:

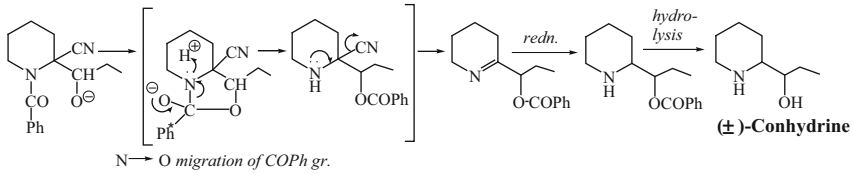


Fig. 17.20 Synthesis of (±)-conhydrine

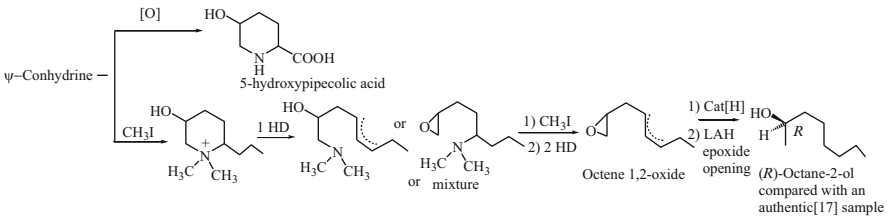


Fig. 17.21 Some important reactions of ψ-conhydrine

17.4.1 Structure. Stereochemistry. Conformation

Some important reactions of ψ-conhydrine are shown in Fig. 17.21. It possesses (2*S*,5*S*)-5-hydroxy-2-propylpiperidine structure.

X-Ray crystallographic study of ψ-conhydrine hydrobromide showed that OH and *n*-propyl groups are *trans* oriented (Fig. 17.22) and intermolecular hydrogen bonding exists. Since (*R*)-octane-2-ol is formed (Fig. 17.21), ψ-conhydrine should have *S* configuration at C5, and the 2-*n*-propyl being *anti* to OH group C2 should also have *S* configuration [26, 27]. *N*-Methyl-ψ- conhydrine, m.p. 157 °C, $[\alpha]_D$ +25 (MeOH) has also been isolated from South African *Conium* species [2]. Its ^{1}H NMR ($CDCl_3$, 300 MHz) parameters were compatible only with a chair conformation of the piperidine ring. The appearance of H5a as a septuplet at δ 3.70 [J Hz (coupled proton) 9 (6a), 4.5 (6e), 9 (4a), 4.5 (4e)] showed conclusively that H5 has an axial orientation [2] (Fig. 17.22). The triequatorial conformer (**E**) will have $-\Delta G^\circ \sim 5$ kcal/mole, since the corresponding flipped triaxial conformer (**A**) entails *syn*-axial interactions (kcal/mole) between *n*-propyl and H (twice) (~2.2) (see Fig. 17.4), 1NMe and OH (~2), and 1NMe and H (0.8). Hence, the triequatorial conformer (**E**) will have >99.9 % population in equilibrium and will be anancomeric and almost rigid.

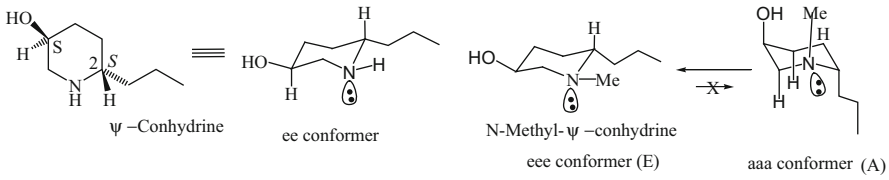


Fig. 17.22 Conformations of pseudoconhydrine and *N*-methylpseudoconhydrine

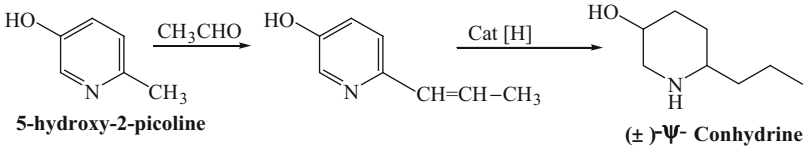


Fig. 17.23 Synthesis of (±)-pseudoconhydrine

17.4.2 Synthesis of (±)-ψ-Conhydrine

(±)-ψ-Conhydrine has been synthesized starting from 5-hydroxy-2-picoline in two steps (Fig. 17.23).

17.4.3 Synthesis of Optically Active (+)-N-Methylpseudoconhydrine

The synthesis by Shono et al. [28] is delineated in Fig. 17.24. The Anodic transformation of the L-Lysine derivative to optically active piperidine skeleton, and hence an enantioselective synthesis of (+)-*N*-methylconiine, was reported earlier by Shono et al. [29]. Their strategy consists of diastereoselective introduction of R^- to the α-position of the anodically prepared key chiral intermediate (**A**) through the influence of a substituent on the chiral α′-position, followed by elimination of the α′ substituent (Fig. 17.24). Thus, the formal enantioselective introduction of R^- to the α-position is achieved. The acid (**B**) was converted to the optically active (+)-*N*-methylconiine in three steps by anodic oxidation followed by successive reduction with $NaBH_4$ and $LiAlH_4$. The synthesis of (2*S*,5*S*)-(+)-*N*-methylpseudoconhydrine applying this methodology is outlined in this figure. The synthesis of its unnatural enantiomer, (2*R*,5*R*)-(−)-*N*-methylpseudoconhydrine, starting from (**A**) and following the same methodology, but changing the sequence of steps to some extent, has also been reported [28]. These syntheses are particularly interesting since some natural piperidine alkaloids have been known [28, 29] to be formed from L-lysine.

(−)-Pseudoconhydrine has been synthesized by the stereoselective rearrangement of a suitably substituted proline derivative [30].

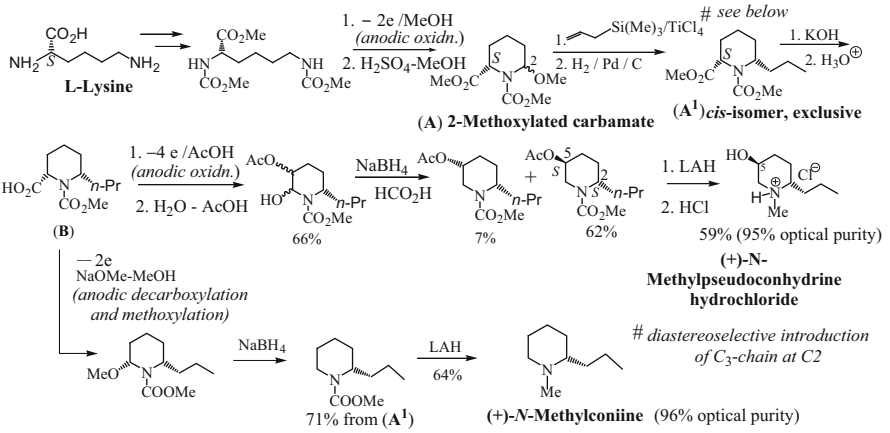


Fig. 17.24 Synthesis of (+)-*N*-methylpseudoconhydrine and (+)- *N*-methylconiine

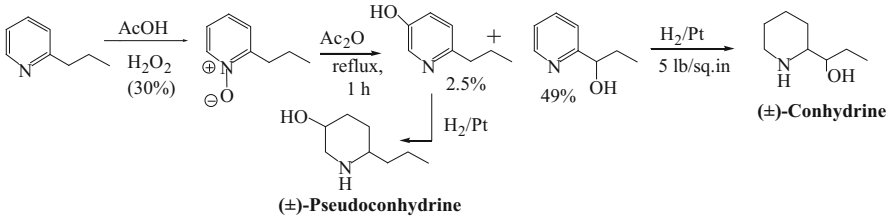


Fig. 17.25 Laboratory analogy for the biosynthesis of conhydrine and pseudoconhydrine

17.5 Laboratory Analogy of the Biosynthesis of Conhydrine and ψ-Conhydrine

The site of oxidation of both conhydrine and ψ conhydrine is β with respect to nitrogen of coniine. This observation led Robinson to suggest a kind of oriented oxidation and a common intermediate precursor. However, a laboratory analogy of this conjecture has been shown by Gobindachari et al. [31]. 2-*n*-Propylpyridine-*N*-oxide on treatment with acetic anhydride and subsequent work-up gave 2-1′-hydroxypropylpyridine (49 %) and 5-hydroxy-2-propylpyridine (2.5 %). On reduction the former gave (±)-conhydrine while the latter gave (±)-ψ-conhydrine. This product profile not only showed the oriented rearrangement but on reduction gave (±)-conhydrine and (±)-ψ-conhydrine. Hence, the entire process constitutes the synthesis of (±)-conhydrine and (±)-ψ-conhydrine [31] (Fig. 17.25).

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Chapter 18
Nicotine (*Pyridine-Pyrrolidine Alkaloid, Derived from* L-*Aspartic Acid and* L-*Ornithine*)

"Giving up smoking is the easiest thing in the world, I know because I've done it thousands of times"

Mark Twain

18.1 Occurrence and Introduction

Nicotine [1–3], the principal alkaloid of the plant *Nicotiana tabacum* Linn (Fam. Solanaceae), was first isolated in 1828 by Posselt and Reimann. It also occurs in other *Nicotiana* species. An interesting story rolls down through centuries: a Spanish man called Rodrigo de Jerez, who learnt about smoking from native Americans, started smoking "cylindrical rolls" of tobacco leaves in Portugal and puffed off smoke through nostrils and mouth. He was identified as a man with evil spirit and was put to prison. After several years of imprisonment he came out, and to his astonishment he found that a large number of people were already addicted to smoking.

Jean Nicot de Villemain, a French diplomat, introduced tobacco in France in 1560, long before the isolation of nicotine from tobacco leaves. The genus of the plant later has been named *Nicotiana* in his honor by Carl von Linné. Most of the tobacco products are prepared from *Nicotiana tabacum*. Natural nicotine is a levorotatory oily alkaloid, $C_{10}H_{14}N_2$, b.p. 247 °C, $[\alpha]_D$ –169 (neat), –154 (*c*. 4, EtOH); dipicrate, m.p. 222–223 °C. The specific rotation of nicotine depends upon the concentration and temperature and varies with the polarity of the solvent (*vide* Sect. 2.2.8).

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18.2 Structure Determination and Absolute Configuration

The structure and stereochemistry of (–)-nicotine have been deduced by some degradation and conversion reactions and identification of the products (Figs. 18.1 and 18.2). One of the products was shown to be (–)-*N*-methylproline {= (−)-hygrinic acid [4]} by chemical correlation with (–)-proline of known structure and absolute configuration [4] (Fig. 18.2). Further, formation of *N*-methylproline showed the site of attachment between pyridine and pyrrolidine rings. The stereostructure of (−)-nicotine is thus represented as (**1**) [1-methyl-2*S*-(3-pyridyl)-pyrrolidine] or [3-(1-methylpyrrolidin-2*S*-yl) pyridine] having (*S*) configuration at its only chiral center. The stepwise conversion of (–)-nicotine to (–)-*N*-methylproline may be explained on the basis of the structure (**1**) for (–)-nicotine (Fig. 18.2).

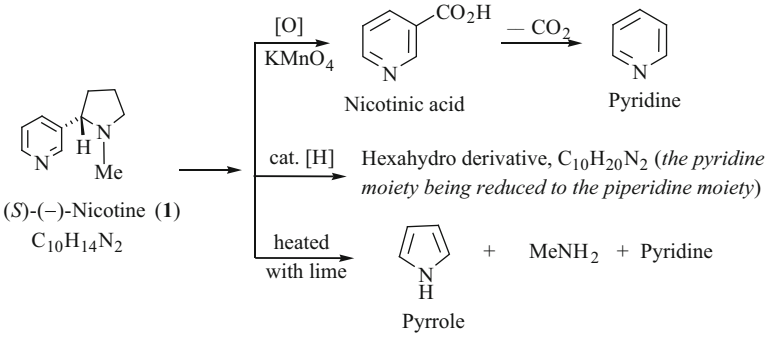


Fig. 18.1 Degradations of (–)-nicotine

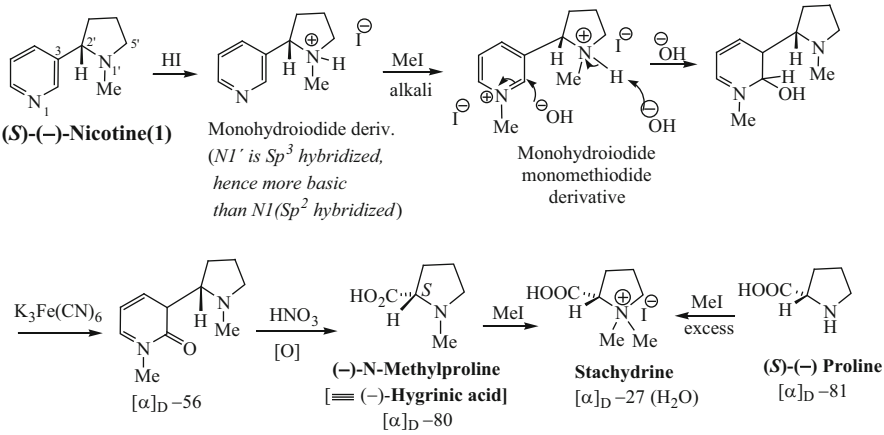


Fig. 18.2 Absolute configuration of (–)-nicotine and (–)-hygrinic acid by chemical correlation with (*S*)-(–)-proline [4]

18.3 Absolute Configuration from Optical Rotatory Dispersion Studies

The absolute configuration (*S*) of the only asymmetric center of nicotine may be ascertained directly from a comparison of the *optical rotatory dispersion* (ORD) curves (in EtOH) of (–)-nicotine and some analogues, (–)-anabasine and (–)-cotinine (the major metabolite of (–)-nicotine), all of them having similar shape [5] (Table 18.1). They all exhibit negative Cotton effect having the highest wavelength extremum appearing as a minimum (or trough) at 273 nm. The peaks appear at wavelengths corresponding to the strong absorption bands (ε ~25,000) of the pyridine ring at 270, 263, and 256 nm (as in 3-methylpyridine). However, the magnitude of the specific rotations at any given wavelength varied in the ORD curves of the compounds (see [4, 5] for the curves). The extra peak at 222 nm in the ORD curve of (–)-cotinine is due to the lactam group having strong additional absorption in 200–220 nm region (Table 18.1) (cf. 1-methyl-2-pyrrolidone, log ε 3.46).

(–)-Proline and (–)-pipecolic acid, each having a weakly absorbing carboxyl group at 200–210 nm (ε 40–70), exhibit a steeply descending negative plain curve (no Cotton effect), thus indicating the L-(*S*)-configuration of the α-carbon of the pyrrolidine and piperidine rings, respectively. The ORD curves of (–)-nicotine, (–)-anabasine, and (–)-cotinine are the results of the algebraic sum of the curves of the pyridine ring and the (–)-proline/piperidine residue.

The above correlation of the negative (or positive) Cotton effect and the absolute configuration (*S*) [or (*R*)] appears to be generally useful for the assignment of the absolute configuration of the α-carbon of pyrrolidine, piperidine, or tetrahydroisoquinoline alkaloids [4, 5].

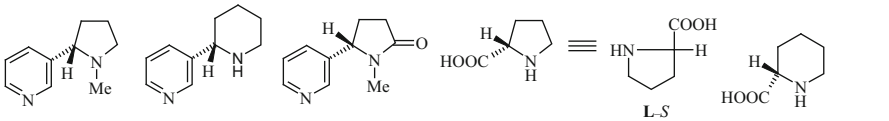


Table 18.1 Extrema (nm) in ORD curves

	Trough	Peak	Peak	Peak	Peak	Cotton effect sign
S-(–)-Nicotine	273	269	259	253		Negative
S-(–)-Anabasine	273	268		254		Negative
S-(–)-Cotinine	273	268	256		222	Negative

18.4 Synthesis

A number of total syntheses of nicotine have been reported.

(i) Späth and Bretschneider [6] in 1928 reported its total synthesis, as outlined in Fig. 18.3. It shows how the synthesis was achieved even in 1920s, using reactions needing readily available common reagents.

Some more syntheses (ii–vi) are briefly discussed and outlined here. These will illustrate how different synthetic methodologies are being developed with the advent of different catalysts and reagents.

(ii) (±)-Nicotine has been synthesized by reduction of an iminium moiety (>C=N^+R_2 or >C=N^+HR) by cyanoborohydride [7], which is capable of reducing >C=O as well as iminium group. At pH 6–7, the reduction of>C=O is negligible, while that of iminium group is appreciable. Fortuitously, the

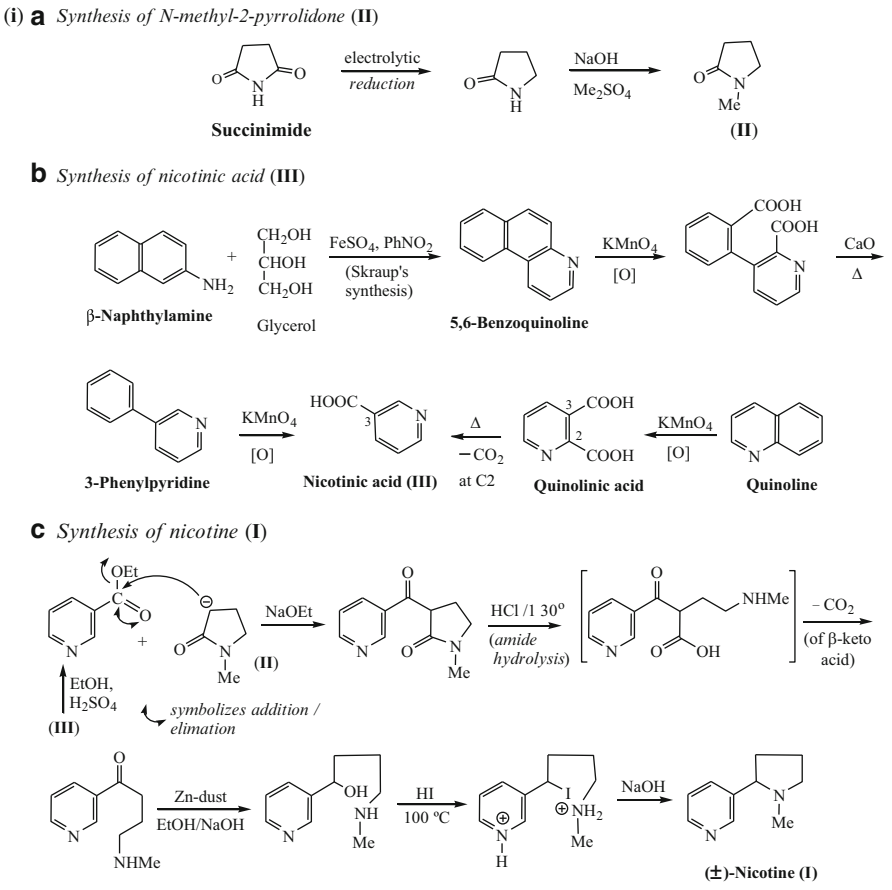


Fig. 18.3 Synthesis of (±) nicotine by Späth and Bretschneider (1928)

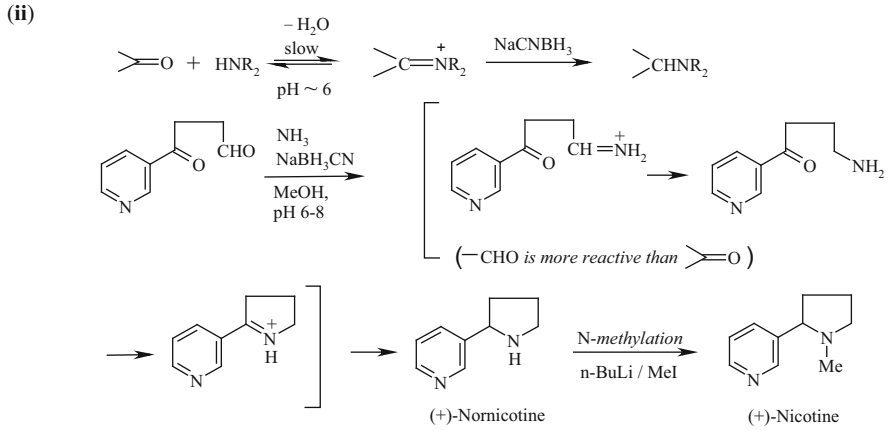


Fig. 18.4 Synthesis of (±)-nicotine (**1971**) [7]

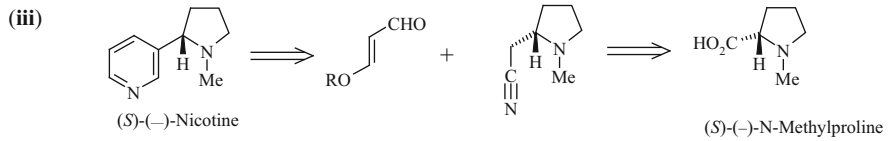


Fig. 18.5 Retrosynthetic strategy for (*S*)-(–)-nicotine synthesis using a chiral synthon

optimum pH for iminium formation is ~6. Thus, it was conceivable that an aldehyde or a ketone could be reductively aminated by treating the carbonyl compound with an amine at pH ~ 6 in the presence of sodium cyanoborohydride ($NaBH_3CN$) (Fig. 18.4). Racemic nicotine has been resolved in most cases *via* the formation of diastereomeric salts using (–)-tartaric acid.

(iii) *Synthesis of optically active nicotine using a chiral synthon*. Chavdarian [8] employed (*S*)-(–)-proline as the chiral source and extended its COOH group into a handle from which pyridine ring could be constructed (retrosynthetic approach, Fig. 18.5).

N-Methyl-(*S*)-L-prolinol (A) is prepared from L-proline by the routes (a) and (b); the route (b) is found to be slightly better (Fig. 18.6). The hydroxymethyl group of (–)-*N*-methylprolinol is converted into the cyanomethyl group by treatment of its chloride with NaCN. The cyanomethyl group is further elaborated for the construction of pyridine ring using 3-ethoxyacrolein to complete the need of the skeletal carbons of pyridine nucleus. Treatment of the cyanomethyl derivative with LDA generates the anion, to which 3-ethoxyacrolein is added to yield the hydroxy compound. The stereoselectivity of this reaction is not significant since the two newly generated chiralities would be lost in the subsequent steps. Cyclization to pyridine nucleus is then achieved with HBr (50 %) to yield the 2-bromo-(–)-nicotine;

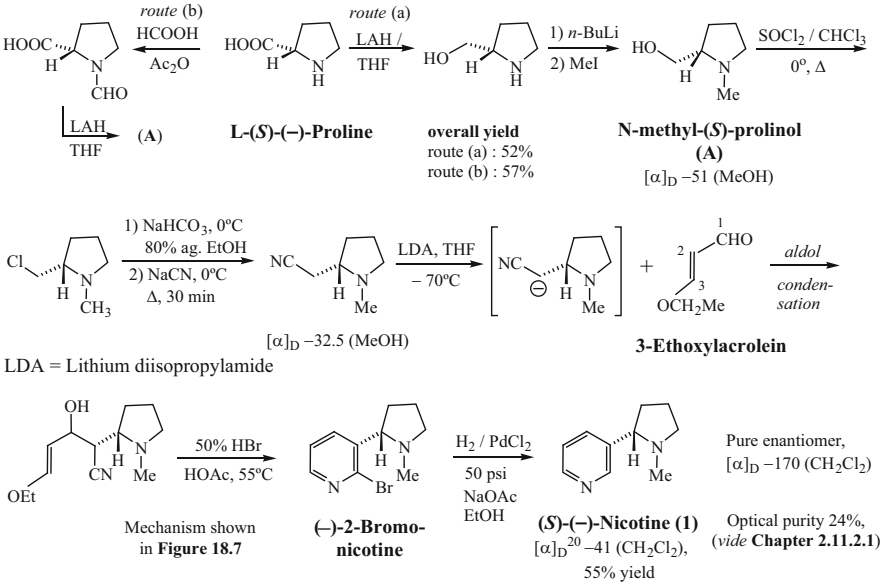


Fig. 18.6 A chiral synthesis of (*S*)-(–)-nicotine (**1982**) [8]

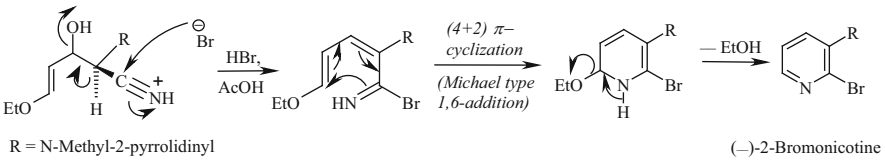


Fig. 18.7 Mechanistic rationale of cyclization to the pyridine nucleus (**1974**) [9]

finally bromine is eliminated by hydrogenation. The optical rotation $[\alpha]_D^{20}$ –41 (CH_2Cl_2) shows the conversion to be ~24 % of the pure enantiomer, $[\alpha]_D^{20}$ –170 (CH_2Cl_2). A plausible mechanism of the cyclization is shown in Fig. 18.7. Such type of cyclization is known in the literature [9]. This is the first synthesis of (*S*)-(–)-nicotine. Following this route (*R*)-(+)-nicotine has been synthesized starting from (*R*)-(+)-proline.

(iv) *An enantiomeric synthesis* [10] *of* (*S*)-(–)-*nicotine* (Fig. 18.8) has been achieved from a chiral homoallylic (*S*)-3-(1-azidobut-3-enyl) pyridine (**B**). The latter is subjected to intramolecular hydroboration and cycloalkylation (mechanism shown) to yield *S*-nornicotine. The latter on ethoxycarbonylation followed by LAH reduction yields (*S*)-(–)-nicotine in 94 % enantiomeric excess.

(v) In 2005, Helmchen and coworkers [11] accomplished an *enantioselective* (>99 % ee) *synthesis of R*-(+) *and* (*S*)-(–)-*nicotine* by Ir-catalyzed asymmetric allylic amination followed by ring closing metathesis (RCM) and recemization-free double bond reduction (Fig. 18.9). (–)Nicotine thus formed

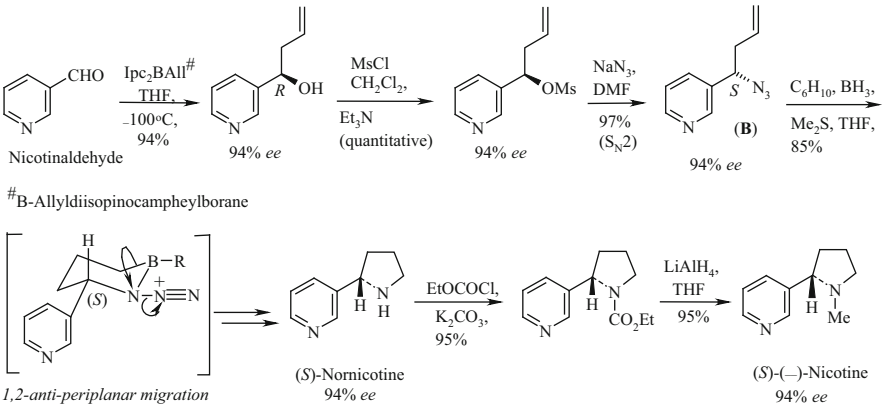


Fig. 18.8 An enantiomeric synthesis of (*S*)-(–)-nicotine (**2001**) [10]

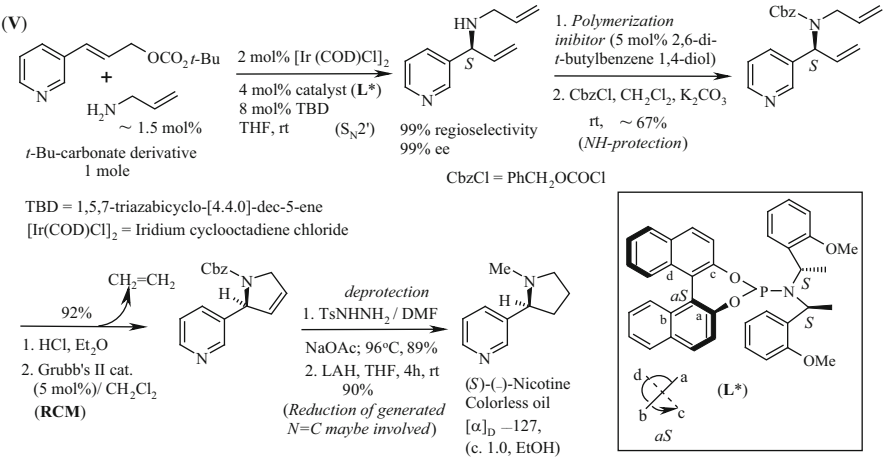


Fig. 18.9 An enantioselective synthesis of (*S*)-(–)-nicotine (**2005**) [11]

is converted into its hydrochloride, m.p. 159–161 °C, and compared with a sample prepared from commercial (*S*)-(–)-nicotine. (*R*)-(+)-Nicotine was also prepared using this route.

(vi) In 2009 a new methodology [12] has been reported for a novel and rapid synthesis of (±)-nicotine and its analogues (from nicotinaldehyde), the potential candidates for the treatment of central nervous system disorders such as *Alzheimer's* and *Parkinson's* diseases. The method involves a highly effective intramolecular [3+2] cycloaddition of a suitably disposed azomethine ylide leading to the construction of the pyrrolidine ring, as outlined in Fig. 18.10, with the plausible mechanistic sequence.

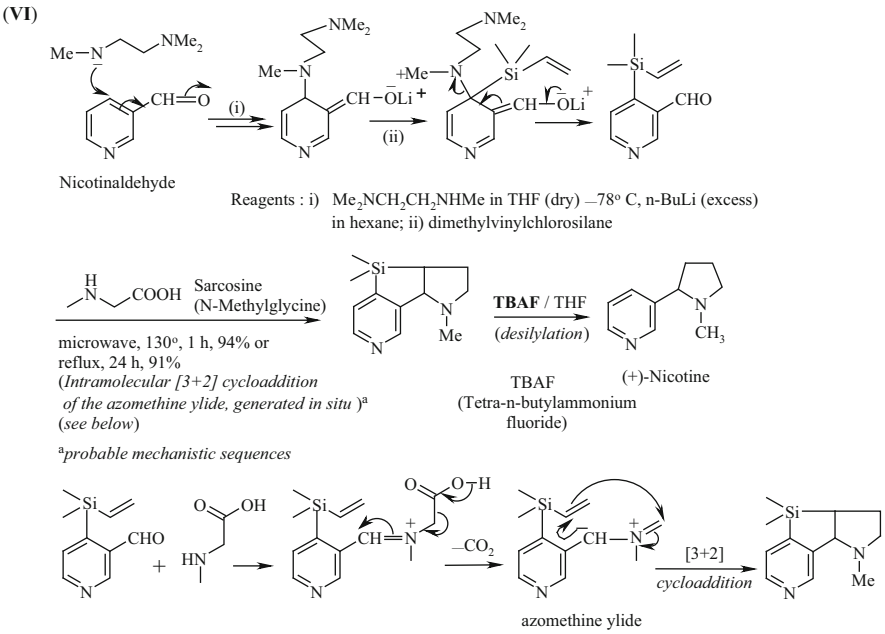
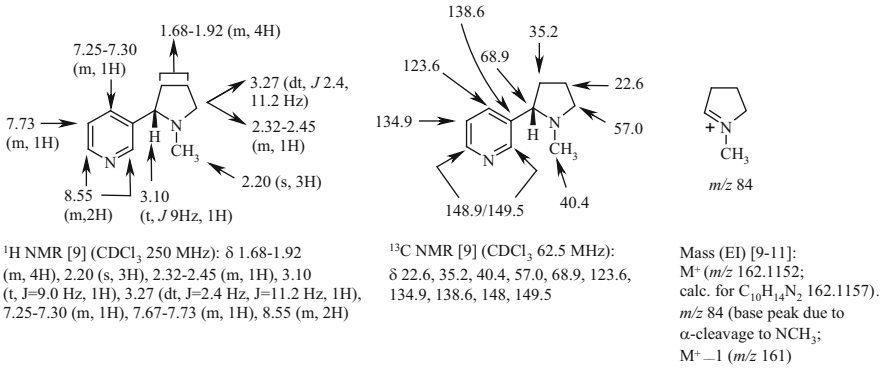


Fig. 18.10 Synthesis of (±)-nicotine using an intramolecular [3+2]cycloaddition reaction (**2009**) [12]



^{1}H NMR [9] ($CDCl_3$ 250 MHz): δ 1.68-1.92 (m, 4H), 2.20 (s, 3H), 2.32-2.45 (m, 1H), 3.10 (t, J=9.0 Hz, 1H), 3.27 (dt, J=2.4 Hz, J=11.2 Hz, 1H), 7.25-7.30 (m, 1H), 7.67-7.73 (m, 1H), 8.55 (m, 2H)

^{13}C NMR [9] ($CDCl_3$ 62.5 MHz): δ 22.6, 35.2, 40.4, 57.0, 68.9, 123.6, 134.9, 138.6, 148, 149.5

Mass (EI) [9-11]: M^+ (*m/z* 162.1152; calc. for $C_{10}H_{14}N_2$ 162.1157). *m/z* 84 (base peak due to α-cleavage to NCH_3; M^+–1 (*m/z* 161)

Fig. 18.11 NMR and spectral data (with assignments) of nicotine

18.5 Spectral Data

The ^{1}H NMR and ^{13}C NMR data and their assignments [11] and the mass spectral [11–14] data of nicotine are given in Fig. 18.11).

18.6 Biosynthesis [15–19]

The biosynthetic path of nicotine has been mapped by using labeled precursors.

(a) The pyridine part of it is derived from nicotinic acid which in turn is formed from 3-phosphoglyceraldehyde (PGA) and L-aspartic acid (Fig. 18.12), and (b) the pyrrolidine ring is generated from L-ornithine (Fig. 18.12) [15, 17–19].

(b) That the formation of *N*-methyl-Δ^1-pyrrolinium cation from L-ornithine goes via a symmetrical intermediate putrescine is proved by the ratio of labeled ^{14}C:^{15}N. During N-methylation and transamination—the responsible enzymes are deceived by radiomers as a consequence of which $^{15}NH_2$ as well as $^{14}NH_2$ are both involved in the transamination and also during N-methylation (Fig. 18.13).

(c) *Formation of nicotine* (Fig. 18.14). Leete suggested [15] that nicotinic acid is activated by reduction at C6 and C3 forming 3,6-dihydronicotinic acid. The reaction is stereospecific. The incoming hydrogen at C6 is entering its *Re*-face shown by label experiment, and the hydrogen introduced at C3 is *cis* on stereoelectronic argument. The hydrogen at C3 is acidic being adjacent to COOH group, and the process of regio- and stereospecific joining of two heterocycles leading to (*S*)-(–)-nicotine is mechanistically delineated in Fig. 18.14.

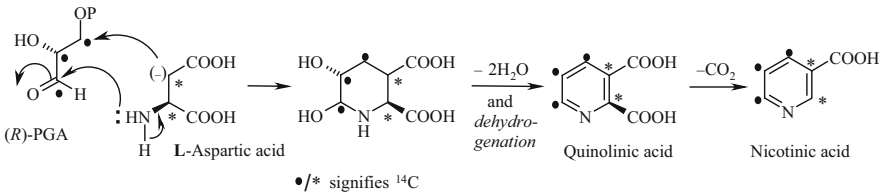


Fig. 18.12 Biosynthesis of nicotinic acid [15]

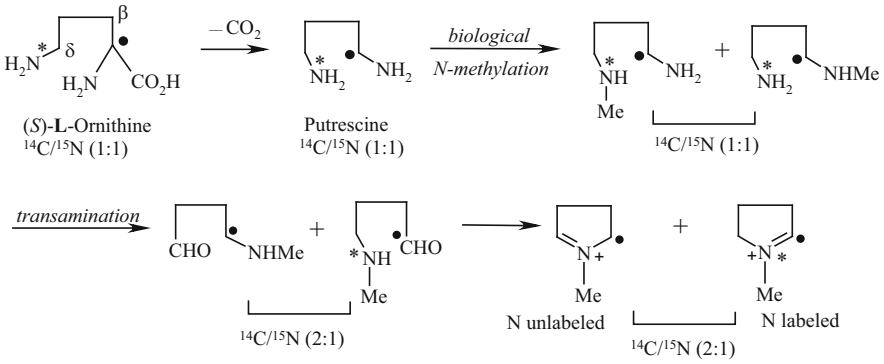


Fig. 18.13 Biosynthesis of pyrrolinium ion

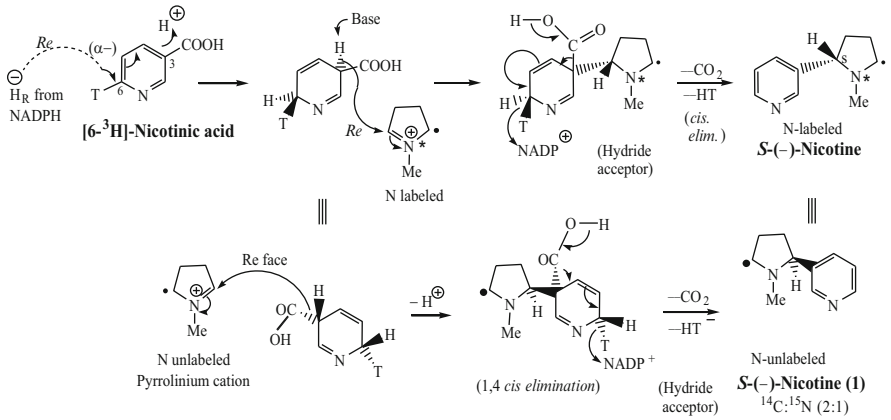


Fig. 18.14 Biosynthesis of (–)-nicotine from nicotinic acid and *N*-methylpyrrolinium ion

18.7 Preferred Molecular Conformation of Natural Nicotine and Synthetic 2-Nicotine and 4-Nicotine [20–23]

It has been established that (–)-nicotine possesses (*S*) configuration (see Sect. 18.2) at its only chiral center C2′ (see structure **1**). ^{1}H NMR spectral analysis [20] of nicotine and its suitable deuterated (pyrrolidine ring protons) analogs provide an unambiguous means of assigning chemical shifts of specific protons and their vicinal coupling constants. The data thus obtained suggest that the pyrrolidine ring attains an envelope conformation (**A**) with the methyl group and the pyridine ring in the preferred equatorial-like orientation having *trans* or *anti* conformation around N1′–C2′ bond (Fig. 18.15) (the relative orientation of pyridine and pyrrolidine rings).

Saturation of H2 led to the 9 ± 2 % NOE of the H2′ resonance whereas saturation of H4 led to its 5 ± 2 % NOE. These observations suggest near orthogonal orientation of the pyrrolidine and pyridine rings in the preferred conformation of nicotine, bringing H2′ closer to H2 than to that of H4. From a careful examination of a molecular model (Dreiding), it appears that this is possible if the bonds H3–H2, H2–N, and N–H6 constitute the front edge in the preferred conformation, while the other three bonds of the pyridine ring are in the rear, as shown in both (**A**) and (**B**). Either conformation also seems to involve minimum steric interaction between axial-like H2′ and H4. However, due to the nonbonded interactions of the axial N-Me group with the proximate hydrogens of the pyrrolidine and pyridine rings, the conformer (**B**) will be less stable than the conformer (**A**) with the NMe group in the equatorial orientation; thus, the conformer (**A**) is the preferred conformer of nicotine.

The pyramidal inversion of the pyrrolidine sp^{3} nitrogen results in the equilibrium with the corresponding more crowded, less preferred gauche conformation (**B**) of the N–Me and pyridine moieties around N1′–C2′ bond with the N1′–Me in the

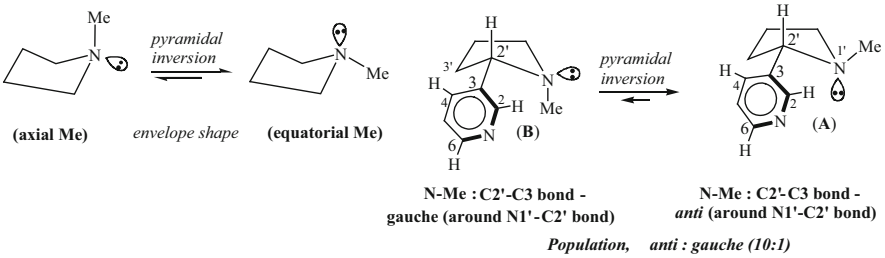


Fig. 18.15 Preferred conformation of (–)-nicotine

axial-like orientation. The increased energy and hence the less stability of the gauche conformer (**B**) is also due to the steric interaction between N1′–Me and H2 (or H4).

A comparison has been made between the solution conformation of natural nicotine (may be called 3-nicotine*) and the solution conformations of its isomeric 2-nicotine* and 4-nicotine*, and their ^{1}H, ^{2}H, ^{13}C, and ^{15}N nuclear magnetic resonances have been studied [22]. *The prefix number in the asterisked compounds 3-nicotine*, *2-nicotine*, *or 4-nicotine designates the pyridine ring position at which the pyrrolidine ring is attached*. Analysis of the long range coupling constants between H2′ and the pyridine protons suggests a perpendicular spatial arrangement of the pyrrolidine and pyridine rings for both nicotine-2 and nicotine-4, and Karplus parameters obtained from the ^{1}H analyses indicate an envelope conformation of the pyrrolidine ring for both with the pyridine ring and the methyl group both equatorial, similar to those for nicotine-3 [22]. The synthesis of these structurally related unnatural nicotinoids has been achieved, and their mass spectrometry along with that of nicotine has been investigated [23].

Molecular orbital calculations on nicotine suggests [21] that the *anti* conformer is favored over the gauche conformer (anti:gauche = 10:1).

The bioactive *pharmacophoric conformation* of nicotine is determined by using conformationally rigid synthetic analogs, elaborating the binding site topography. This study also leads to the anti-conformer (**A**) of nicotine.

18.8 Photochemistry of Nicotine [24]

When a methanolic solution of nicotine is irradiated in the presence of oxygen and the sensitizer methylene blue, as well as also with KCN, the pyrrolidine ring is affected in both cases [24], and the products profile[1] is shown in Fig. 18.16.

[1] In Fig. 18.16 we have shown the natural (–)-nicotine as the starting material and hence the absolute configuration at C2′ as (*S*) for all the optically active products. In the review [24] the authors mentioned nicotine (without sign of rotation) as the starting material and showed (*R*)-configuration for all active products.

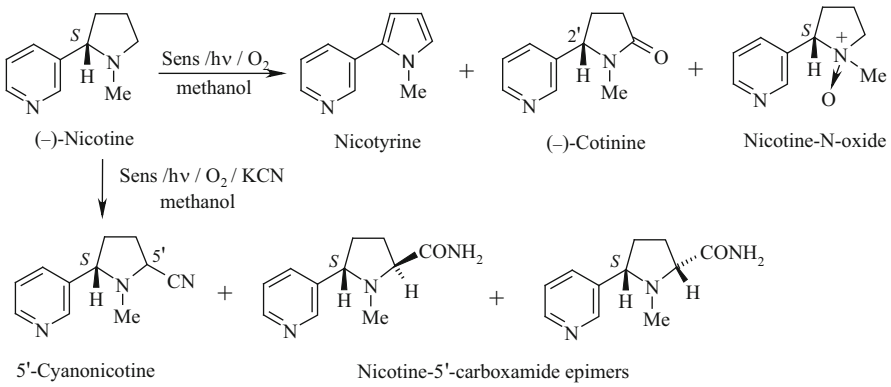


Fig. 18.16 Photochemical reactions of (–)-nicotine

18.9 Composition of Tobacco Smoke [25, 26]

Tobacco smoking is very much discouraged because of its bad effects on health. However, it is interesting to know what changes do take place during tobacco smoking.

Nicotine is present in the tobacco matrix as the salt of tobacco acids (such as malic acid, acetic acid, and formic acid), and its heterocyclic sp^2 and sp^3 nitrogens remain protonated. These salts are decomposed below 300 °C to non-protonated (free base) nicotine, which is monitored by thermal analysis studies (thermogravimetric mass spectrometric analysis TGA-MS). This study requires the heating of the material from room temperature to a selected temperature at a specific heating rate along with the monitoring of the loss of weight of the sample as function of time and temperature. *Basically*, *non-protonated* (–)*-nicotine is released with the retention of configuration of the chiral center in the smoke from the solid matrix* and such release depends on the vapor pressure of the substance and its thermal stability (Fig. 18.17). Further, it has been shown that transfer of nicotine in the smoke from non-protonated nicotine (matrix) as well as protonated nicotine takes place with parallel efficiency when the counter ions of the protonated nicotine are endogenous tobacco carboxylic acids.

Ammonium salts are added to cigarette blend, and it is thought to influence the above equilibrium and to help in releasing nicotine in the smoke. But experimentally no such role of ammonium salts could be documented [25]. They add to the cigarette blend and effect the transfer of nicotine from a puffing cigarette to smoke. It is interesting to note that during puffing of cigarette, peak coal temperature (burning center) can exceed 900 °C, though there is a rapid decrease in the temperature in the rest of the solid matrix and the smoke leaving distance from the coal center.

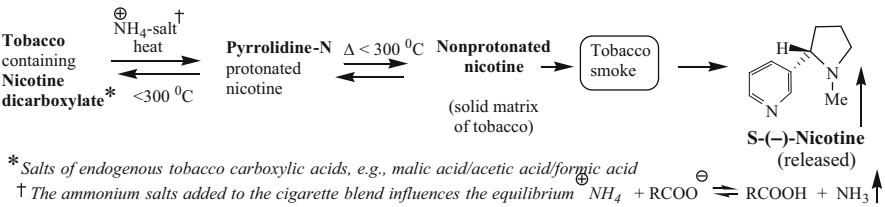


Fig. 18.17 Release of (–)-nicotine in the smoke from the solid tobacco matrix

The stereochemical integrity of (–)-(*S*)-nicotine remains unaltered [25] in cigarette smoke. However, Bowman et al. [27] mentioned a report on the racemization of (–)-nicotine during the course of tobacco smoking due to the conversion of a significant amount of (–)-nicotine to (+)-nicotine, and that (+)-nicotine has a lethality similar to that of (–)-nicotine in its insecticidal property [27]. Smoking cessation and nicotine replacement are still active areas of research.

18.10 Racemization of Natural (–)-Nicotine. Resolution of the Racemic Variety

The earlier method (before 1904) was to heat an acidic solution of (–)-nicotine in a sealed tube at 180–200 °C for several days to a week to get racemic nicotine, yield <30 %. Bowman developed [27] the following convenient method. Refluxing of (–)-nicotine in dry *p*-xylene with NaH in N_2 atmosphere for 6 h afforded (±)-nicotine, yield >93 %, $[\alpha]_D$ 0 (*c* 0.01, EtOH).

R-(+)-Nicotine was obtained [27] from the racemic variety through the diastereomeric salts of di-(*p*-toluoyl)-(–)-tartaric acid and fractional crystallization from absolute ethanol, followed by treatment with base. Resolution can also be done through diastereomeric salts of dibenzoyl-(–)-tartaric acid or MTPA (Mosher's acid, more expensive).

18.11 Bioactivity, Uses, and Therapeutic Potential

Effects of ingesting and smoking cigarettes [26]. Nicotine is a toxic alkaloid. In very low doses it stimulates the central nervous system while in high doses it is extremely harmful and causes paralysis of central nervous system. Once in the bloodstream, it is an extremely deadly poison: a lethal dosage for adults can be 40–60 mg. Death can result if a small child swallows only one cigarette. For an adult the lethal amount would be as little as half of a cigar or three cigarettes if he ingests them. But only a small fraction of nicotine from tobacco is released as such into the

smoke due to chemical processes taking place [25]. Nicotine is highly volatile to yield vapor (flash point 95 °C) and most of the nicotine released is burned off. Still enough can be inhaled to provide the known desired effects. Blood rich in nicotine reaches the brain from the lungs within only seven seconds, where it stimulates the release of chemical messengers like *acetylcholine*, *dopamine*, and *β-endorphine*. They produce feelings of calmness, relaxation, alertness, decreased anxiety, and enhanced pleasure, which give a mildly euphoric state. Memory and concentration are enhanced by increased acetylcholine level. The effects last up to 2 h.

Whereas nicotine exhibits cholinergic activity, this property is either severely attenuated or is lacking entirely for its isomers, 2-nicotine and 4-nicotine (see Sect. 18.7). The nicotinic receptor probably requires a precise spatial arrangement for the atoms responsible for binding at the active site. Thus, the loss in activity of 2-nicotine and 4-nicotine could result either from varying spatial placement of atoms such as the pyridine and pyrrolidine nitrogen atoms, which may bind to precisely situated sites at the receptor, or from electronic changes induced by the structural variation, affecting factors such as basicity. The ^{1}H–^{1}H vicinal and long range coupling constants of the nicotinoids are consistent with virtually identical solution conformations, so that the position of pyridine ring substitution has very little influence, if any, on the conformations of these molecules [22].

All over the globe, tobacco smoking and chewing are highly condemned as it is known to be harmful for health. A high percentage of lung and oral cancer patients have a history of long smoking and tobacco chewing habits, respectively. A lifelong smoker, Walt Disney (1901–1966) died of lung cancer [28]. Statistically, tobacco smoking is associated with shorter life expectancy [29]. However, nicotine and some of its analogues may have beneficial effects in the treatment of *Alzheimer's* disease (AD) and *Parkinson's* disease (PD), attention deficit hyperactivity disorder (ADHD), Tourette syndrome (TS), and in lowering anxiety and depression [30]. Further, the American Association of the Advancement of Science in Washington has made a noticeable observation on the improvement of the PD sufferers and ADHD patients wearing *nicotine patches* or using *nicotine chewing gums*. This encouraging observation advocates a follow-up research studies on ADHD and other neuropsychiatric illnesses.

A few comments and observations on the *neuroprotective effect of tobacco smoking* have been made in brief [31]. Recently, it has been observed that nicotine delays the onset of *Alzheimer's disease*. The mechanism of this surprising observation is not yet known. Another study showed that nornicotine (*N*-demethylnicotine) inhibits the formation of amyloid-β-peptide (**Aβ**) fibrils whose aggregation into neuritic plaques is a principal cause of this devastating disease. Compounds interacting covalently with **Aβ** are now being searched, which may be used for the treatment of Alzheimer's disease. It is predicted that this disease will afflict 22 million people worldwide within next 20 years. However, significant toxicity and psychoactivity of nicotine and nornicotine severely limits their therapeutic potential. Thus, the question remains whether "*A puff a day keeps the plaques away*?" [31]

Cosford and coworkers [32] of SIBIA Neurosciences Inc. achieved the seven-step synthesis of the enantiomerically pure 5-ethynyl-(*S*)-nicotine (~6 %), a light brown oil, $[\alpha]_D$ –164 (*c* 5, EtOH). It has been found to be a novel agonist, an important *anti-Perkinsonian agent* and named SIB-1508Y. It is used as its monomaleic acid salt, an off-white solid, m.p. 152–153 °C. The last part of the synthesis is outlined in Fig. 18.18. An enantioselective reduction of a synthesized imine was followed by N-methylation and crystallization of enantiomerically enriched-5-bromo-(*S*)-nicotine (30 % ee) as the dibenzoyl-L-tartrate. Pure 5-Bromo-(*S*)-nicotine (>99 % ee) thus obtained was subjected to a Pd/C-catalyzed cross coupling with 2-methyl-3-butyn-2-ol, followed by NaH catalyzed deprotection to yield altinicline with no loss of stereochemical integrity at any step. The absolute stereochemistry of enantiomerically pure 5-bromo-(*S*)-nicotine and altinicline is established by the hydrogenation of the former to afford (*S*)-(–)-nicotine. This synthesis was mentioned in a review [33], in which each compound was given (*R*)-configuration by oversight.

Wagner and Comins [34] synthesized SIB-1508Y, also known as *Altinicline*, starting from natural (*S*)-(–)-nicotine in expedient five steps in 32 % overall yield, via a regioselective substitution of the pyridine ring, as outlined in Fig. 18.19.

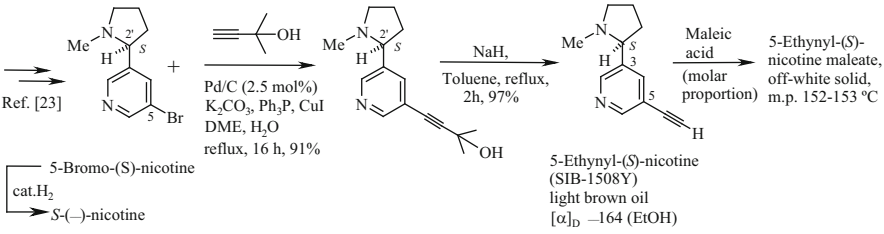


Fig. 18.18 Synthesis of SIB-1508Y from 5-bromo-(*S*)-nicotine [32] (**1998**)

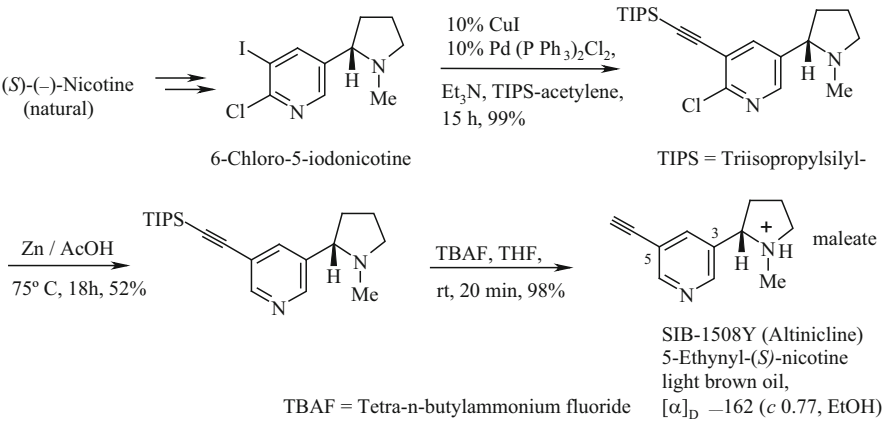


Fig. 18.19 Conversion of (*S*)-(–)-nicotine to SIB-1508Y (altinicline) [34] (**2006**)

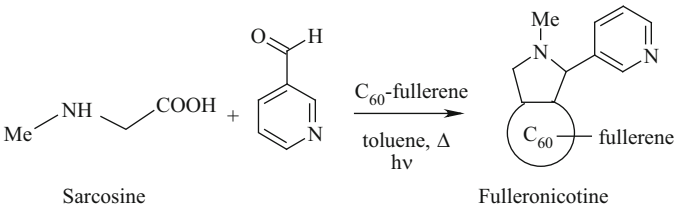


Fig. 18.20 Preparation of fulleronicotine

A fullerene derivative of nicotine, hence named *fulleronicotine*, has been prepared in a single step through a photocycloaddition reaction [35]. The C3′–C4′ bond of pyrrolidine ring is fused to a 6,6-ring juncture of C_{60}-fullerene (Fig. 18.20). Nicotine possesses wide range of biological activities, and its fullerene derivative may influence its toxicity and other biological activities. In this connection it may be of interest to note that 1,3-dipolar cycloadditions of azomethine ylides to C_{60} afforded a water-soluble fullerene derivatives [36].

Earlier, nicotine was used as an *insecticide*. In the villages still dried tobacco leaves are kept within the garments, especially the woolen ones, to repel the insects.

Highly effective treatment for the nicotine addiction forms a major part of research of current interests [37]. Haptens (substances which have a single antigenic determinant) for the generation of antibodies specific for (*S*)-nicotine, (*S*)- and (*R*)-nornicotine, and (*S*)-cotinine—a metabolite of (*S*)-nicotine—have been designed and tested. All these haptens do have the (*S*) configuration and alkyl linker of appropriate length at the pyrrolidine N of N-nor derivatives and the presence of two basic nuclei intact. Recently, a molecule with a linker of ~12 Å in length with an internal amide bond has been found to be close to nicotine in high affinity recognitional features. The mechanism of action has been briefly discussed [37]. Nornicotine is present as both (*R*) and (*S*) forms but only to the extent of 2 % of the total alkaloid content of tobacco.

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Further Reading

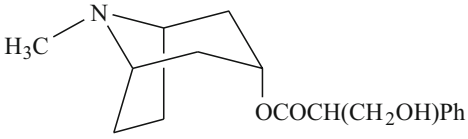
Stefan Berger and Dieter Sicker, 1.1 Nicotine in *Classics in Spectroscopy. Isolation and Structure Elucidation of Natural Products*, WILEY-VCH, **2009**, pp 3-24.

Chapter 19
Atropine [(±)-Hyoscyamine] and Cocaine (*Ornithine-Derived Alkaloids*)

19.1 Introduction

Atropine (≡ tropine tropate), the major alkaloid of jimson weed, *Datura stramonium* (Fam. Solanaceae) and the "deadly nightshade," *Atropa belladonna* (Fam. Solanaceae), is optically inactive, though it contains a chiral center; hence, it occurs as the racemate. In fact (*S*)-(–)-hyoscyamine, m.p. 108–111 °C, $[\alpha]_D$ –22 (50 % ethanol), present almost exclusively in the optically active form in the fresh leaves of the *Datura* and *Hyoscyamus* (*H. niger*) species (Fam. Solanaceae), partly racemizes to atropine during isolation. Atropine may thus be regarded as (±)-hyoscyamine. *Atropa belladonna* is associated with an interesting folklore. Fashionable ladies during renaissance used to add a drop of *belladonna* extract to their eyes to look glossy, bright, and attractive; "bella donna" are Italian words meaning beautiful lady [1].

19.2 Stereostructural Elucidation



Atropine (1)
[(–)-Hyoscyamine]

The structure of atropine has been elaborated through its degradations (Fig. 19.1) and syntheses. Formation of (**a**) and (**b**) suggests that atropine is an ester of tropine and tropic acid; hence, atropine is tropine tropate. The reaction and degradation products of atropine are outlined in Fig. 19.1.

S.K. Talapatra and B. Talapatra, *Chemistry of Plant Natural Products*,
DOI 10.1007/978-3-642-45410-3_19, © Springer-Verlag Berlin Heidelberg 2015

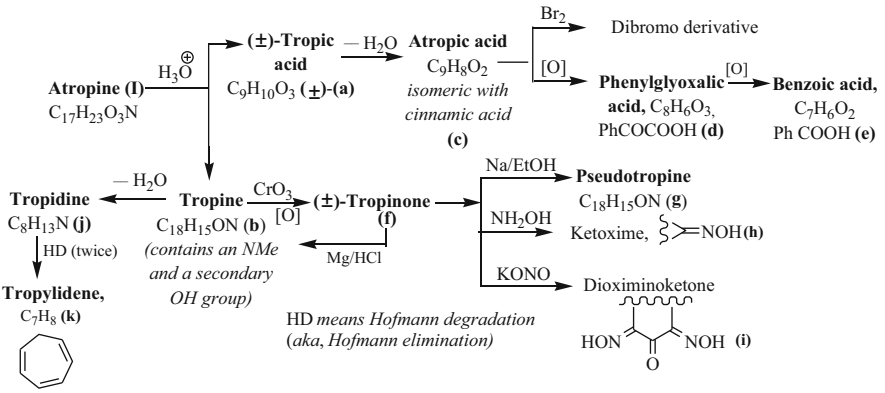


Fig. 19.1 Reactions and degradation products of atropine

19.2.1 Structure, Synthesis, and Absolute Configuration of (–)-Tropic acid (a)

Tropic acid (**a**), the hydrolysis product of atropine (**1**), upon dehydration gave atropic acid (**c**), which is nonidentical but isomeric with cinnamic acid (**c′**). Consequently, tropic acid could be represented by either (**a**) or (**a′**) (Fig. 19.2). The structure (**a**) of tropic acid has been proved by its synthesis (Fig. 19.3).

In the molecule of atropic acid, the electron withdrawing substituent (COOH) (both by resonance and inductive effects) being attached to the olefinic C2 transfers some electronic charge from C3 to the inner σ orbitals of C2 and distorts the π-molecular orbital as shown in Fig. 19.4. The electrophile (here $H^{\oplus}$) will attack the position with largest concentration of charge (at C2) in the bonding orbital and the nucleophile Cl^{-} interacts with the antibonding orbital at C3 leading to the formation of the *anti-Markonikov addition product* 2-chloromethylphenylacetic acid.

19.2.1.1 Absolute Configuration of (–)-Tropic acid, the Hydrolyzed Product of (–)-Hyoscyamine

Freudenberg [2] on the basis of the dextroshifts in the rotational values of (–)-mandelic acid and (–)-atrolactic acid in their respective esters suggested the absolute configuration of (–)-tropic acid (Fig. 19.5) which exhibits similar dextroshift in its ester. However, it was an empirical approach.

Fodor [3] later confirmed the absolute configuration (*S*) for (−)-tropic acid by indirectly correlating it with (−)-alanine as shown in Fig. 19.6. This correlation shows that quite disparate kind of chiral centers may be brought together by synthesis into one compound and or its enantiomer. Curtius reaction which occurs

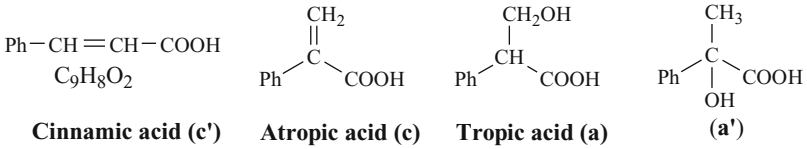


Fig. 19.2 Structure of tropic acid

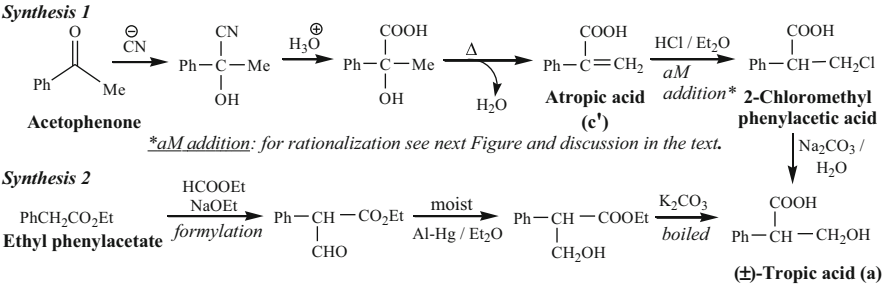


Fig. 19.3 Synthesis of (±)-tropic acid

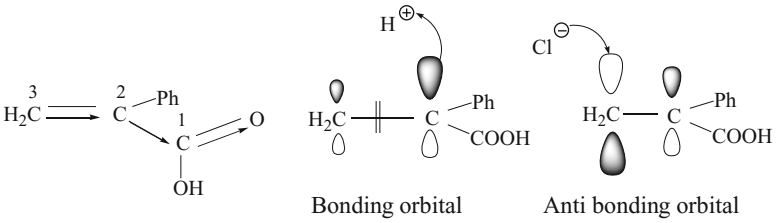


Fig. 19.4 Electron withdrawing effect on position of electrophilic attack

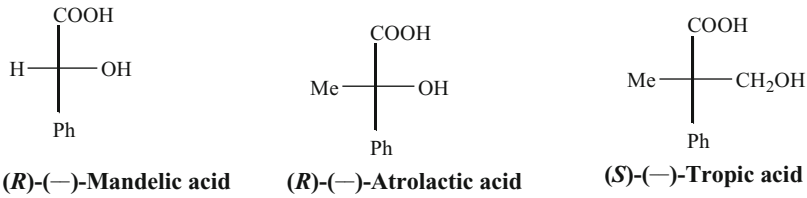


Fig. 19.5 Absolute configuration of (–)-tropic acid from rotational difference studies

with retention of configuration of the migratory group with very few exceptions has been employed to convert COOH group to NH_2 group. (*S*)-(+)-α-Phenylpropionic acid is correlated to the chiral standard L-(+)-alanine having absolute configuration (*S*). In the above relationship the chiral carbon atom has not been touched, and the

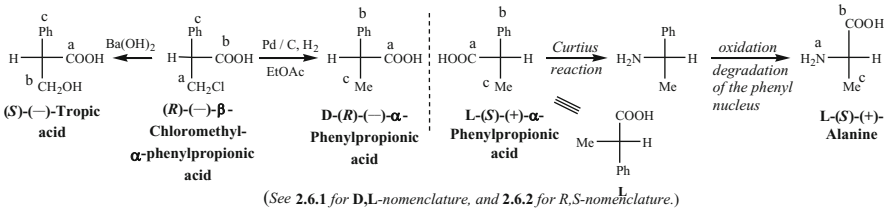


Fig. 19.6 Absolute configuration of (–)-tropic acid by correlation with L-(+)-alanine

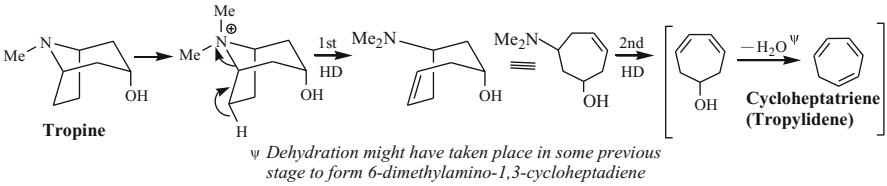


Fig. 19.7 Hofmann degradation (or elimination) products of tropine

sense of the priority sequence of the ligands in both the compounds is same. The enantiomer of (+)-α-phenylpropionic acid, *i.e.*, (*R*)-(–)-α-phenylpropionic acid, has been correlated to (–)-tropic acid by way of their direct relationship with a common compound, (*R*)-(–)-β-chloro-α-phenylpropionic acid. In doing so the chiral center has not been touched, but the sense of the priority sequence of the ligands around the chiral carbon is changed in (–)-tropic acid with respect to its precursor, (*R*)-(–)-β-chloromethyl-α-phenylpripionic acid, specifying the absolute configuration (*S*) to (–)-tropic acid [3] (Fig. 19.6).

19.2.2 Structure of Tropine

From the degradation products of tropine (Fig. 19.1), the latter is suggested to contain a secondary alcoholic OH group and a tertiary NMe group. Two Hofmann degradations are needed to eliminate nitrogen completely from the molecule. Formation of a 7-membered triene, tropylidene [(**k**), Fig. 19.1], requires a bicyclic system containing one 7-membered carbocyclic system and a nitrogen as a part of a bicyclic system, but not at the ring juncture that would have needed 3 HDs for complete elimination of nitrogen. Further nitrogen carries a Me group. The cyclic triene formation can be explained as shown in Fig. 19.7.

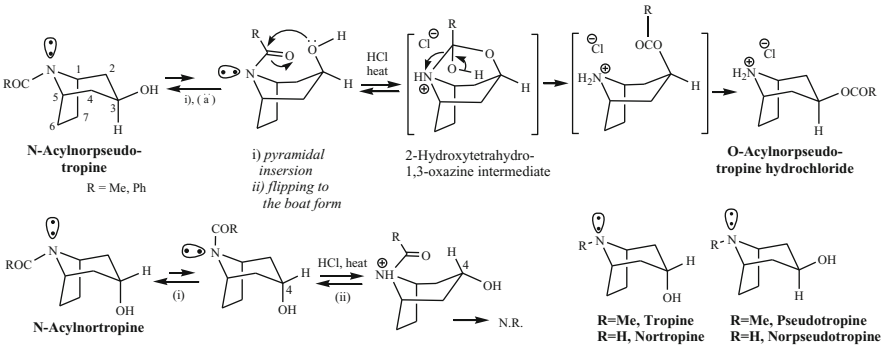


Fig. 19.8 N→O Acyl migration in acylpseudotropine

19.2.3 Configuration of the Hydroxyl Group of Tropine and Pseudotropine

The stereospecific N→O acyl migration was attempted in cases of *N*-acylnorpseudotropine and *N*-acylnortropine by heating with HCl. In the former case, the intramolecular rearrangement took place to *yield O*-acylnorpseudotropine hydrochloride, while under the same condition *N*-acylnortropine failed to rearrange (for conformations see Sect. 19.2.4). This behavior serves as the evidence for *cis* and *trans* disposition of the 3-OH group with respect to the nitrogen bridge in norpseudotropine and nortropine, respectively (Fig. 19.8).

19.2.4 Conformation of Tropine-Pseudotropine System [4–6]

It has been shown in Fig. 19.8 that the piperidinol ring in both tropine and pseudotropine exists almost exclusively in the chair conformation with very little percentage of boat conformation in equilibrium (cf., anancomeric system). The N-acyl (like NMe) group which exists in the equatorial orientation can assume axial orientation in equilibrium by pyramidal inversion. Flipping of the right half of the chair (piperidinol ring) in equilibrium brings the N-acyl group in the bowsprit position, and the equilibrium is shifted by demand of the reaction (N→O acyl migration). The 5-membered pyrrolidine ring is formed in tropine-pseudotropine system by fusion of C6 and C7 through axial bonds of the piperidine ring at C5 and C1, respectively.

Tropine and pseudotropine exhibit ν_{max} (free OH stretching maxima) at 3,626 and 3,621 cm^{-1}, respectively, in quite dilute solutions (2×10^{-3} M solution) in CCl_4. Thus, in such a dilute solution the bonded O–H stretching absorption is completely eliminated in both the alcohols, and the free O–H stretching band of tropine occurs at a slightly higher frequency (5 cm^{-1} in CCl_4) [4]. The absence of

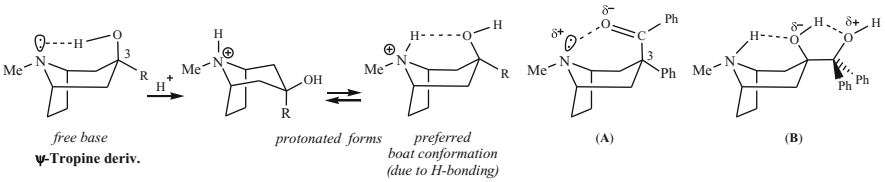


Fig. 19.9 Suggested conformation aspects of a ψ-tropine molecule (free base and protonated forms)

any detectable intramolecular hydrogen bonding indicates that the piperidinol ring of the tropine system must exist in a chair conformation, and the percent of molecules in a boat conformation must be smaller (<2 %) than can be detected by IR. The bonded hydroxyl absorption was observed at a much more concentrated solution (≥0.023 M) in CS_2. This is due to intermolecular hydrogen bonding between N and OH of different molecules, because such bonded OH stretching band appears at high concentration only.

A combination of proton magnetic resonance spectroscopic and dipole-moment measurements [5] confirms that tropanes exist with the piperidine ring in a chair conformation and with the N-methyl substituent predominantly equatorial. A 3α-substituent causes considerable ring distortion.

Carbon-13 NMR studies [6] later provided evidence for the presence of non-chair (boat) conformations and presence of intramolecular H-bonding in some tropane derivatives, with 3β-OH or 3β-yl ketone, e.g., 3α-phenyltropan-3β-yl-phenyl ketone (**A**) and 3α-hydroxydiphenylmethyltropan-3β-ol (**B**) (Fig. 19.9). This was verified by comparing the effects of protonation. In these tropane derivatives conformational change from chair to boat is exemplified.

19.3 Synthesis of Tropinone

Tropinone (**3**) is a key member of the tropane alkaloids, isolated from various plants of the family Solanaceae, viz. *Atropa belladonna* and *Datura stramonium* (jimson weed). Tropinone is a pivotal synthetic target because from it one could make a number of other congener alkaloids, *viz*., *tropine* (**2**), *ψ-tropine* (**2a**), *atropine* (**1**), *tropacocaine* (*ψ-tropine benzoate*), *ecgonine*, and *cocaine* (**7**).

19.3.1 Willstätter's Synthesis

In fact, the structure of tropinone, the oxidation product of tropine, was confirmed by its first synthesis by Richard Willstätter (NL 1915) in 1903 (Fig. 19.10) [7]. Though a roundabout one, *this synthesis will remain as one of the milestones*

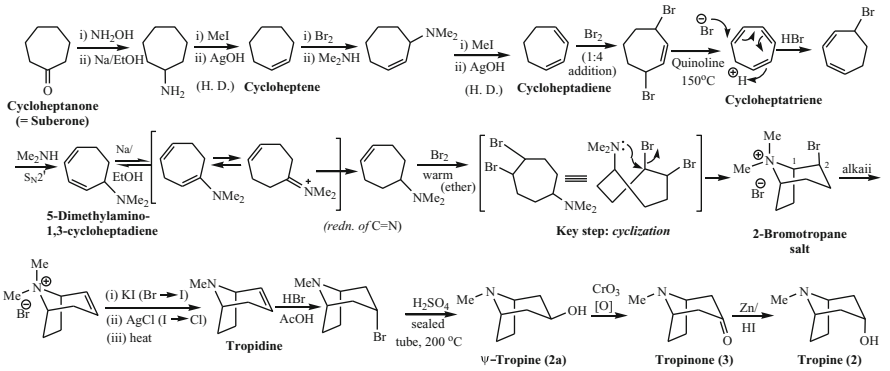


Fig. 19.10 Willstätter's total synthesis of tropine via tropinone (1903)

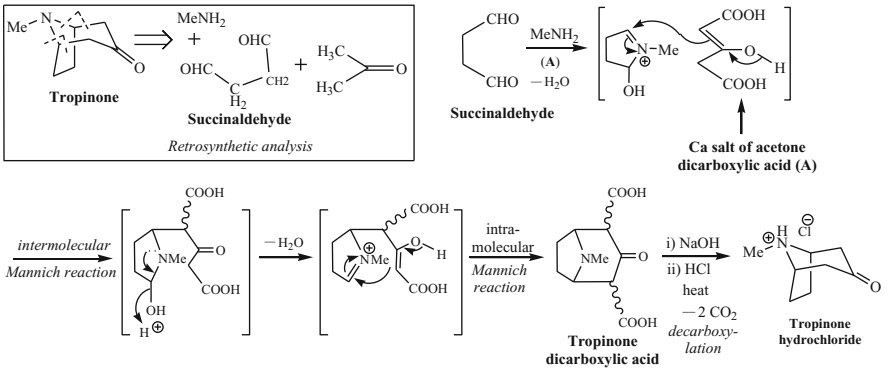


Fig. 19.11 Robinson's biomimetic total synthesis of tropinone hydrochloride (1917) [8]

in organic synthesis, *particularly if we project ourselves back to 19th century*. The key step in this synthesis is a truly historic reaction: a double bond dibromination primed a cyclization to generate the 2-bromotropane salt, which constitutes the first preparation of a tropane salt. All other steps are straightforward being predictable and are rationalized in terms of their stereochemistry and conformation, whenever applicable.

19.3.2 Robinson's Synthesis

Willstätter's synthesis of tropinone has been to some extent overshadowed by Robinson's one most brilliant synthesis (Fig. 19.11) [8], in which he selected the synthons on the basis of the symmetrical structure by dissecting the molecule on both sides of NMe and $CH–CH_2$ in the 6-membered ring as shown in the box of the

Fig. 19.11. This synthesis (1917), being close to Nature's way of synthesis—in a biogenetic fashion, marks the *beginning of the biomimetic synthesis as well as a multicomponent atom economic synthesis* [9] of organic molecules. Thus, a simple synthesis reflects the genius of Robinson.

Tropinone has been reduced to tropine and pseudotropine using different reducing agents.

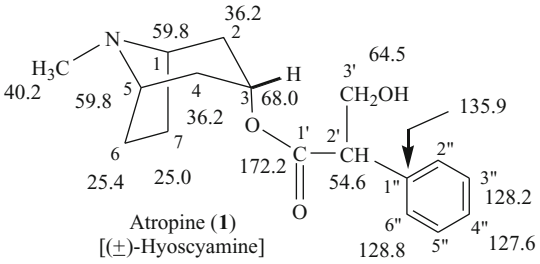
19.4 Spectral Data of Atropine

UV (EtOH): λ_{max} 246, 251.6, 257, 263.5, and 271 nm (ε 148, 175, 210, 143, and 25, respectively)

IR (KBr): ν_{max} 3,070 (H-bonded OH), 2,810 (N–CH_3 stretch), 1,725 (ester carbonyl), 1,155, 1,030 (C–O–C), 770, 725, and 690 (monosubstituted Ar) cm^{-1}

^{1}H NMR ($CDCl_3$): δ 7.23 (5H, brs, Ar*H*s), 4.96 (1H, t, *H*3), 3.9 (1H, m, *H*2′), 2.93 (2H, bs, *H*1 and *H*5), 2.34 (3H, s, NC*H*$_3$), 1.66 (8H, m, *H*2,4,6,7)

^{13}C NMR data(δ-values, $CDCl_3$) of atropine [6, 10] are shown in the structure (**1**)



Atropine (**1**)
[(±)-Hyoscyamine]

Because of the presence of a local plane of symmetry (**C_S**) (disregarding the C2′ chiral center two bonds away) in the tropine part of the molecule, the carbons at (1 and 5), (2 and 4), and (6 and 7) behave like enantiotopic atoms, and hence the protons and carbons at these paired positions are like isochronous and have very close δ-values [10]. Earlier, each of these three pairs was reported to have same δ_C values [6]. However, because of the entirely free rotation of the phenyl group around C1″–C2′ bond, δ_C of the carbons at the *ortho* positions 2″ and 6″ and *meta* positions 3″ and 5″ are found to be the same.

19.5 Biosynthesis of Tropine [10–13]

The *N*-methylpyrrolidinium part of tropine comes from ornithine, a nonprotein amino acid as described in Sect. 19.6 (Fig. 19.12). Several proposals regarding the rest part of tropinone, the immediate precursor of tropine, have been put forward. On critical analysis of these proposals, asking for experimental supports, Hemscheidt [13] arrived at the conclusion of accepting Robinson's general hypothesis of tropane biosynthesis [8] (Fig. 19.12). A nucleophilic attack on C2 of pyrrolidinium ion by acetone dicarboxylic acid, generation of pyrrolidinium ion on the less substituted side by dehydrogenation ($NADP^+$), intramolecular nucleophilic attack on the pyrrolidinium ion, hydrolysis, and decarboxylation generates tropinone (Fig. 19.12). Stereospecific reduction of tropinone by NADPH-dependent tropinone reductase (TRI) yields tropine, while enzyme TRII reduces tropinone to pseudotropine.

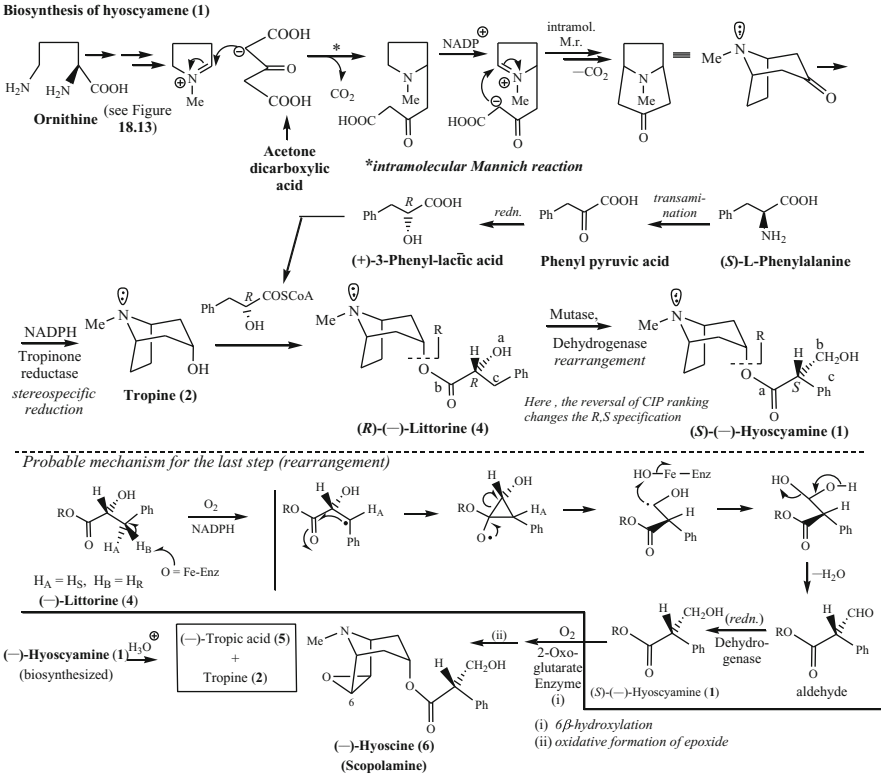


Fig. 19.12 Biosynthesis of (–)-hyoscyamine (**1**) via tropinone (**3**), tropine (**2**), and littorine (**4**). Biosynthesis of (*S*)-(–)-tropic acid (**5**) and (–)-hyoscine (**6**) via (–)-hyoscyamine (**1**)

19.6 Biosynthesis of (−)-Tropic acid and (–)-Hyoscyamine [10–12]

By label experiment it has been shown that *R*-(+)-3-phenyl-(1,3-$^{13}C_2$)-lactic acid is the precursor for (–)-tropic acid part of the alkaloid. However, ^{13}C NMR spectra of (–)-tropic acid obtained from labeled (–)-hyoscyamine upon hydrolysis showed that the observed ^{13}C-^{13}C coupling needs the rearrangement of the carbon connectivity of phenyllactic acid during its conversion to (–)-tropic acid moiety. Interestingly, (–)-tropic acid does not enter into the biogenetic esterification leading to the alkaloid formation. Thus, 3-phenyllactic acid esterifies tropine to form another natural alkaloid (–)-littorine prior to the (intramolecular isomerization) rearrangement of the 3-phenyllactic acid to tropic acid, demonstrating thereby that free tropic acid is not an intermediate, rather littorine is the intermediate precursor of (–)-hyoscyamine, and for that matter atropine. (–)-Hyoscyamine, thus biosynthesized, on hydrolysis furnishes (–)-tropic acid [13]. The rearrangement of the acid part of littorine may be rationalized as shown in Fig. 19.12. By labeling experiment it has been shown that H_R is stereospecifically removed from C3.

19.7 Use of Atropine

Atropine, the racemic form of hyoscyamine, is widely used for the dilatation of eye pupils in the treatment of various diseases of eyes (Chap. 33).

19.8 Scopolamine (6)

Two other important tropane alkaloids are scopolamine [≡ (–)-hyoscine (**6**)] (Fig. 19.12) and cocaine. Scopolamine (the racemic form is called atroscine) co-occurs with (–)-hyoscyamine (**1**). It finds application prior to surgical operation under general anesthesia to arrest salival and mucus secretions.

19.9 Cocaine (7)

19.9.1 Introduction

The stimulant and hunger suppressant properties of *coca* have been known for centuries. The alkaloid cocaine was first isolated [14] from the leaves of *Erythroxylon coca* (Fam. Erythroxylaceae) (*coca*) by the German chemist Friedrich

Gaedcke in 1855, and named it erythroxyline. Niemann [15] isolated this alkaloid from Peruvian *coca* leaves as colorless transparent prisms in 1860. In his PhD dissertation (preserved in British Museum) he worte, "Its solutions have an alkaline reaction and a bitter taste; it promotes the flow of saliva and leaves a peculiar numbness, followed by a sense of cold, when applied to the tongue." Niemann named it "cocaine" from "coca" + suffix "ine." Because of its use as a local anesthetic a suffix "caine" was later used to name synthetic local anesthetics. Cocaine occurs in smaller amounts in some other species of *Erythroxylon*.

19.9.2 Synthesis of Cocaine

The elucidation of the structure of cocaine and its first synthesis were achieved by Willstätter [16] in 1898. It can be synthesized from tropinone [17] in few steps as shown in Fig. 19.13. A total synthesis [18] is schematically outlined in Fig. 19.14.

19.9.3 Biosynthesis of Cocaine

As evident from feeding experiments, biosynthesis of cocaine is schematically outlined in Fig. 19.15 [19–22]. Two acetyl-CoA units are added to the *N*-methyl-Δ′-pyrrolinium cation (see Fig. 18.13). The first addition is a Mannich-like reaction

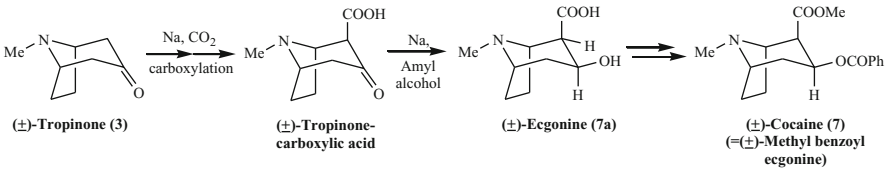


Fig. 19.13 Synthesis of (±)-cocaine (**7**) from tropinone (**3**)

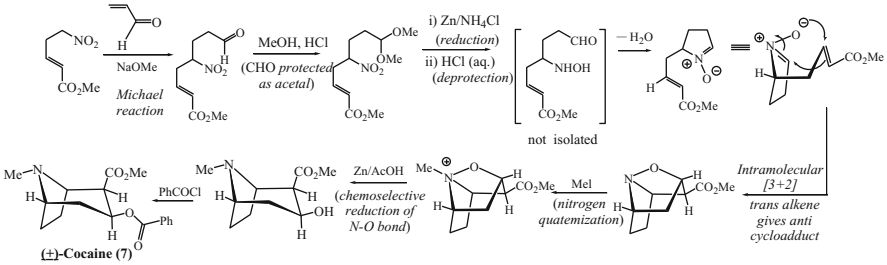


Fig. 19.14 Total synthesis of (±)-cocaine (**7**)

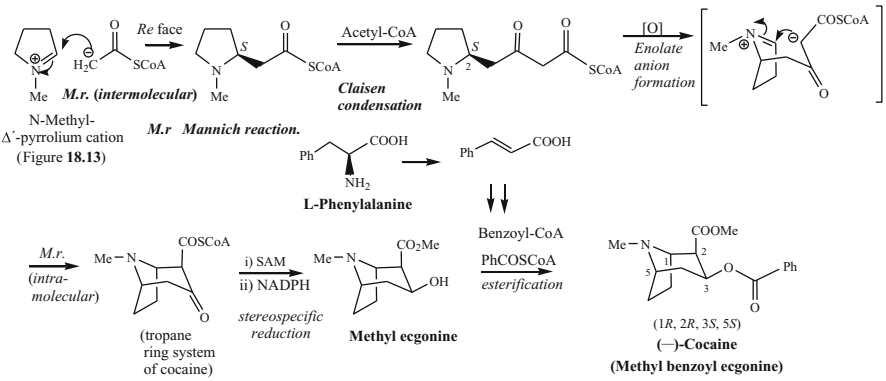


Fig. 19.15 Biosynthesis of cocaine

with the carbanion from acetyl-CoA acting as a nucleophile attacking on the *Re* face of the pyrrolinium cation acting as the electrophile to produce the (±)-2-substituted pyrrolidine. The second addition occurs through a Claisen condensation. In the biosynthesis of cocaine, however, only the (*S*)-enantiomer can cyclize to form the tropane ring system of cocaine [19]. The stereoselectivity of this reaction was further investigated through the study of prochiral methylene hydrogen discrimination [20]. This is due to the asymmetric induction of the chiral center C2 of the 2-substituted pyrrolidine [21]. This process occurs through an oxidation regenerating the intermediate pyrrolinium cation, formation of an enolate anion, followed by an intramolecular Mannich reaction. The tropane ring system thus formed undergoes hydrolysis, SAM-dependent methylation and stereospecific reduction with NADPH to form methyl ecgonine. The latter is then benzoylated by benzoyl-CoA to form (–)-cocaine. Benzoyl-CoA required in the last step is biosynthesized from L-phenylalanine via cinnamic acid [21, 23].

19.9.4 Biological Activities and Uses

Cocaine stimulates central nervous system, and increases the physical endurance. It possesses local anesthetic properties, and is used during some operation of ear, throat and nose, carried out under local anesthesia. It was also used as toothache drops.

The major disadvantages of its use are its intense vasoconstrictor activity (narrowing of the blood vessels) and its potential for cardiovascular toxicity. The functionalities of cocaine required for its activity were evaluated to be the aromatic carboxylic acid ester and the amino group, separated by a lipophilic hydrocarbon chain. Cocaine has since been largely replaced in Western medicine by more simple synthetic local anesthetics (developed from the cocaine structure based on its active

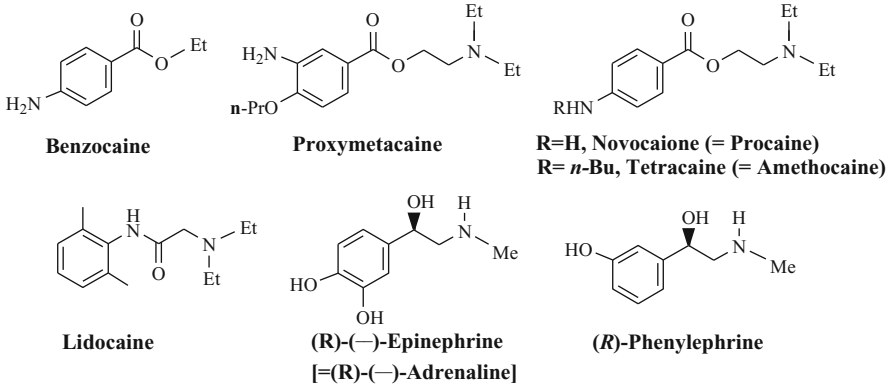


Fig. 19.16 Some synthetic local anesthetics and vasoconstrictors

functionalities), such as *benzococaine*, *proxymetacaine*, *lidocaine*, and *tetracaine* (Fig. 19.16), though cocaine remains available for use, if specified. If vasoconstriction is desired for a procedure (as it reduces bleeding), the anesthetic is combined with a vasoconstrictor such as (*R*)-(–)-*epinephrine or* (*R*)-*phenylephrine* (Fig. 19.16). Cocaine is currently prescribed for use as a local anesthetic for mouth and lung cancers in Australia.

Cocaine gives a sense of euphoric happiness, but it causes addiction, which is a great global concern. A number of cocaine analogs has been synthesized to study the binding characteristics at cocaine receptor [23]. Sigmond Freud was impressed by the ability of cocaine to stimulate the central nervous system. He treated his morphine-addicted colleague with cocaine. It was a successful attempt but his colleague became the first identified cocaine addict of the world (1884).

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Chapter 20
Ephedrine and Pseudoephedrine (C_6–C_1 Part Derived from L-Phenylalanine and Nitrogen Derived by Transamination)

20.1 Introduction

(−)-Ephedrine, m.p. 33–42 °C (depending on the water content) and its diastereomer (+)-pseudoephedrine (also written as (+)-ψ-ephedrine) occur in various *Ephedra* species [1] (Fam. Ephedraceae). (−)-Ephedrine was first isolated from the Chinese drug "Ma Huang" in 1885 and was purified in 1887 by Nagai who also obtained pseudoephedrine from the same source. The drug Ma Huang is reported to be *Ephedra sinica* as well as a mixture of *E. equisetina*, *E. sinica*, and *E. distachya*. *Ephedra* belongs to the order Ephedrales, of which it is a single genus [2]. In some literature, the genus *Ephedra* has been included in the family Gnetaceae [3]. The *E. sinica* plant extracts containing (−)-ephedrine have been used for nearly 5,000 years in Chinese folk medicine. The genus *Ephedra* is not confined to China and Japan but has a much wider geographical distribution [1, 3, 4]. Ephedrine and ψ-ephedrine are also obtained from other sources [1].

20.2 Structure

The structure of (−)-ephedrine has been elucidated by degradation reactions (Fig. 20.1) and confirmed by synthesis. Hofmann degradation has been very useful in its structural elucidation. Only a few degradative reactions will be discussed. The structure was first suggested by Ladenburg [5]. The stereostructures of natural (−)-ephedrine and (+)-ψ-ephedrine are shown in Fig. 20.1.

Formation of compounds (a), (b), (d), and (e) suggested the presence of a monosubstituted benzene ring with a C_3-side chain in ephedrine; (b), (d), and (e) further suggested that the ephedrine side chain contains a benzylic hydroxyl group with an adjacent amino function, since (d) is formed as a Hofmann degradation product. The presence of –NHMe group is supported by the formation of $MeNH_2$ (c). All the products suggested structure (1) for ephedrine without

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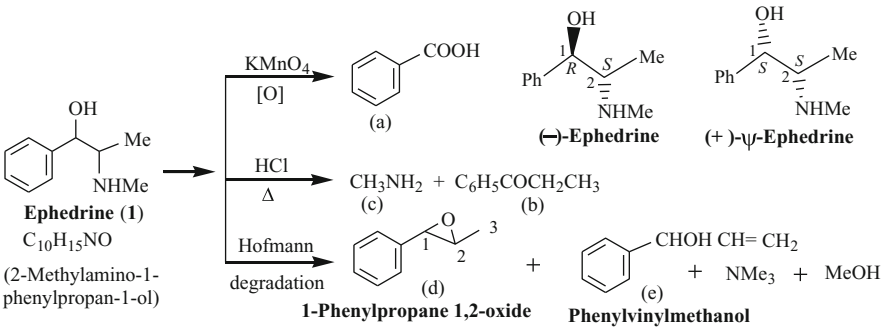


Fig. 20.1 A few degradative reactions of ephedrine

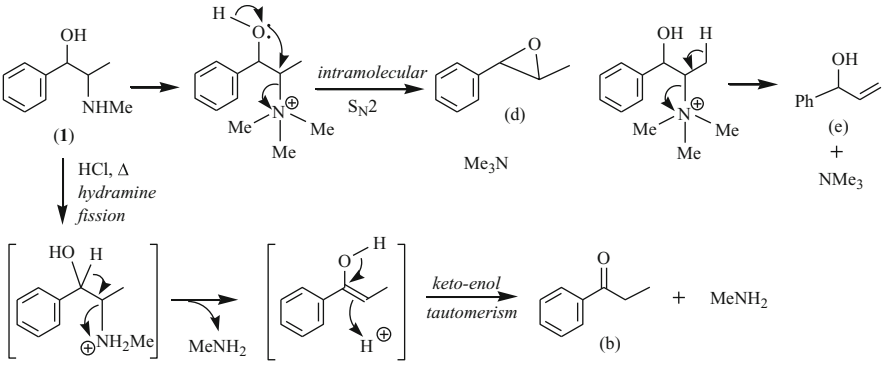


Fig. 20.2 Hofmann degradation and hydramine fission products of ephedrine

stereochemical description. The formation of Hofmann degradation products as well as the hydramine fission [6] products has been shown in Fig. 20.2.

20.3 Relative and Absolute Stereochemistry of *Ephedra* Alkaloids

Formation of chiral cyclic compounds/intermediates with ligands attached to the chiral centers, keeping the stereochemistry of the centers intact, may sometimes be of help in ascertaining the relative stereochemistry of the diastereomers. One way by which the relative stereochemistry of ephedrine and ψ-ephedrine was determined is discussed in the sequel.

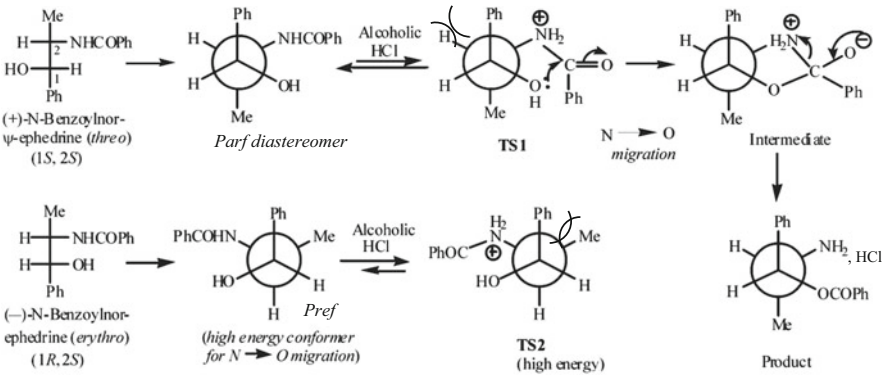


Fig. 20.3 N → O migration in *N*-benzoylnor-ψ-ephedrine (*threo*) and its absence in *N*-benzoylnorephedrine (*erythro*)

20.3.1 Relative Stereochemistry [7]

N-Benzoylnor-ψ-ephedrine upon treatment with alcoholic hydrogen chloride is rapidly converted into the hydrochloride of the *O*-benzoyl derivative, whereas *N*-benzoylnorephedrine remains unchanged under the same condition. This difference can be explained in the following way (Fig. 20.3). The facile reaction of *N*-benzoylnor-ψ-ephedrine involves N → O migration of benzoyl (PhCO) group in the transition state (**TS1**), when the benzamido and hydroxyl groups are gauche, and the bulky methyl and phenyl groups are *anti*. In case of the *N*-benzoylnorephedrine, in the transition state (**TS2**) for the attempted N → O migration, the bulky phenyl and methyl groups become crowded together in the gauche conformation making the activation energy too high for the migration to take place under such condition. Thus, the relative stereochemistries of ψ-ephedrine and ephedrine are suggested to be *threo* and *erythro*, respectively (Fig. 20.3). Such migration has also been observed in the other direction (O → N) in the presence of a weak acid.

Comparison of the basicity of ephedrine and ψ-ephedrine also leads to their *erythro* and *threo* configurations, respectively, as explained in Sect. 20.4.

20.3.2 Absolute Configuration

Once the absolute configuration of one chiral center is known that automatically fixes the absolute stereochemistry of the adjacent chiral center, the relative stereochemistry is known. Synthesis of (−)-ephedrine and (−)-ψ-ephedrine from D-(−)-mandelic acid (Fig. 20.4) leads to their absolute configurations (1*R*, 2*S*) and (1*R*, 2*R*), respectively.

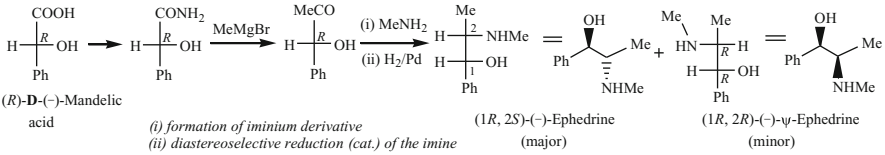


Fig. 20.4 Chemical conversion of (R)-(−)-mandelic acid into (−)-ephedrine and (−)-ψ-ephedrine

Table 20.1 pK_a values of ephedrine and related compounds [8]

	pK_a [a]	pK_a difference	Temp. (°C)
(−)-Ephedrine (**1**)	9.14	0.08	22
(+)-ψ-Ephedrine (**2**)	9.22		22
(−)-*N*-Methylephedrine	8.50	0.31	27
(+)-*N*-Methyl-ψ-ephedrine	8.81		27

[a]Determined in 80 % aqueous methyl cellosolve. A stronger base will have a higher pK_a value

20.4 Basicity of Ephedrine and Related Compounds

From Table 20.1 [8] it is concluded that ephedrine (**1**) (the most populated conformer **1a**) and its *N*-methyl derivative are weaker bases than ψ-ephedrine (**2**) (the most populated conformer **2a**) and its *N*-methyl derivative, respectively. This also leads to the *erythro* configuration of ephedrine (**1**) and its *N*-methyl derivative and *threo* configuration of ψ-ephedrine (**2**) and its *N*-methyl derivative (Fig. 20.5). The increased basicity of (**2**) may be ascribed to stabilization of its conjugate acid as shown in (**2b**) by the H-bonding between N–H and O, i.e., (>HN^+–H···OH–), and also by electrostatic attraction between unlike charges on the N and O (partial charge) atoms. It is to be noted that *the formation of H-bond in the ephedrinium ion is hindered by the repulsion of the phenyl and methyl groups (structure* **1b***), while there is no such repulsive interaction in the ψ-ephedrinium ion (***2b***).* If H-bonding between N and O–H, i.e., (>HN···HO–), is operative in the partially eclipsed form of the free base, its basicity in this form will be absent because of nonavailability of the lone pair of nitrogen. Moreover, this form will be destabilized because of electrostatic repulsion of O and N lone pairs.

The decreased basicity of both **1** and **2** on N-methylation is probably due to the decrease of the positive charge on N due to the inductive effect of the additional methyl group and thus weakening the H-bonding of their conjugate acids and decreasing their stability.

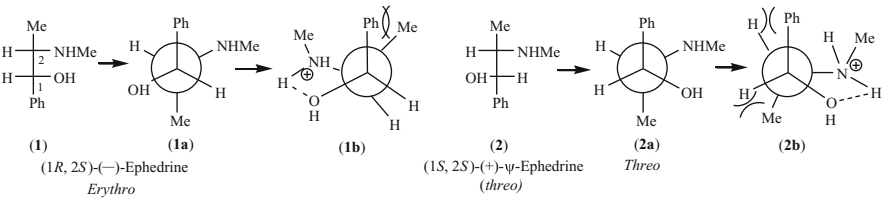


Fig. 20.5 Ephedrine and ψ-ephedrine and their conjugate acids [8]

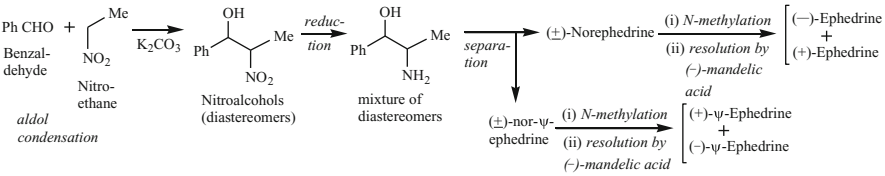


Fig. 20.6 Synthesis of (±)-ephedrine and (±)-ψ-ephedrine and their resolution into active enantiomers [9, 10]

20.5 Synthesis of (±)-Ephedrine and (±)-ψ-Ephedrine and Their Resolution

Several syntheses of *Ephedra* alkaloids have appeared in the literature. In fact, for the establishment of their absolute stereostructures, a four-step reported synthesis has already been mentioned from D-(−)-mandelic acid (Fig. 20.4). A simple synthesis of (±)-ephedrine and (±)-pseudoephedrine and their subsequent resolution to the optically active varieties [9, 10] are shown in Fig. 20.6. Benzaldehyde was allowed to react with nitroethane in the presence of potassium carbonate when a diastereomeric mixture of nitro alcohols is formed through aldol condensation. The latter on reduction yielded (±)-norephedrine as well as (±)-nor-ψ-ephedrine. Each racemate upon separation, N-methylation, and subsequent resolution gave the corresponding optically active enantiomers.

Stereoselective synthesis of (±)-ephedrine was achieved [11] by Roger Adams in 1928. Bromination of propiophenone followed by reaction with methylamine provided the logical synthetic precursor (±)-α-methylaminopropiophenone (Fig. 20.7). Catalytic reduction of this aminoketone hydrochloride with hydrogen over platinum gives (±)-ephedrine almost exclusively in over 90 % yield. Thus, this is a stereoselective synthesis of ephedrine. However, reduction of the aminoketone with sodium amalgam gives a mixture of (±)-ψ-ephedrine (which predominates slightly) and (±)-ephedrine. Thus, this synthesis is largely non-stereoselective. The reactions are summarized in Fig. 20.7.

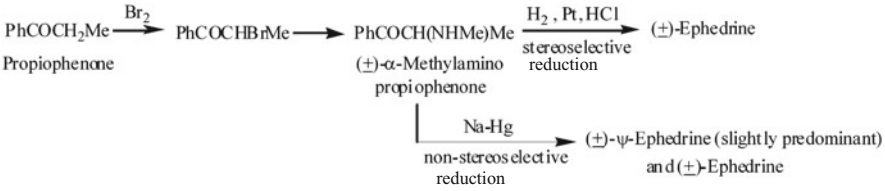


Fig. 20.7 Stereoselective synthesis of (±)-ephedrine [11]

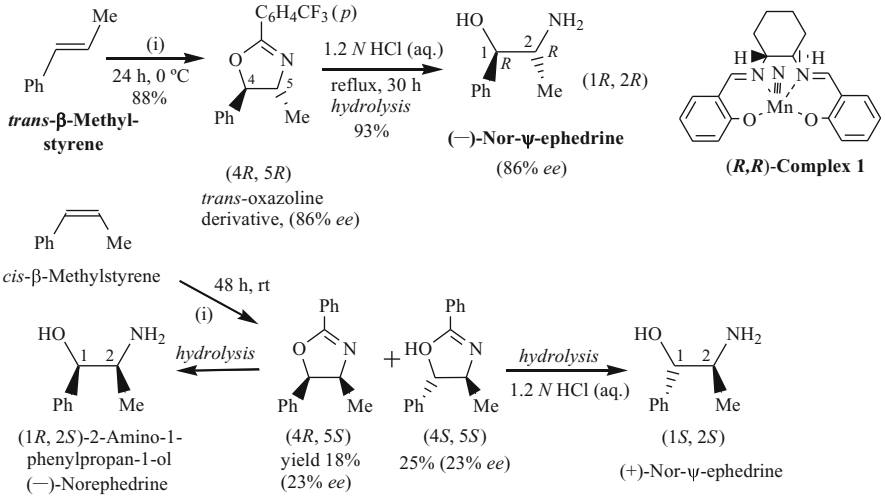


Fig. 20.8 Asymmetric synthesis of (−)-nor-ψ-ephedrine, (+)-nor-ψ-ephedrine, and (−)-norephedrine [12]

20.6 Asymmetric Synthesis of Ephedrine Derivatives via Chiral 2-Oxazolines

A new highly enantioselective synthetic method for chiral 2-oxazolines has been developed [12] by the transfer of N1 unit to olefins using a reagent controlled three-component coupling. *This is the first example of a [2 + 2 + 1] type asymmetric synthesis of oxazolines*. The components are olefins, chiral *nitridomanganese complex* 1, a source of N, and an acid chloride (Fig. 20.8).

Of the several attempted conditions employing *p*-(trifluoromethyl) benzoyl chloride in the reaction, *trans*-β-methylstyrene was found to react smoothly and stereoselectively under condition (i) to give the corresponding *trans*-(4*R*,5R)-oxazoline derivative in 88 % yield with 86 % *ee*. The oxazoline derivative on hydrolysis under reflux with 1.2 *N* HCl gave (−)-nor-ψ-ephedrine in 93 % yield

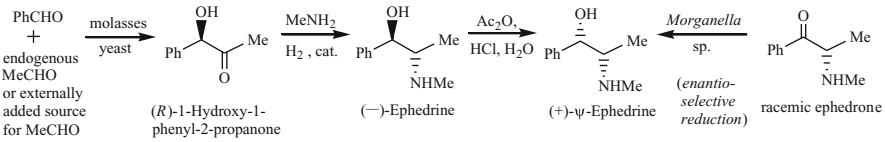


Fig. 20.9 Industrial preparation of (−)-ephedrine [13, 14]

(86 % *ee*). Likewise, *cis*-β-methylstyrene was stereoselectively converted to (−)-norephedrine and (+)-norpseudoephedrine (in the aforesaid two steps), both in poor yields and much less *ee*'s (Fig. 20.8). The latter is produced by the hydrolysis of the (4*S*,5*S*)-*trans*-oxazoline derivative, formed in the first step as a result of partial epimerization at the benzylic position under the reaction condition.

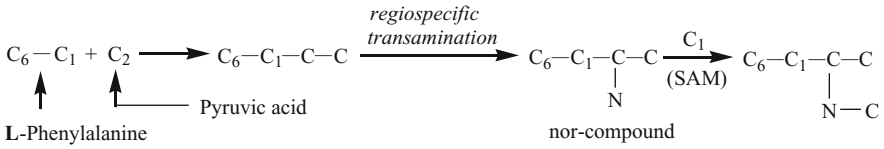
20.7 Industrial Preparation of Optically Active Ephedrine [13, 14] (Combination of Biotechnology and Chemical Steps)

In 1920 it was observed that fermenting yeast is capable of catalyzing stereoselective acyloin condensation of benzaldehyde with endogenous acetaldehyde to form (*R*)-1-hydroxy-1-phenyl-2-propanone. The latter could be subjected to reductive methylation via iminium derivative to yield (−)-ephedrine. (−)-Ephedrine could be stereoisomerized with aqueous HCl in the presence of Ac_2O (Fig. 20.9). This is one of the first few useful processes in which large scale microbial transformation to an optically active intermediate is followed by chemical conversion to the desired product. This serves as an early example where a combination of biotechnology and chemical synthetic steps yields the desired product. Today (−)-ephedrine is prepared by this method in large scales.

20.8 Biosynthesis of *Ephedra* Alkaloids [15–18]

Leete [15] in 1958 demonstrated by feeding experiments using 3-^{14}C(±)-phenylalanine that its C_6–C_1 unit is incorporated in (+)-norpseudoephedrine in *Catha edulis*; so phenylalanine is a direct precursor of the alkaloid. However, Shibata et al. [16] showed by biosynthetic experiments with *Ephedra distachya* (no incorporation of 2-^{14}C(±)-phenylalanine into (−)-ephedrine) that the skeletal alkaloidal N is not supplied by L-phenylalanine, as suggested by Leete earlier. They found that benzoate (carboxyl-^{14}C), benzaldehyde (carbonyl-^{14}C), and cinnamate (3-^{14}C) were incorporated into (−)-ephedrine. The skeletal alkaloidal nitrogen

comes from transamination after the basic carbon connectivity is over [17, 18]. Thus, the formation of the skeletal structure of the *Ephedra* alkaloids is outlined below.



During the biosynthesis of *Ephedra* alkaloids (Fig. 20.10), L-phenylalanine undergoes side chain truncation via cinnamic acid to give benzoic acid (C_6–C_1) [16]. The origin of the remaining C_2 unit of the *Ephedra* alkaloid skeleton remained obscure for >30 years till Spenser et al. identified the CH_3CO moiety of pyruvic acid as its source in the four alkaloids in *E. geradiana sikkimensis* [17]. Biosynthetic experiments done by them by feeding the growing plants with suitable ^{13}C labeled precursors [17, 18] led to the following conclusion. Benzoic acid, rather than benzaldehyde, was found to be incorporated. Benzoic acid formed gets activated as the thioester, benzoyl coenzyme A. Through the intermediacy of the activated acetaldehyde (C_2-unit) generated from pyruvic acid by its decarboxylation effected by the thiamine pyrophosphate (TPP) (see Sect. 3.4), the C_2-unit is added to C_6–C_1 unit by its nucleophilic attack on benzoyl coenzyme A. The resulting 1,2-diketone, 1-phenylpropan-1,2-dione undergoes transamination at the more electrophilic carbonyl group to form the α-ketoamine, (−)-canthinone. The latter then undergoes

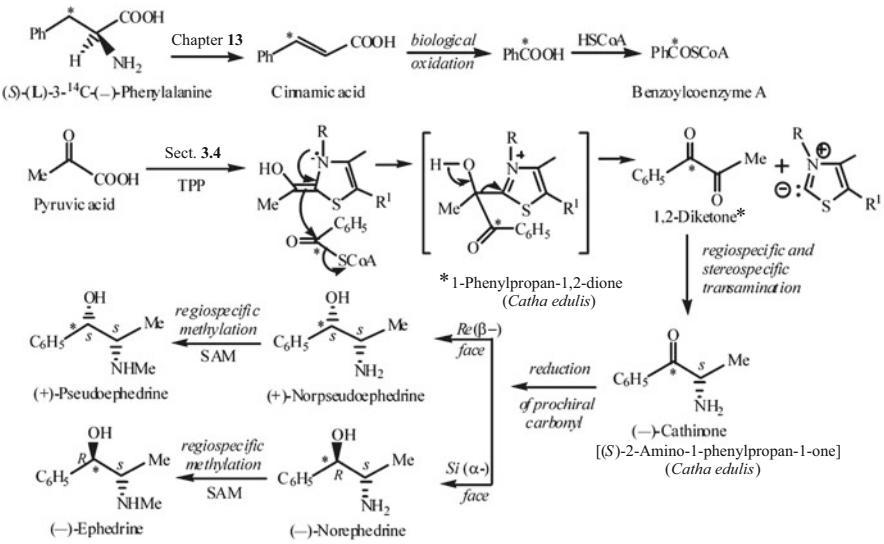


Fig. 20.10 Biosynthesis of *Ephedra* alkaloids

carbonyl reduction from *Si* face as well as *Re* face to form the diastereomers, (−)-norephedrine, and (+)-norpseudoephedrine, respectively. They get methylated regiospecifically by SAM on the NH_2 group forming (−)-ephedrine and (+)-pseudoephedrine. Since the corresponding noralkaloids are also obtained, the obvious conclusion is that the methylation takes place at the final stage. The entire biosynthetic sequence is delineated in Fig. 20.10.

20.9 Conversion of Ephedrine to Methamphetamine, a Well-Known Psychostimulant Drug

Ephedrine (or pseudoephedrine) was converted to methamphetamine first by Nagai [19] in 1893 by reduction with Na and liq. ammonia. This reaction later on became known as Birch reduction.

Another synthesis of methamphetamine involves reductive amination of phenylacetone as outlined below:

$$\underset{\text{Phenylacetone}}{PhCH_2COMe} + \underset{\text{Methylamine}}{MeNH_2} \rightarrow \underset{\text{Schiff base}}{PhCH_2C(Me)=NMe} \rightarrow \underset{(\pm)-\text{Methamphetamine}}{PhCH_2CH(Me)NHMe}$$

Methamphetamine (IUPAC name: 2-methylamino-1-phenylpropane) is a psychostimulant drug. It increases alertness, concentration, and energy in low doses, but has high potential for abuse and addiction [20, 21]. It triggers a cascading release of dopamine, norepinephrine, and serotonin in the brain. It is approved for the treatment of ADHD and exogenous obesity. Its chronic abuse can result in prolonged psychiatric disorders, as well as in increased neurotoxicity. Addicts may experience psychosis resembling schizophrenia. Acute abuse can often lead to cardiovascular damage.

Methamphetamine was widely distributed across ranks and divisions to German military forces during the ***World War II***. *Adolf Hitler* might have been given it by intravenous injections from 1942 until his death, which developed his Parkinson-like symptoms.

The production of methamphetamine has been legally prohibited in 1983 in the USA. Even the purchase of ephedrine and pseudoephedrine in amounts has also been restricted legally by an Act of 2005 in the USA [20].

20.10 Bioactivity and Applications

The use of Ma-Huang in Chinese medicine dates back to nearly 5,000 years. Its active principal component (−)-ephedrine possesses physiological properties close to adrenaline [=(*R*)-ArCHOHCH$_2$NHMe, where Ar = 3,4-dihydroxyphenyl]. Its adrenergic property is manifested by its capability to raise the arterial blood

pressure. It is sympathomimetic and acts on sympathetic nerves. It relieves bronchial spasms, nasal, and chest congestion and is used for the treatment of allergy-like hay fever. Pseudoephedrine has similar effects, but to a considerably less extent. In traditional Chinese medicine it has been used in the treatment of asthma and bronchitis for centuries. It is a heart stimulant and diaphoretic agent (perspiration producer) and increases secretion. It promotes thermogenesis [21], a physiological process of releasing fatty acids from stored fat cells and their conversion to energy and heat; this is called the burning of fat. This last physiological property allures endurance athletes like bodybuilders, heavy-weight lifters, and discus and javelin throwers to use this drug unadvisedly. Ephedrine in combination with caffeine shows the ability to increase the energy expenditure and is used to reduce weight.

(−)-Ephedrine acts as antiobesity (lipolytic) agent. The drug ephedrine consists of a mixture of the natural (1*R*, 2*S*)-(−)- as well as its unnatural (1*S*, 2*R*)-(+)-enantiomer . The (−)-variety has direct activity on receptor while its enantiomer has indirect activity. (+)-ψ-Ephedrine and its unnatural (−)-enantiomer have no such direct effect [22].

A recent review [23] discusses the synthetic methodology to access chiral heterocycles of various types derived from *Ephedra* alkaloids for their use as asymmetric inductors (chiral auxiliaries) (Chap. 32) and the biological activity of some of them.

Ephedrine borane acts as a Lewis acid catalyst for carbonyl addition, reduction, and hydroboration.

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Chapter 21
Pilocarpine and Isopilocarpine (L-Histidine-Derived Imidazole Alkaloids)

21.1 Introduction and Structure [1, 2]

(+)-Pilocarpine is the most important imidazole alkaloid because it exhibits extensive pharmacological properties. It co-occurs with its diastereomer (+)-isopilocarpine in the leaves of various South American *Pilocarpus* species (Fam. Rutaceae). The dried leaves of some *Pilocarpus* species, *P. jaborandi*, *P. macrophyllus*, and *P. pennatifolium* are commonly known as "jaborandi." *P. macrophyllus* is the major source of pilocarpine in which it occurs to the extent of 0.5 % along with other congener alkaloids, raising the total alkaloid content to ~1 %. Pilocarpine and isopilocarpine are generally obtained as viscous liquids, but both can also be obtained as low melting hygroscopic crystals.

Pilocarpine, first isolated in 1875, generally obtained as a viscous liquid, is odorless, colorless, and optically active. It forms crystals, m.p. 34 °C, $[\alpha]_D$ + 100.5 (H_2O). It is insoluble in cold alkali, but it dissolves on heating. Acidification of its alkaline solution regenerates it, a behavior typical of lactones, which reveals the nature of both oxygen atoms in its molecule. The structure of pilocarpine, proposed in 1900, was confirmed by chemical degradative studies [1, 2] (Fig. 21.1).

Regeneration of pilocarpine and isomerization to isopilocarpine and formation of imidazole derivatives, isopilopic, homoisopilopic, and the tricarboxylic acids, are consistent with structure (**1**) for pilocarpine/isopilocarpine without any specification of the stereochemistry.

21.2 Relative and Absolute Stereochemistry of Pilocarpine [3]

The relative and absolute configurations at the two chiral centers were settled by the quasi-racemate method and by correlation with molecules of known structure and stereochemistry [3].

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DOI 10.1007/978-3-642-45410-3_21, © Springer-Verlag Berlin Heidelberg 2015

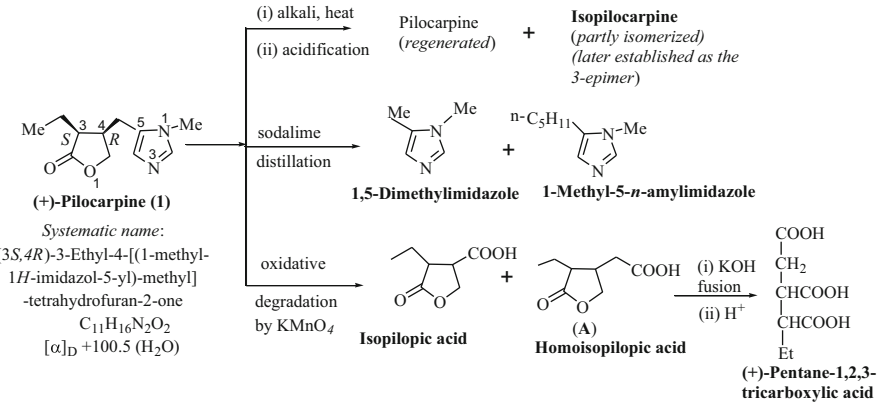


Fig. 21.1 Degradation products of pilocarpine

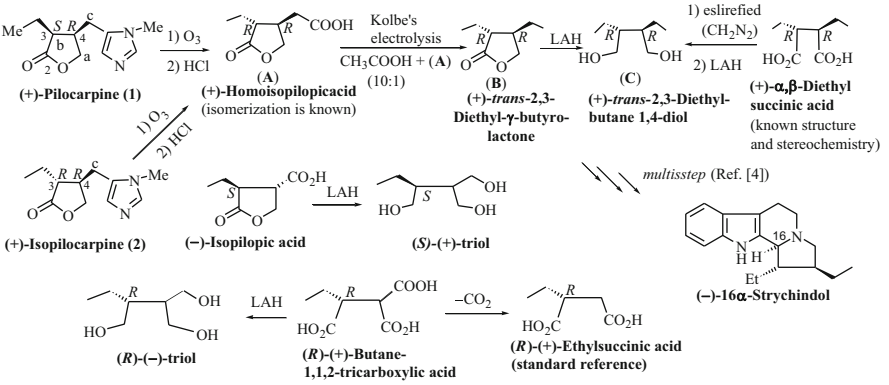


Fig. 21.2 Relative and absolute stereochemistry of (+)-pilocarpine and (+)-isopilocarpine [2, 3]

From the measurements of optical rotations of the two alkaloids (+)-pilocarpine and (+)-isopilocarpine and their derivatives, Preobrashenski suggested that in pilocarpine the two substituents at C3 and C4 are in *cis* relationship while in isopilocarpine they are *trans*. This is the reason for easy epimerization of one center of pilocarpine to yield predominantly the more stable *trans* isomer, isopilocarpine, when the former is heated with alkali followed by usual acidification (Fig. 21.1). Again, (+)-homoisopilopic acid (**A**) is obtained as an oxidative degradation product of both pilocarpine and isopilocarpine (Fig. 21.2). Compound (**A**) is converted first to (+)-*trans*-2,3-diethyl-4-butanolide (**B**) (also named (+)-*trans*-2,3-diethyl-γ-butyrolactone) and then to (+)-*trans*-2,3-diethylbutane 1,4-diol (**C**), which is identical with the (+)-*trans*-diol prepared from (+)-(*R*,*R*)-α,-β-diethylsuccinic acid with two ethyl groups in *trans* disposition. Hence, homoisopilopic acid has the lactonic substituents *trans* with (3*R*,4*R*) configuration,

and pilocarpine has the lactonic substituents *cis* with (3*S*,4*R*) configuration. In pilocarpine C3, being α to the lactonic C=O, is undergoing epimerization to give (**A**) (Fig. 21.2). The absolute stereochemistry of compounds (**B**) and (**C**) has also been settled by a multistep conversion of (**B**) into (−)-16α-strychindol [4], a degradation product of strychnine of known structure and absolute stereochemistry.

In another correlation work (−)-isopilopic acid [the enantiomer of (+)-isopilopic acid obtained by $KMnO_4$ oxidation of (**1**) or (**2**)] is converted to a (*S*)-(+)-triol. Its enantiomer (*R*)-(−)-triol has been obtained by LAH reduction of (*R*)-(+)-butane-1,1,2-tricarboxylic acid; the latter was correlated with *R*-(+)-ethylsuccinic acid of known stereochemistry (Fig. 21.2) [3]. The stereostructures of (+)-pilocarpine and (+)-isopilocarpine as deduced from the above correlation studies are shown in Fig. 21.2. The absolute stereochemistry of (+)-pilocarpine has been confirmed by X-ray analysis [5].

21.3 Syntheses of Pilocarpine and Isopilocarpine

The first synthesis of (±)-pilocarpine and (±)-isopilocarpine via homopilopic acid was reported in 1936 by Preobrashenski et al. [6]. Due to extensive pharmacological properties (see Sect. 21.6), considerable efforts have been devoted to the synthesis of pilocarpine. Till 2002 nine independent syntheses have been reported of which four syntheses have been schematically represented here (Figs. 21.4, 21.5, 21.6, and 21.7) [7–10]. For the references of other syntheses, [10] may be consulted.

21.3.1 Synthesis by DeGraw [7]

In the synthesis of (±)-pilocarpine by DeGraw, the key intermediate is homopilopic acid obtained from furfural (Fig. 21.3). The homopilopic acid is then converted to (±)-pilocarpine using modified Preobrashenski's method. The steps in this synthesis are portrayed in this figure.

21.3.2 Synthesis by Noordam et al. [8]

A stereoselective synthesis of (+)-pilocarpine and (+)-isopilocarpine, starting from L-histidine, has been reported by Noordam et al. [8] (Fig. 21.4). The amino group is diazotized to yield the hydroxyl compound. The latter is converted to a bromo derivative via 4-nitrobenzenesulphonyl derivative when Walden inversion takes place. After N-methylation it suffers another inversion when alkylated with

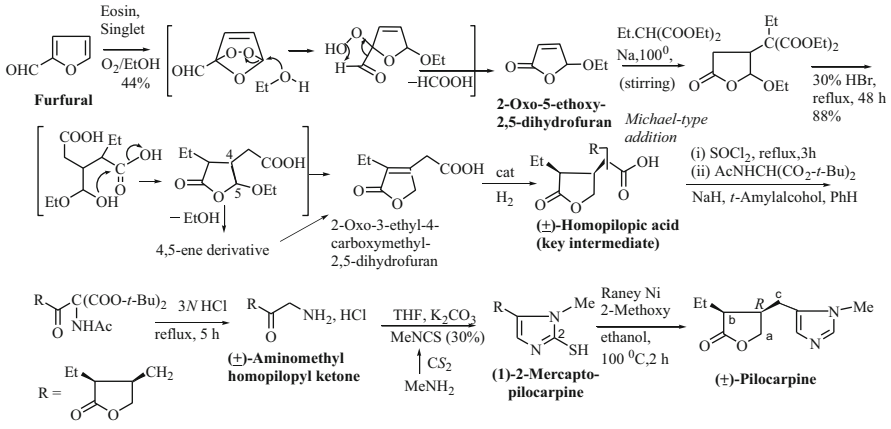


Fig. 21.3 Synthesis of (±)-pilocarpine by DeGraw [7]

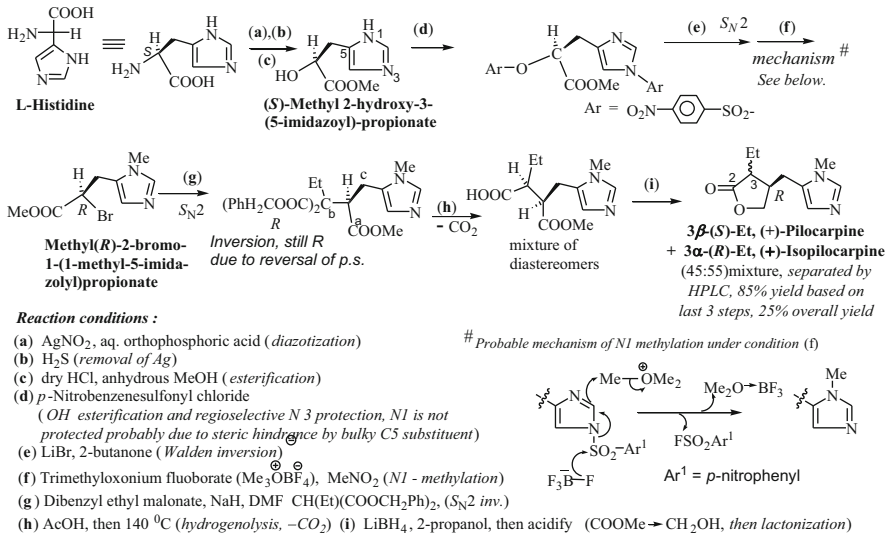


Fig. 21.4 Synthesis of (+)-pilocarpine and (+)-isopilocarpine by Noordam et al. [8]

dibenzyl ethylmalonate. Alkylation is usually a bimolecular nucleophilic substitution accompanied by Walden inversion. Hydrogenolysis debenzylates the product. Decarboxylation followed by $LiBH_4$ reduction yields the hydroxyacid which undergoes lactonization to yield a 1:1 mixture of (+)-pilocarpine and (+)-isopilocarpine in 85 % yield on the basis of the last three steps (Fig. 21.4) and 25 % overall yield.

21.3.3 Synthesis by Büchi et al. [9]

Pilocarpine deceptively looks like a simple molecule to synthesize. But the stereospecific construction of the imidazole moiety *cis* to the ethyl group on the butyrolactone ring in pilocarpine represents a challenge that has led to many independent syntheses. None of them produce the alkaloid without its C3 epimer, isopilocarpine. Büchi et al. have reported the first synthesis of pilocarpine *unaccompanied by isopilocarpine* [9] (Fig. 21.5). In this process, the starting material is commercially available as 2-acetylbutyrolactone, which has been converted to homopilopic aldehyde. The aldehyde group is then extended chemically for an imidazole moiety leading to pilocarpine formation. The reaction condition as well as the strategy of the synthesis is such that no epimerization at the potential C3 is allowed to form its diastereoisomer isopilocarpine, a product always found to be accompanied with pilocarpine in its synthesis till this report [9]. The reactions are delineated in Fig. 21.5.

Mechanism of the conversion of (C) to Pilocarpine is quite interesting. The proposed stepwise mechanistic rationale for the conversion of the aldimine of (**C**) to pilocarpine through a number of reversible steps terminating in an irreversible step is outlined in Fig. 21.6

We include here the highly enantioselective formal synthesis by Zhang et al. (Sect. 21.3.4).

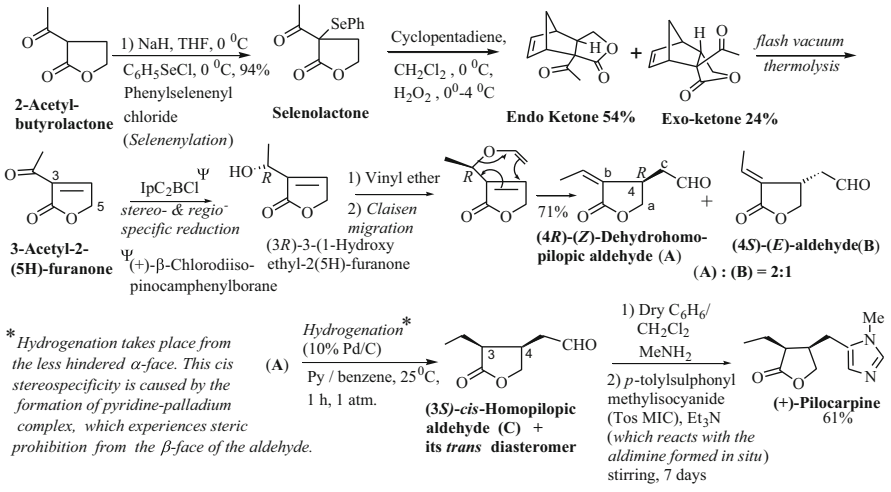


Fig. 21.5 Synthesis of (+)-pilocarpine by Büchi et al. [9]

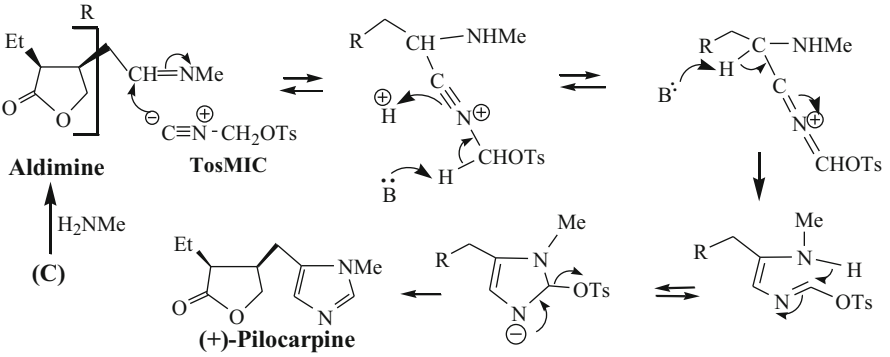


Fig. 21.6 Proposed stepwise mechanism of conversion of the aldimine from (**C**) to (+)-pilocarpine

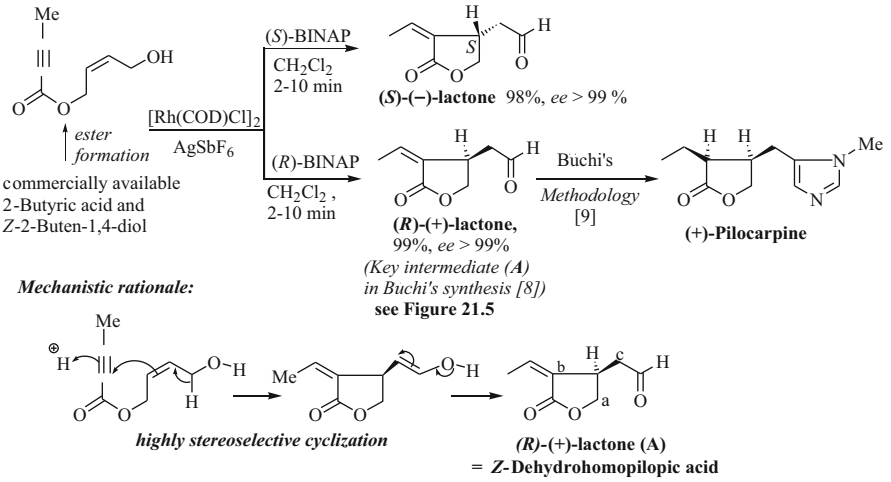


Fig. 21.7 Atom-economic enantioselective synthesis of (4*R*)-(*Z*)-(+)-dehydrohomopilopic acid (**A**) [10]

21.3.4 Chirospecific Synthesis by Zhang et al. [10]

Till 2002 eleven syntheses [10] of pilocarpine including Rapoport's 1993 synthesis [11, 12], of which five are chiral/chirospecific, have been reported. Zhang has reported [10] an *atom economic highly enantioselective synthesis* of the lactonic part [4*R*-(*Z*)-dehydrohomopilopic aldehyde (compound **A** of Fig. 21.5)] of (+)-pilocarpine using asymmetric Rh(1)-catalyzed intramolecular Alder-ene reaction in an excellent chemical yield and enantiomeric excess (Fig. 21.7). The aldehyde was then converted to (+)-pilocarpine using Büchi's method [9], and thus this synthesis constitutes a formal total synthesis of (+)-pilocarpine.

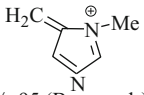
21.4 Spectral Data of Pilocarpine and Isopilocarpine

Pilocarpine [9]

UV (EtOH) λ_{max} 217 (end absorption).

IR ($CHCl_3$) ν_{max} 2,995, 1,770 (butyrolactonic C=O), 1,500, 1,180 cm^{-1}.

^{1}H NMR ($CDCl_3$) δ 1.12 (t, 3H, *J* 7.2 Hz), 1.58 (m, 1H), 1.92 (m, 1H), 2.42 (dd, 1H, *J* 15.7, 11.5 Hz), 2.68 (m, 2H), 2.83 (m, 1H), 3.57 (s, 1H), 4.11 (dd, 1H, *J* 9.1, 2.5 Hz), 4.20 (ddd, 1H, *J* 9.1, 5.4, 1.8 Hz), 6.81 (br s, 1H), 7.43 (br s, 1H).



m/z 95 (Base peak)

MS, *m/z* (relative intensity) 208 (M^+, 8), 95 ($M^+ - 113$) (100 %).

Isopilocarpine [12]

^{1}H NMR δ 1.00 (t, 3H, *J* 7.5 Hz), 1.72 (m, 2H, *J* 5.9 Hz), 2.27 (q, 1H, *J* 7.1 Hz), 2.66 (m, 2H), 2.81 (q, 1H), 3.56 (s, 3H), 3.89 (q, 1H, *J* 9.3 Hz), 4.39 (q, 1H, *J* 9.3 Hz), 6.78 (s, 1H), 7.40 (s, 1H).

(+) Pilocarpine hydrochloride, [11], m.p. 200–201 °C, recrystallized from ethanol/acetone, $[\alpha]_D^{22}$ + 88(*c* 2.0, H_2O)

^{1}H NMR (D_2O) δ 0.90 (t, *J* 7.5, 3H, CH_3), 1.45 (m, 1H, CH_2), 1.65 (m, 1H, CH_2), 2.52 (dd, *J* 16.1, 11.4, 1H, CH_2), 2.73–2.80 (m, 2H, CH and CH_2), 2.96 (m, 1H, OCH_2), 4.24 (dd, *J* 9.6, 5.8, 1H, OCH_2), 7.16 (*s*, 1H, CH), 8.48 (s, 1H, CH).

^{13}C NMR (free base of (+)-(**1**); $CDCl_3$, δ 12.1(q), 18.2(t), 21.2(t), 31.3(q), 37.2(d), 44.8(d), 69.8(t), 126.9(d), 128.5(a), 138.2(d), 177.8(s).

21.5 Biogenesis of Pilocarpidine and Pilocarpine

In the absence of definitive experimental evidence by way of label incorporation from the precursor to the product, the biogenetic path for pilocarpine and isopilocarpine formation could not be ascertained.

However, a suggestion was put forward by Boit [13], as well as by Leete [14], that pilocarpine is formed from acetoacetic acid (**A**) (Fig. 21.8) and the phosphate ester (**B**) *of a histidine-derived imidazole alcoholic compound* by sequential aldol condensation, dehydration, lactonization, reduction of the acetyl carbonyl, stereospecific reduction of the lactonic double bond, and N-methylation (Fig. 21.8). It has been suggested [15] that esterification may take place prior to intramolecular aldol condensation, as shown in the latter part of this figure. In the final step N-methylation is done by SAM. This has been proved by labeling experiment, which showed that methylation takes place in the leaves [2] and not in the roots. Thus, *N*-norpilocarpine

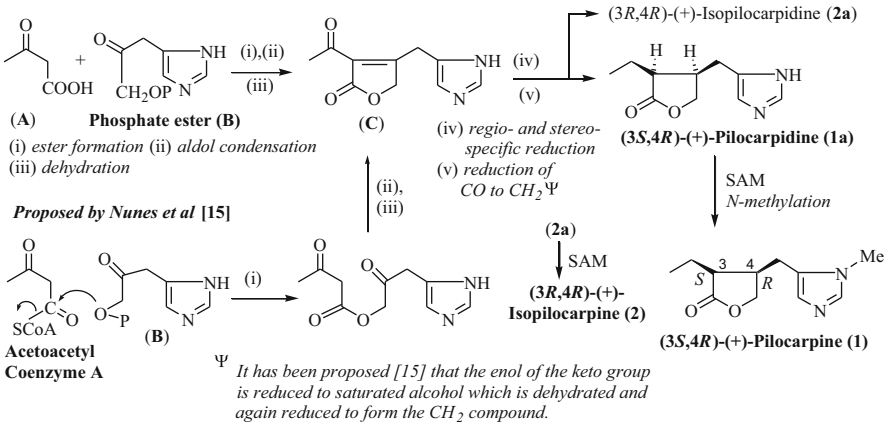


Fig. 21.8 Proposed biogenesis of pilocarpidine (**1a**), pilocarpine (**1**) isopilocarpidine, and isopilocarpine

called pilocarpidine which has been isolated from the roots of *Pilocarpus jaborandi* is translocated to the leaves where it is N-methylated by SAM to form pilocarpine.

21.6 Bioactivities and Uses [1, 2, 7]

Pilocarpine acts as the peripheral stimulant of the para-sympathetic system. Its pharmacological properties also include diaphoretic effects and miotic action to counteract the mydriatic effect of atropine. It increases the secretion of sweat, saliva, tears, and mucus. It is used to treat dry mouth (xerostomia) caused by chemotherapy and radiotherapy in people with head and neck cancer or with Sjogren's syndrome—a condition that affects the immune system and causes dryness of eyes and mouth or certain parts of the body. Its diaphoretic behavior relieves the kidneys and removes the toxic materials. It is used in nephritis [1] and is currently the drug of choice in the *treatment of narrow and wide angle glaucoma, because it decreases the intraocular pressure* and can be administered for long periods without side effects [7].

Pilocarpine comes as a tablet (oral formulation) to be taken by mouth. Ophthalmic pilocarpine (protonated) comes as an eye-drop solution to instill in the eyes or as an eye gel to apply to the eyes. Its bioavailability is low. Pilocarpine is reported to stimulate the growth of hair and is therefore used in hair lotions [1]. Studies on the activity of compounds that are structurally related to pilocarpine reveal that any structural modification of the pilocarpine molecule causes a drastic reduction or complete loss of its biological activity.

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Chapter 22
Papaverine (L-Tyrosine-Derived Alkaloid)

22.1 Occurrence

Papaverine, m.p. 147 °C a benzylisoquinoline alkaloid, co-occurs with morphine and other related alkaloids in opium (*Papaver somniferum*, Fam. Papaveraceae) [1]. It is present in opium to the extent of 0.5–1 %, and its other companion alkaloids are codeine, thebaine, etc. (Chap. 23). It was first isolated from the mother liquor of morphine in 1848.

22.2 Structure Determination

The structure of papaverine was established by Goldschmidt and his coworkers in the later part of nineteenth century (1883–1885). Some of the relevant degradative reaction products leading to the correct structure of papaverine are given in Fig. 22.1.

Formation of (**a**) suggests papaverine to be a tertiary base. Zeisel experiment shows the presence of four phenolic methoxyl groups in its molecule. The formation of (**b**) (≡tetrademethylpapaverine) supports this contention. Degradation products (**c**), (**d**), (**e**), and (**f**) suggest that methoxyl groups are located in two different rings and they are *ortho* to each other. Compound (**e**) is supposed to be formed from a 3,4-dimethoxybenzyl moiety of the molecule, while (**f**) and (**g**) point to the presence of an isoquinoline moiety, and the pyridine part (isoquinoline) is attached to three contiguous carbons (two from the benzene part of the isoquinoline) and the other one outside the ring.

Papaverine was the first opium alkaloid, the structure of which had been fully elucidated as (**1**), based on these degradation products in 1888.

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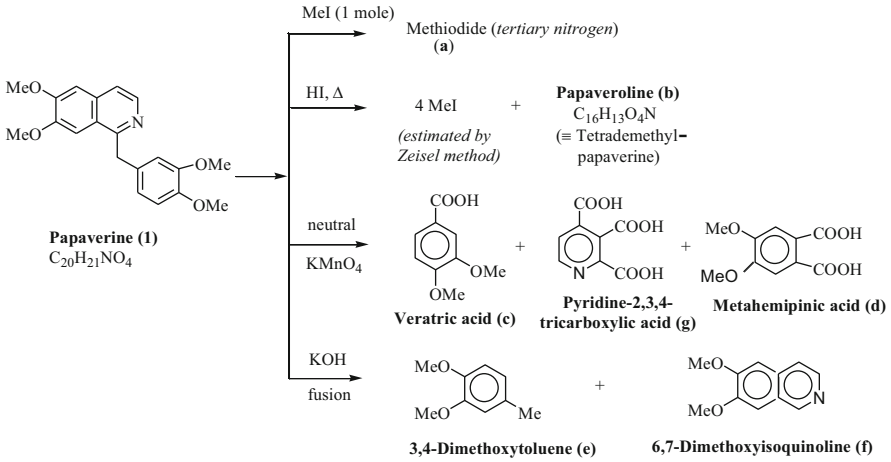

Fig. 22.1 Degradation products of papaverine (**1**)

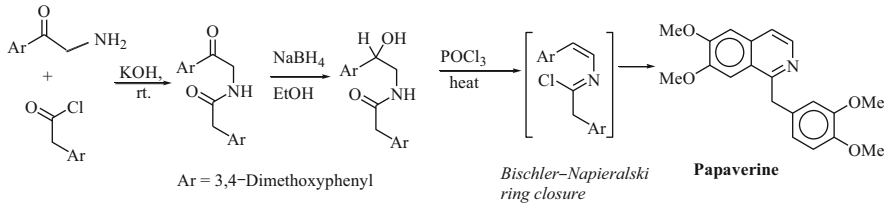

Fig. 22.2 Pictet and Gams synthesis of papaverine (1909)

22.3 Synthesis of Papaverine

Synthesis 1 The structure of papaverine was confirmed through its first synthesis by Pictet and Gamble in 1909, the essential steps of which are outlined in Fig. 22.2.

Many modifications in both reaction conditions and reagents have been introduced in a number of syntheses of papaverine. Two other syntheses will be schematically presented, the last step of which is also based on Bischler–Napieralski (Augustus Bischler, 1865–1957, and B. Napieralsky—a doctoral student of Bischler) ring closure [2]. In fact, this ring closure method has been employed for the synthesis of various isoquinoline alkaloids.

Synthesis 2 Bide and Wilkinson [3] achieved the synthesis of papaverine in seven steps with good yields, as outlined in Fig. 22.3. This synthesis is reminiscent of the biosynthetic route to papaverine (see Fig. 22.7).

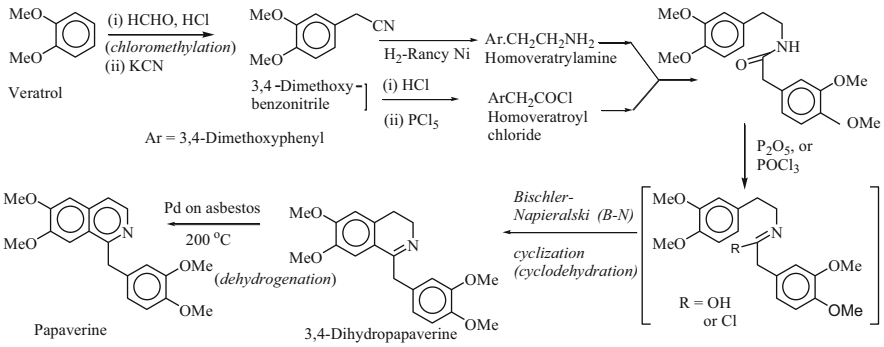

Fig. 22.3 Synthesis of papaverine by Bide and Wilkinson (1945)

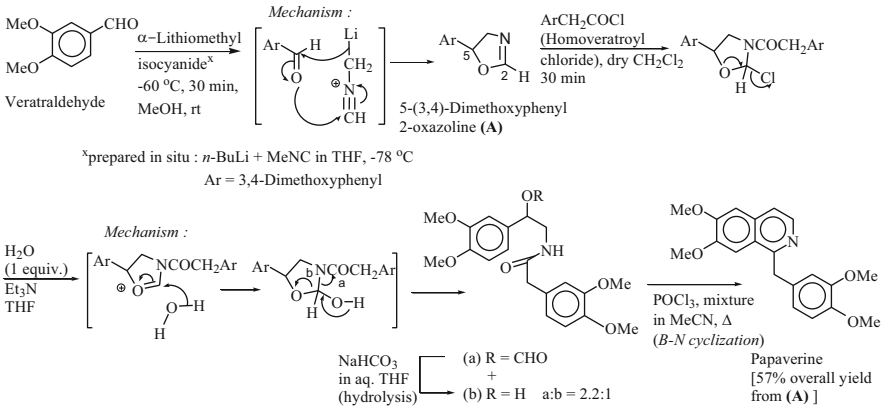

Fig. 22.4 Synthesis of papaverine via oxazoline derivative (1981)

Synthesis 3 A synthesis of papaverine (1981) using methoxyphenyloxazoline as the key intermediate has appeared in the literature [4] which is delineated in Fig. 22.4.

22.4 Pavine, An Interesting Rearranged Product from Papaverine

Papaverine upon reduction with tin and hydrochloric acid yields norlaudanosine, the expected 1,2,3,4-tetrahydroderivative via the 1,2-dihydrocompound, and a rearranged tetracyclic product pavine. The formation of pavine can be well explained mechanistically (Fig. 22.5). Pavine was obtained in 1886, and later it

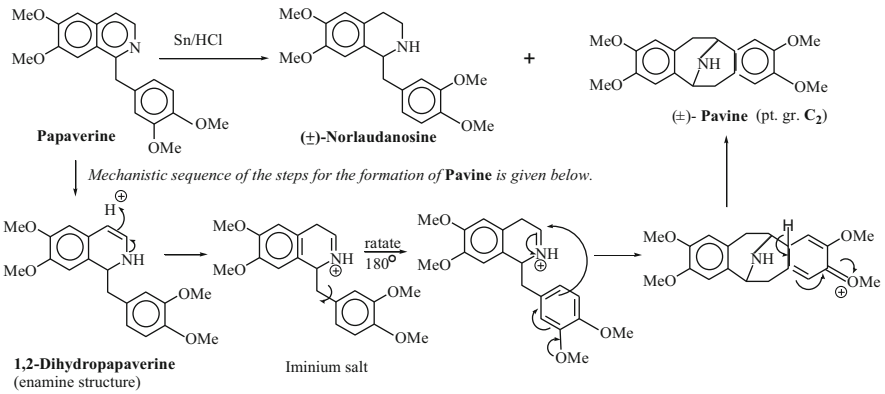

Fig. 22.5 Reduction products of papaverine

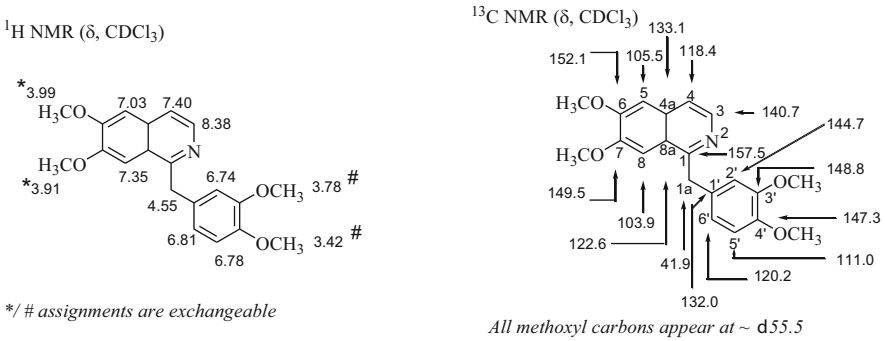

Fig. 22.6 ^{1}H NMR and ^{13}C NMR spectral data of papaverine

has been shown to possess a modified benzylisoquinoline structure. (−)-Argemonine, an alkaloid isolated from some *Argemone* species (Fam. Papaveraceae), has been shown to be identical with (−)-*N*-methylpavine.

22.5 NMR Spectral Data of Papaverine [5] (Fig. 22.6)

22.6 Biosynthesis of Papaverine [7] (Fig. 22.7)

In the biosynthesis of papaverine having a 1-benzylisoquinoline skeleton, initially two molecules of L-*tyrosine* are involved. *Papaver somniferum* plants were fed with (±)-[2-^{14}C]tyrosine, and the original activity of the isolated papaverine was shown

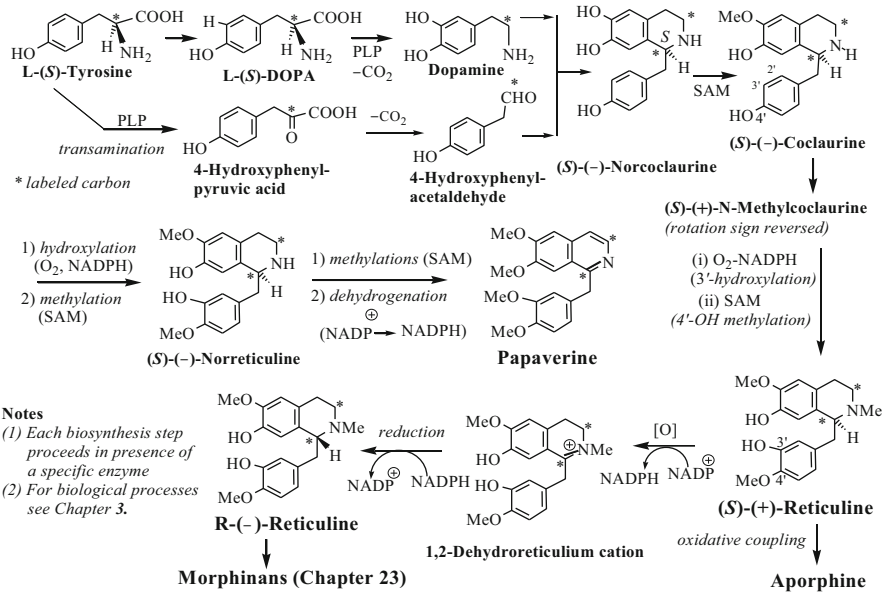

Fig. 22.7 Biosynthesis of papaverine and isomerization of (*S*)-reticuline to (*R*)-reticuline

to be divided equally between the two carbon atoms marked with *; this result establishes the biogenesis of papaverine from two molecules of tyrosine (Fig. 22.7) [7]. The oxidation pattern of both aromatic rings is identical in the final product papaverine. They are generated from the same precursor L-tyrosine; the hydroxylation on the aromatic rings takes place at different stages (Fig. 22.7). From labeled experiments showing the selective incorporation of the assumed precursors//intermediates, it has been settled that the upper part of the alkaloid comes from 3,4-dihydroxyphenylethylamine (*dopamine*), formed from L-tyrosine, via hydroxylation (L-DOPA) and decarboxylation. For the lower part, L-tyrosine is converted to *4-hydroxyphenylacetaldehyde* via transamination (4-hydroxyphenylpyruvic acid) and decarboxylation. The two fragments, the amine and the aldehyde, then condense to form an imine, followed by cyclization in the Pictet–Spengler fashion to form (*S*)-(−)-*norcoclaurine*, and thus the alkaloid status is attained. (*S*)-Norcoclaurine undergoes regiospecific methylation to form (*S*)-(−)-*coclaurine*, followed by hydroxylation and another regiospecific methylation to yield *(S)-(−)-norreticuline, identified as the key intermediate in the biosynthesis of papaverine*. Final stages include full methylation and dehydrogenation to convert tetrahydroisoquinoline to isoquinoline, and thus papaverine is biosynthesized.

It is interesting to note that (*S*)-(−)-*coclaurine undergoes methylation (by SAM) to form (S)-(+)-N-methylcoclaurine, in which the sign of rotation is reversed. It may be mentioned that morphine and its congeners (morphinans) (Chap. 23) are biosynthesized from (R)-(−)-reticuline, whereas aporphines and proaporphines*

are biosynthesized from (S)-(+)-reticuline and its congeners. Perhaps (S)-recticuline undergoes biological oxidation and reduction to epimerize the only chiral centre to form (R)-recticuline (Fig. 22.7), which then slips into the biosynthesis of morphine alkaloids. Alternatively, during cyclization in the Pictet–Spengler fashion the (*R*)-epimer may also be formed.

22.7 Bioactivity and Uses in Human Health [8]

Papaverine is a smooth muscle relaxant. It is used to treat spasms of the gastrointestinal tract, bile ducts, ureter, visceral spasm, vasospasm—especially those involving the heart and the brain, and occasionally in the treatment of erectile dysfunction in men.

It is also a vasodilator causing blood vessels in patients with circulation problems, to expand, thereby increasing blood flow, and is used to treat problems resulting from poor blood circulation. It is used as a cerebral and coronary vasodilator, balloon angioplasty, and coronary artery bypass surgery. It controls high blood pressure, but like other hypotensive medicines, does not cure it. Papaverine hydrochloride is available in intravenous, intramuscular, and orally administered formulations.

Papaverine is also present in combination with other opium alkaloids like morphine, codeine as salts in a percentage similar to that of opium or modified for a given application.

Side Effects Frequent side effects of papaverine treatment include ventricular tachycardia, constipation, increased transaminase and alkaline phosphatase levels, vertigo, and also some other effects. Its rare side effects include sweating, flushing (feeling warmth), headache, tiredness, dizziness, loss of appetite, constipation, stomach pain, upset of stomach, diarrhea, and skin rash. Moreover, it is habit forming and should not be taken more often and in large doses or for a longer period.

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Chapter 23
Morphine. Codeine. Thebaine: *Modified Benzyltetrahydroisoquinoline Alkaloids*

23.1 Historical Background. Occurrence

The word "analgesic" is almost synonymous with morphine, the major alkaloid of "opium." Morphine has no peer till date in controlling the excruciating pain, especially of postsurgical patients, and in intensive burn and severe fracture cases. Opium is the rubbery viscous exudate that oozes out from the sacrificed or pricked unripe seed capsules of opium poppy, *Papaver somniferum* Linn. (Papaveraceae). The exudates, when collected and dried, and pressed into bricks, form the opium of commerce. The use of opium in the form of tincture has been known for thousands of years. Babylonians and Egyptians recorded the use of opium preparations as an effective pain reliever. Hippocrates, Dioscorides, Galen, and other physicians wrote about its miraculous power "to lull all pain and anger, and bring relief to every sorrow." [1] The seventeenth century pioneer of English medicine, Thomas Sydenham wrote, "Among the remedies which it has pleased Almighty God to give to man to relieve his sufferings, none is so universal and so efficacious as opium" [1]. The use of opium to induce mystical or spiritual experiences has been known since antiquity.

Opium is a prodigious source for at least 20 alkaloids; many of them are structurally related to morphine and are known as morphine alkaloids. They are structurally more complicated compared to other congener alkaloids like papaverine (Chap. 22). Morphine was first isolated from opium in the pure form in 1803/1805 by Friedrich W Sertürner [1], an apothecary in Paderborn, Germany. This event marked the beginning of alkaloid chemistry. Because of its profound sedative action, the compound was named after *Morpheus* the Greek God of dreams and the son of sleep. In the opium morphine occurs to the extent of 10–20 %, while three other well-known congener alkaloids, *codeine, thebaine* and *papaverine* occur to the extent of 0.2–0.8, 0.2–1.01 and 0.5–1.0 % respectively.

The earlier users might not be so much aware of the addictivity and enslaving character of opium; perhaps they enjoyed the "most pleasant Elysian fields of sensation," [2]. In 1821 Thomas de Quincey, a brilliant student of Oxford, published

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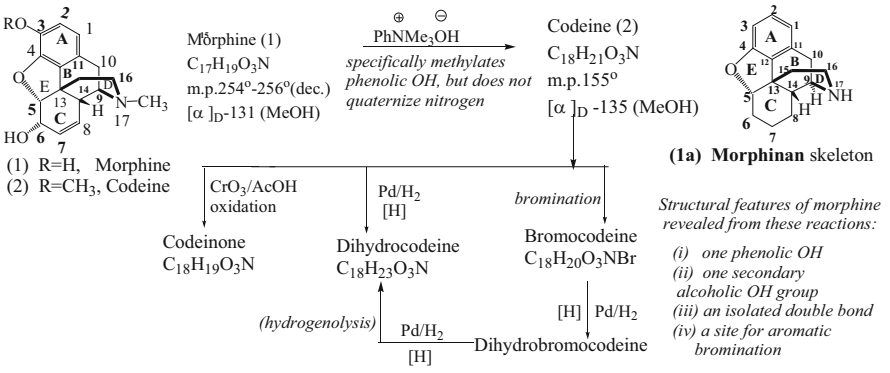
an article in a magazine in 1821, "Confession of an English Opium Eater", which contains for the first time the details of opium addiction, its dangerous withdrawal effect, the physiological and psychological dependence on this drug, and a "tale of a life dragged down from promise to depravity" [1, 3]. He wrote, "Here was a panacea . . . for all human woes, here was the secret of happiness, about which philosophers had disputed for so many ages, at once discovered; happiness might now be bought for a penny . . . portable ecstasies might be had corked up in a pint bottle; and peace of mind could be sent down in gallons by the mail-coach" [3]. The beginning of the use of hypodermic syringe in ~1853 gave a great booster to the medical treatment, but unfortunately, at the same time, started the increase of the number of self-injecting drug abusers—which is of great concern even today all over the globe.

Scientists are making efforts to discover a drug which will share the best part of morphine (miraculous pain killer) and shun the worst part of it (addictive liability, depressing effect on respiration, nausea, constipation, etc.). It has been found that in human body morphine is metabolized into two glucuronides, morphine-3-glucuronide (M3G, phenolic OH is involved) and morphine-6-glucuronide (M6G, alcoholic OH is involved). These are eliminated from the body. Studies showed that the opium receptor has much greater affinity for M6G showing analgesic property and low affinity for M3G [4]. Thus M3G has no analgesic property. M6G causes much less nausea amongst the postoperative patients and possesses almost parallel analgesic effect of morphine. Phase III trial of M6G is in progress [4]. A simple first synthesis of morphine-3,6β-di-D-glucuronide (M3,6diG) from morphine in fair yield has been reported [5].

In absence of adequate instrumental facilities – intuition, keen observations, chemical wisdom, insight, and logic were the main weapons with which chemists used to give heroic fights to win the structural details of unknown complex compounds. It is really amazing to find how chemists could arrive at the correct structures of complex molecules like morphine, strychnine (Chap. 27), and others in prespectroscopic facilities days, especially before the advent of NMR spectroscopy and mass spectrometry. Sir Robert Robinson (NL 1947) and John M. Gulland arrived at the correct structure (**1**) (Fig. 23.1) of morphine in 1925 by collecting



For better view of the numberings of morphine and codeine see p.801.

Fig. 23.1 Some reactions of morphine and codeine demonstrating the nature of their oxygen functions

from experiments the morphine-derived structural fragments and joining them with chemical arguments [6, 7]. The structure (**1**) of morphine was confirmed by its synthesis in 1952 by M. Gates et al. [8–10]. The numbering of the carbons in morphine is shown in the stereostructure (**1**). In terms of the basic morphinan skeleton (**1a**) (Fig. 23.1), morphine is named as 4,5-epoxy-7,8-didehydro-17-methylmorphinan-3,6-diol.

23.2 Structure Determination [11, 12]

For proper and immediate understanding of the designed reactions and the products leading to the structural elucidation of morphine, we have first presented its stereostructure (**1**). The students would be able to correlate the pieces of chemical evidence with the assigned structure – the treatment is equivalent to a picture puzzle where the pieces are put together looking at the picture to complete the same. The systematic development of structure, though absorbing and fascinating, is somewhat difficult and needs more space for detailed discussion, in case of morphine [13] or other complex natural products. However, with the help of Figs. 23.1, 23.2, 23.3, 23.4, 23.5, 23.6, 23.7, and 23.8 describing the reactions along with inferences, we have tried to elaborate the structure of morphine, and in some places (*cf.* elaboration of the basic skeleton) brief discussions have been made. Scientists working on such complex molecules during the years devoid of spectroscopic comforts (till 1940 or so) were gifted persons.

23.2.1 Oxygen Functions

The relationship of morphine (**1**) and its congener, codeine, is shown in Fig. 23.1. Both morphine and codeine form crystalline methiodides, phosphates, and sulphates. Morphine contains a phenolic OH group which can be methylated by trimethylanilinium hydroxide to give codeine (Fig. 23.1). Hence, codeine, with the protected phenolic group as the methyl ether, is a more suitable starting material for degradative studies. It forms a monoacetate showing the presence of an alcoholic OH group. It is a secondary alcohol capable of being oxidized to a ketone, codeinone, $C_{18}H_{19}O_3N$. The other oxygen, being inert to most of the common reagents, is assumed to be involved in an ether linkage.

23.2.2 Nitrogen Function

Codeine requires one mole of CH_3I to form the methiodide, prior to Hofmann degradation indicating the tertiary nature of the nitrogen. The presence of an

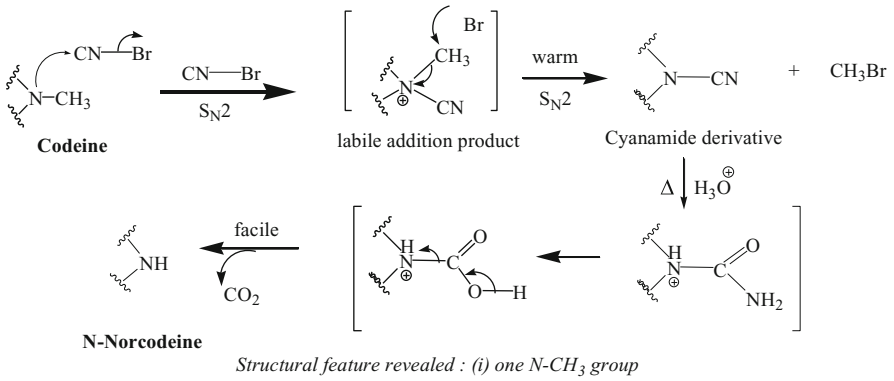
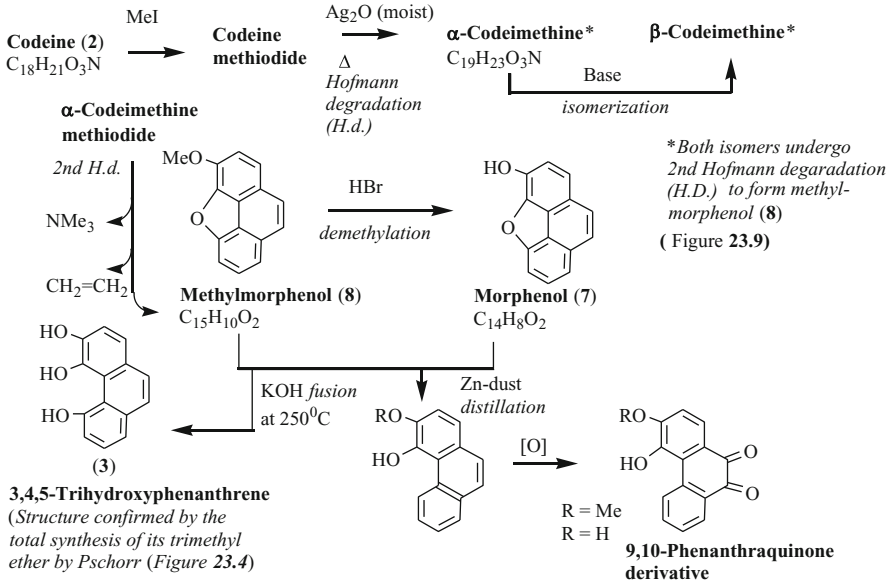


Fig. 23.2 von Braun degradation: conversion of codeine to N-norcodeine (only N function shown)



Structural features (of codeine) revealed:

(i) nitrogen is tertiary in nature and is a part of a ring, since two H.D.s are needed to form the nitrogen free compound, methyl morphenol
(ii) presence of a hydrophenanthrene ring system
(iii) 3,4,5-trioxygenated pattern
(iv) a probable presence of an ether linkage between C4 and C5
(v) unsubstituted nature of 9- and 10-positions in (7) or (8)

Fig. 23.3 Conversion of codeine to phenanthrene derivatives

N–CH_3 group in codeine is confirmed by subjecting it to the von Braun degradation involving reaction with cyanogen bromide, followed by acid hydrolysis of the resulting cyanamide derivative to yield N-norcodeine (Fig. 23.2). Two Hofmann degradations are necessary to form a nitrogen free product, methylmorphenol $C_{15}H_{10}O_2$ (**8**), so the nitrogen atom is present as a part of a ring (Fig. 23.3). Methanol is also a product to the extent of maximum 8 %. Methylmorphenol (**8**) upon treatment with HBr gets demethylated to form morphenol (**7**), $C_{14}H_8O_2$.

23.2.3 Presence of a Double Bond. Bromination

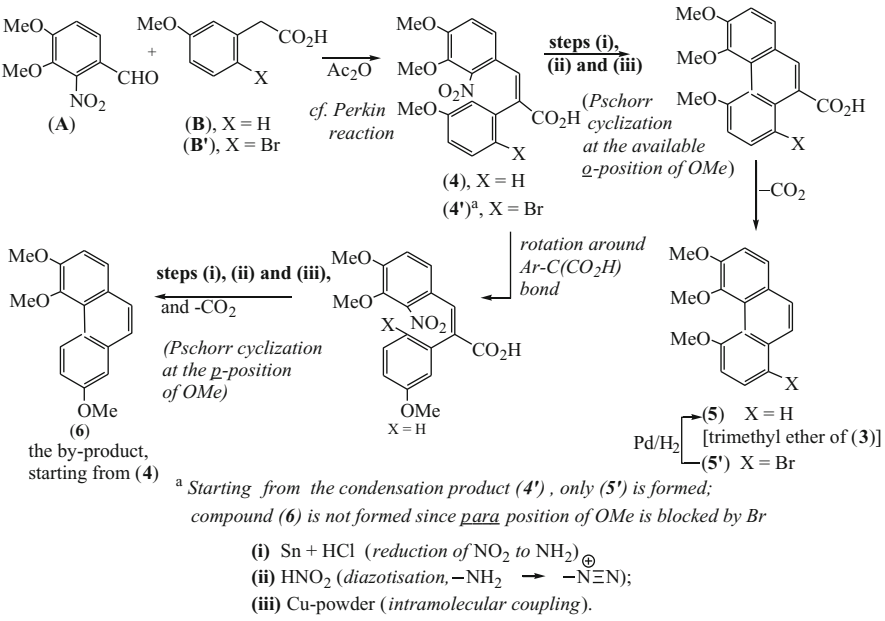
Codeine on hydrogenation gives dihydrocodeine. Monobromocodeine, obtained by bromination of codeine, on careful hydrogenation gives dihydrobromocodeine which on further hydrogenation gives dihydrocodeine, showing that codeine possesses a double bond and a position available for aromatic bromination (Fig. 23.1).

23.2.4 Basic Skeleton, Oxygenation Pattern, Part Structures

The idea about the basic skeleton is obtained by Zn-dust distillation of morphenol (**7**) and methylmorphenol (**8**) separately, when phenanthrene derivatives are obtained (Fig. 23.4). The locations of the oxygen functions have been determined by KOH fusion of morphenol. Morphenol is a monohydroxy compound but shows the presence of two oxygen atoms in its molecular composition $C_{14}H_{18}O_2$. The formation of 3,4,5-trihydroxyphenanthrene (**3**) on KOH fusion suggests the presence of an oxide bridge between C4 and C5 in morphenol and, for that matter, in methylmorphenol, morphine, and codeine.

The fact that the C9 and C10 positions of the phenanthrene derivatives are unsubstituted has been shown by their oxidation to the phenanthraquinones without loss of carbon atoms. The structure of 3,4,5-trihydroxyphenanthrene (**3**) (Fig. 23.3) is confirmed by synthesizing 3,4,5-trimethoxyphenanthrene (**5**), the trimethyl ether of (**3**) (Fig. 23.4) via (**4**) by a method designed by Robert Pschorr (1868-1930), which came to be known as Pschorr's phenanthrene synthesis, Incidentally, it may be mentioned that the conversion of morphine and codeine into phenanthrene derivatives marked the beginning of phenanthrene chemistry (Chap. 31).

In order to avoid confusion regarding the oxygenation pattern due to the formation of the undesired by-product (**6**) in the above synthesis (Fig. 23.4), compound (**A**) is condensed with (**B′**) (X = Br) to form the compound (**4′**) which is subjected to the same sequence of reactions to give the bromo derivative (**5′**). Catalytic hydrogenation of (**5′**) gives (**5**) (Fig. 23.4). In view of the oxygenation pattern of



[a] *Starting from the condensation product (**4'**), only (**5'**) is formed; compound (**6**) is not formed since para position of OMe is blocked by Br*

(i) Sn + HCl (*reduction of* NO_2 *to* NH_2)
(ii) HNO_2 (*diazotisation,* $-NH_2 \rightarrow -N\equiv N$);
(iii) Cu-powder (*intramolecular coupling*).

Fig. 23.4 Pschorr synthesis of 3,4,5-trimethoxyphenanthrene (5) and 3,4,7-trimethoxyphenanthrene (6)

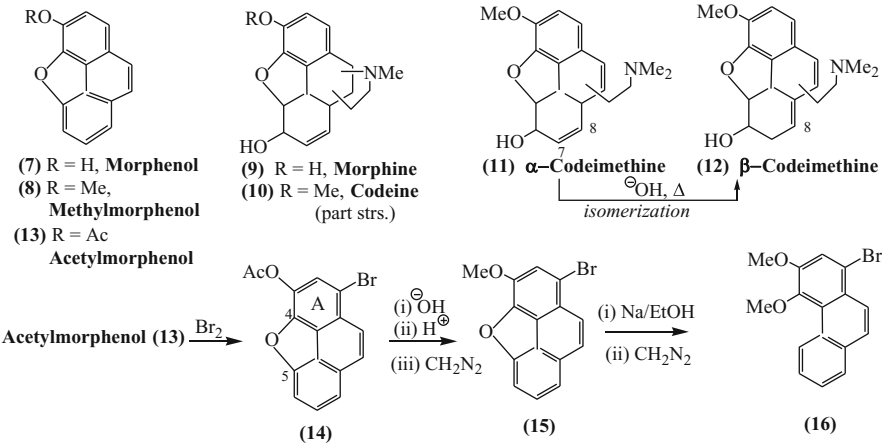


Fig. 23.5 Partial structures of morphine, codeine, their reaction products, and the structures of phenanthrene derivatives

the trimethoxyphenanthrene (**5**)—morphenol and methylmorphenol are represented as (**7**) and (**8**). respectively. Hence, the part structures of morphine, codeine, α-codeimethine, and β-codeimethine may be represented as (**9**), (**10**), (**11**), (**12**), respectively (Fig. 23.5).

23.2.5 *Locations of the Ether Oxygen, Double Bond, and Alcoholic OH*

Bromination of codeine gives 1-bromocodeine. Again, bromination of acetylmorphenol (**13**) gives a bromo compound (**14**), which has subsequently been converted to 1-bromo-3,4-dimethoxyphenanthrene (**16**) *via* compound (**15**) (Fig. 23.5). The bromination, in each case, takes place *para* to the ether linkage; hence, one hand of the ether is attached to C4 of the ring **A**. Compound (**16**) has been synthesized; it is also obtained from 1-bromocodeine by degradation. The other hand of the ether has to be linked to the only available position C5, making a 5-membered furan ring. The secondary OH in codeine or morphine is located at C6—as stated in the sequel, a fact also substantiated by the formation of (**3**) by KOH fusion of morphenol (Fig. 23.3).

The isolated double bond can be at (i) C7, C8 or (ii) C8, C14. Since α-codeimethine, upon treatment with alkali, isomerizes to β-codeimethine, the double bond in the former, and hence in codeine or morphine, must be at C7, C8—isomerizable to C8, C14. The OH being at C6, the double bond at C6, C7 would make it an enol, tautomerizing to the ketone.

The expulsion of two more carbons during 2nd Hofmann degradation of morphine methiodide and codeinone methiodide in addition to $N(CH_3)_3$ demands a special feature for codeine and, for that matter, of morphine. Moreover, methiodides of morphine, codeinone, and thebaine upon treatment with acetic anhydride and sodium acetate undergo reactions resembling the two Hofmann degradations to form 3,4-diacetoxyphenanthrene, 4,6-diacetoxy-3-methoxyphenanthrene, and 4-acetoxy-3,6-dimethoxyphenanthrene, respectively (Fig. 23.6). Robinson realized that aromatization was the driving force behind such extrusion during Hofmann degradation or Ac_2O/NaOAc reaction; for mechanism see Fig. 23.10. In case of morphine

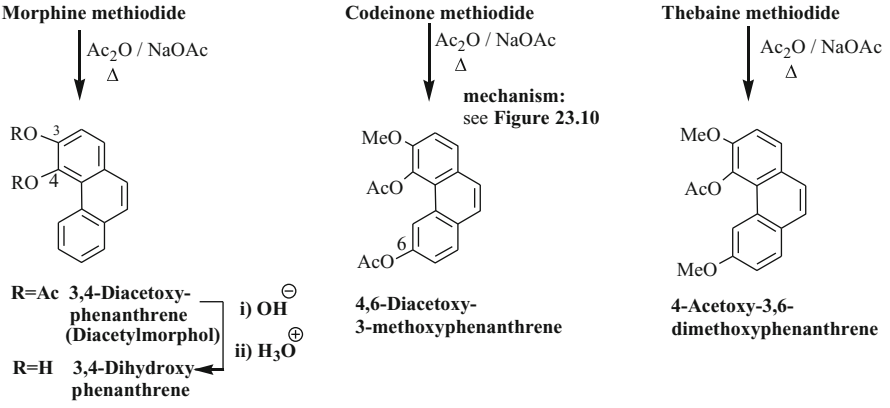


Fig. 23.6 Conversion of methiodides of morphine, codeinone, and thebaine to phenanthrene derivatives

methiodide, the alcoholic OH group at C6 is lost through the elimination of the elements of water, while in codeinone methiodide the carbonyl at C6 forms enol acetate to facilitate the aromatization. The identification of these products confirms that the alcoholic OH group in morphine and codeine and a methoxyl group in thebaine are located at C6, and the ether oxygen is linked to C4 and C5.

23.2.6 Location of Ethanamine Bridge

In an attempt to locate the attachment of the N end of the ethanamine bridge –CH_2–CH_2–NMe– at C9 or C10, a low temperature oxidation of codeine with $K_2Cr_2O_7/H_2SO_4$ was carried out to give a hydroxycodeine (**17**) (Fig. 23.7) in a low yield (~ 10 %). Hydroxycodeine methiodide (**18**) on Hofmann degradation gave a keto compound (**19**) suggesting that OH and N are attached to the same carbon atom. Both OH and N must be located either at C9 [*cf.* (**18**)] or at C10 generating an enolic double bond between C9 and C10 during Hofmann degradation. In the literature for convenience, the attachment of nitrogen is shown at C9, which has been later found to be true (Fig. 23.7). However, no positive evidence was available till the synthesis of morphine for the site of attachment of nitrogen at C9. We may now consider the problem of anchoring the –NMe–CH_2–CH_2 chain to various carbons in the hydrophenanthrene skeleton of morphine or codeine as shown in the expressions (**K**). Thus, apart from (**1**), structures (**L**) and (**M**) are also possible.

Since some codeine and morphine derivatives give phenanthrene derivatives under various conditions, especially during dehydrogenation, as expected, the end CH_2 (C15) of the ethanamine chain should be connected to a tertiary centre, like

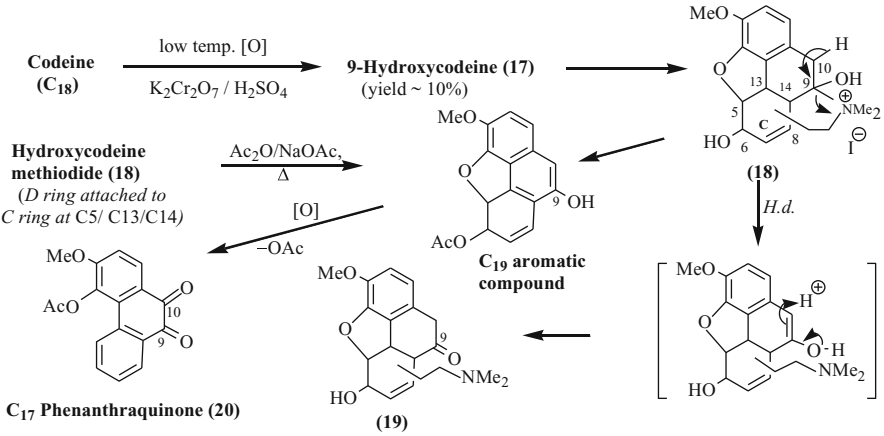


Fig. 23.7 Conversion of codeine to its 9-keto derivative (part structure) (19) C_{17} phenanthraquinone (20)

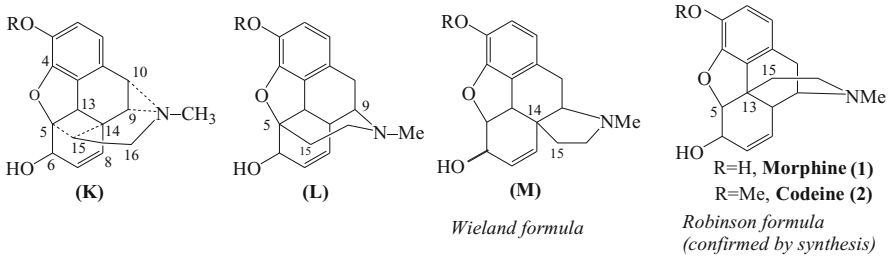
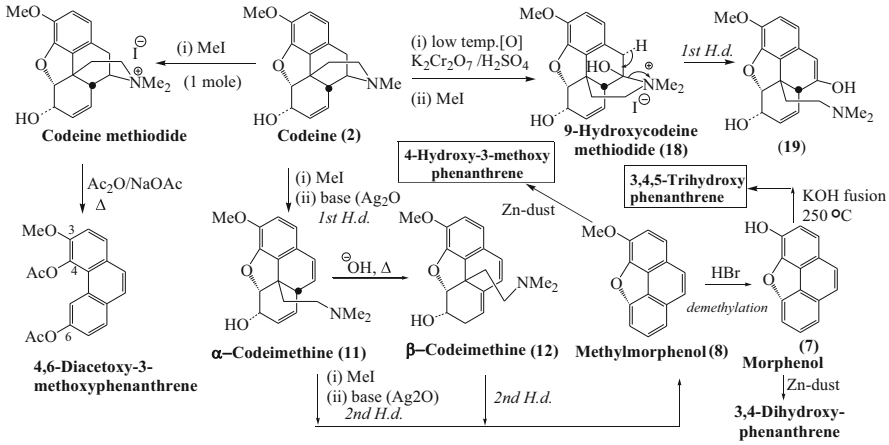


Fig. 23.8 Proposed gross structures for morphine (R=H) and codeine (R=Me)



Note: *Mechanism of some reactions are given in* **Figure 23.10**

Fig. 23.9 Some pertinent reactions of codeine delineated in terms of its stereostructure (2)

C5, C13, or C14, as it gets expelled during aromatization. The CH_2 end of the chain may not be attached to C7 or C8 for geometric reasons (Bredt's rule violation), to C5 for steric strain, and to C14 which will not allow isomerization of α-codeimethine to β-codeimethine. A plausible mechanistic pathway for the conversion of codeinone methiodide to 4,6-diacetoxy-3-methoxyphenanthrene (Fig. 23.5) is shown in Fig. 23.10, in terms of the accepted structure and stereochemistry of codeine.

Attachment of the CH_2 end to C13 was revealed later from Rapoport's work (Fig. 23.7). Several structures (Fig. 23.8) were proposed by different authors, of which Robinson's proposed structure (**1**) for morphine turned out to be the correct one, which received confirmation by its total synthesis—first achieved by Marshall Gates et al. [8–10] and subsequently by others [11, 12, 14].

Thus, some of the important reaction products [11, 12] pertaining to the structural elucidation of codeine (**2**) are delineated in Fig. 23.9 in terms of its established structures and stereochemistry.

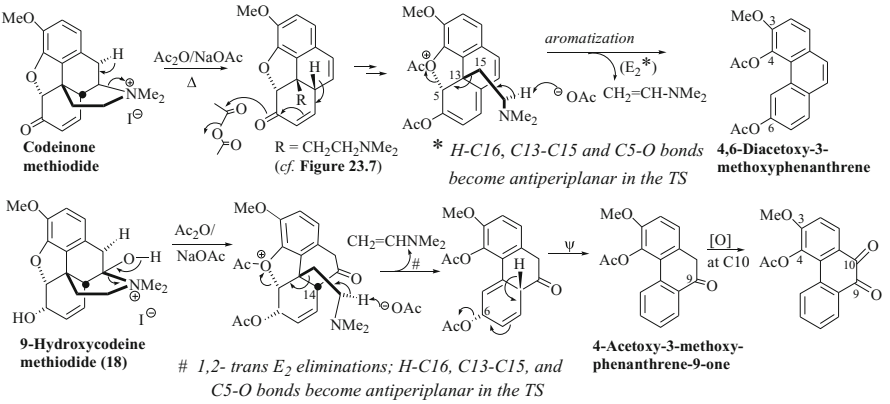


Fig. 23.10 Mechanistic pathways for the conversion of codeinone methiodide and 9-hydroxycodeine methiodide to the corresponding phenanthrene derivatives

23.3 Synthesis of Morphine

The fascinating carbon scaffolds of natural products have always allured synthetic organic chemists to reconstruct them in the laboratory. Morphine (**1**) is one such molecule whose structure was derived from careful scrutiny of the degradation products, and finally confirmed by its total synthesis. Since the appearance of its first synthesis by Marshall Gates [8–10], several other syntheses of this molecule have been published. They all offer excellent chemistry. The first synthesis by Gates [8–10] was achieved by classical chemistry and published in 1950. The second synthesis by Ginsberg [14], also based on classical chemistry, was published in 1954. The first synthesis will be discussed here briefly. A brief discussion of the most recent (2006) enantioselective synthesis of (−)-morphine by Parker *at al*. [15], based on radical cyclization, will follow.

23.3.1 Retrosynthetic Analysis and Strategy by M. Gates et al. [8–10]

The general features of the synthesis of morphine by M.Gates et al. are illustrated in a retrosynthetic format in Fig. 23.11. From the retrosynthetic analysis it appears that the properly substituted naphthaquinone (**A**) could be provided with a cyanomethyl group through Michael addition at the right location for its future use. Diels–Alder angular fusion of a C_4-diene to (**B**) could give a *cis* adduct, a hydrophenanthrene system. The latter epimerizes through enolization to the more stable *trans* adduct (**C**), which upon reductive cyclization and N-methylation would yield the 16-keto compound (**D**). Compound (**D**) upon LAH reduction would give (**E**), which upon

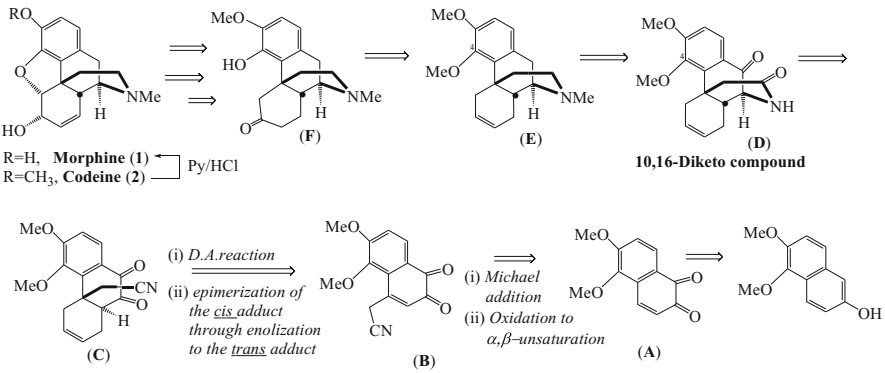


Fig. 23.11 Retrosynthetic analysis of morphine

***i) First Part: Synthesis of the trans adduct* (D) *via* (B) *and* (C)**

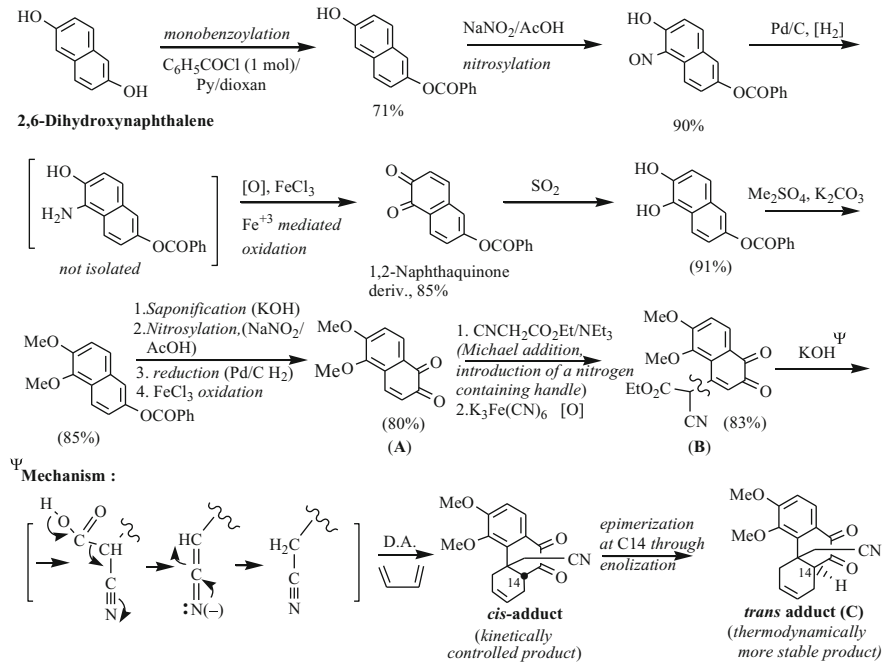


Fig. 23.12 Synthesis of the key synthon (A) and its conversion to (C) (a hydrophenanthrene nucleus with a nitrogenous handle) via (B)

hydration, selective demethylation at C4, and oxidation will yield the 6-keto derivative (**F**). The latter may be subjected to successive bromination (1,7-di-bromination), DNPH formation (when epimerization at C14, formation of 7,8 double bond also takes place), regeneration of the 6-ketone, bromination to get 1,5,7-tribromo derivative, and a few more reactions involving an intramolecular

nucleophilic displacement of bromine at C5 would lead to the formation of the ether linkage between C4 and C5. Finally, removal of bromine at C1 and stereospecific reduction by LAH to form 6-α-ol would lead to codeine (**2**), which undergoes demethylation to give morphine (**1**).

23.3.2 *Synthesis of (±)-Morphine by Gates et al. (Figs. 23.12, 23.13, 23.14, and 23.15)*

The achieved total synthesis of (±)-morphine based on this retrosynthetic analysis is delineated in three parts in Figs. 23.12, 23.13, 23.14, and 23.15. Although in each step after generation of chiral center/s, invariably the (±)-variety was formed; the absolute configuration of the chiral center/s of the natural product is shown which also allows the relationship of the chiral centers as they are built during the synthesis.

***ii) Second Part: Synthesis of the basic tetracyclic skeleton* (E).**

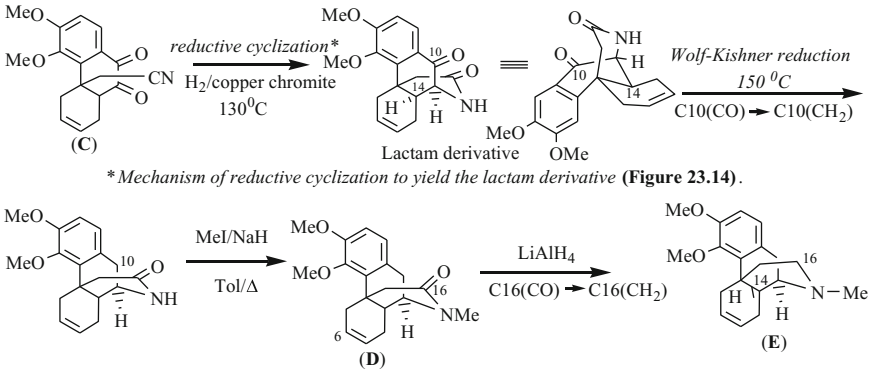


Fig. 23.13 Formation of the basic tetracyclic skeleton of morphine (with wrong stereochemistry at C14)

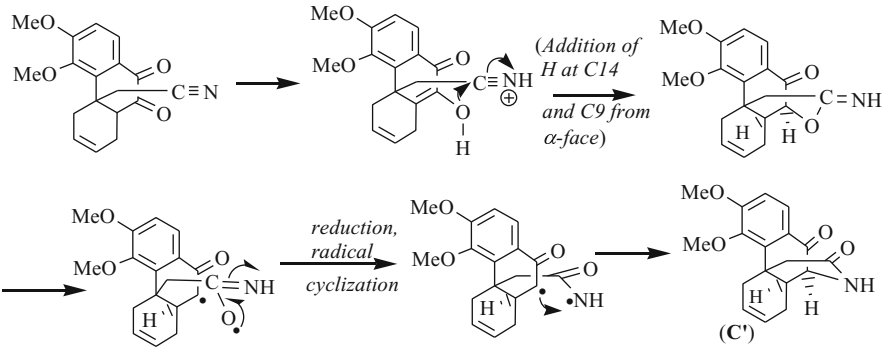


Fig. 23.14 Mechanism of the lactam formation

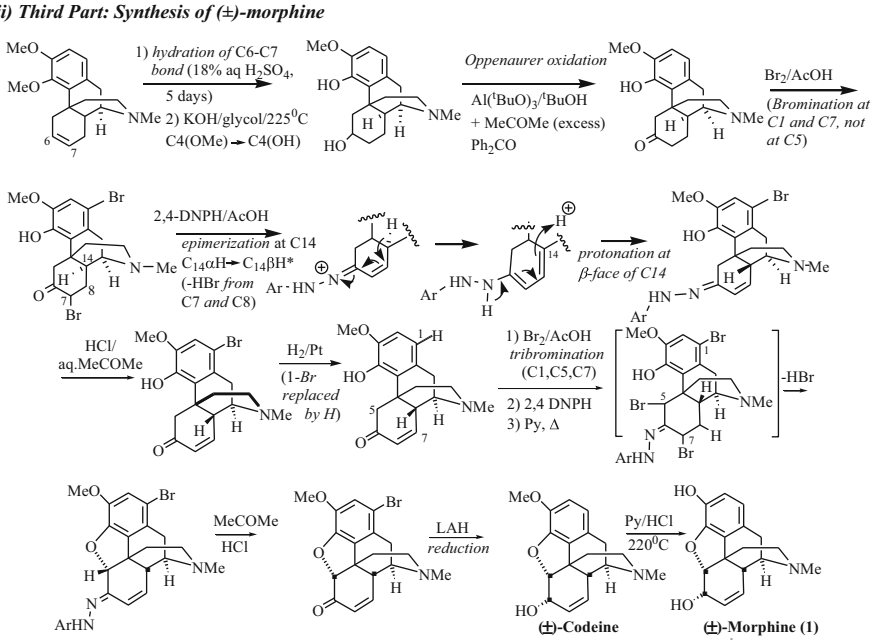


Fig. 23.15 Total synthesis of (±)-codeine and (±)-morphine (Gates) [9, 10]

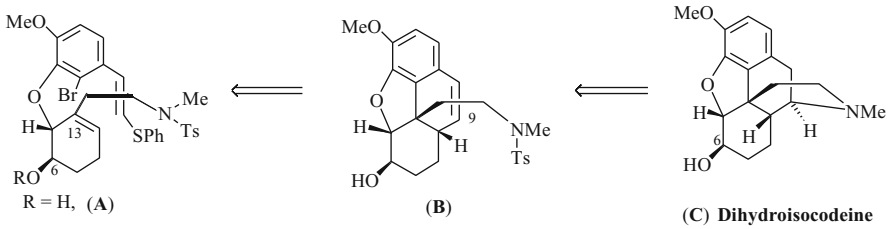


Fig. 23.16 Retrosynthetic analysis of the key steps in the synthesis of morphine [15]

23.3.3 Enantioselective Synthesis of (−)-Morphine

Parker and Fokas [15] have reported in 2006 a formal enantioselective synthesis of (−)-dihydrocodeinone and its conversion into (−)-morphine, applying a radical cyclization approach. The key steps planned are shown in a retrosynthetic format in Fig. 23.16.

In (**A**), the potential C_6 and C_{13} (of morphine) bear a hydroxyl group (wrong stereochemistry) and an aminoethyl side chain, respectively. The aryl radical derived from the bromoarene (**A**) would give the tetracyclic intermediate (**B**).

The latter could be converted to (**C**) by deprotection and formation of C9-N bond. This planning has been implemented and the subsequent steps leading to (−)-dihydroisocodeine (**C**), and previous steps to arrive at (**A**), are delineated in Fig. 23.17 with pertinent comments whenever necessary. Some steps have been mechanistically explained later.

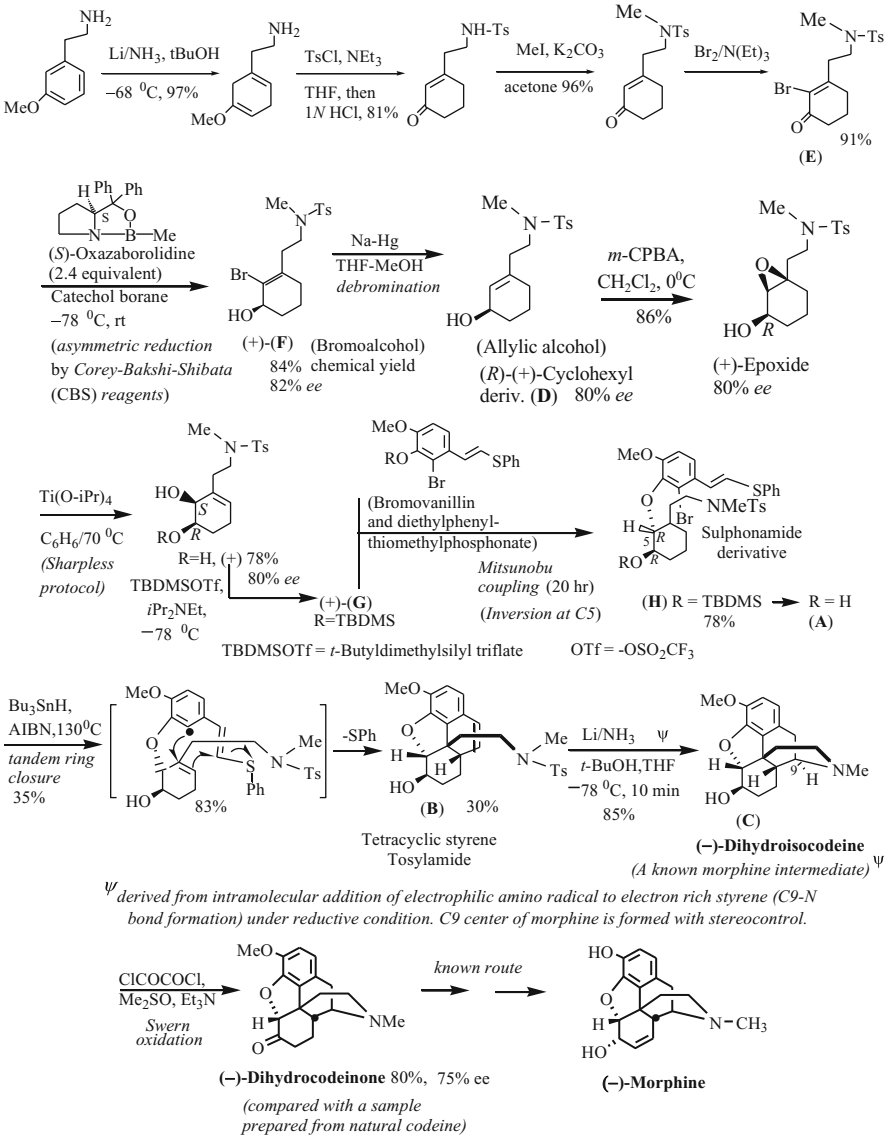


Fig. 23.17 Synthesis of (−)-dihydrocodeine and a formal total synthesis of (−)-dihydrocodeinone and (−)-morphine [15]

Parker et al. [15] selected the starting material (*R*)-(+)-cyclohexenyl derivate (**D**) (Fig. 23.17) as a source of chirality. Through a few steps discussed in the sequel, they converted (**A**) into a fully substituted substrate (**B**) having direct correspondence of functionality and structure with the target molecule (−)-dihydroisocodeine (**C**) from which (−)-morphine has been synthesized through steps delineated in Fig. 23.17.

23.3.3.1 Some Comments and Mechanistic Explanations

(1) The enantiomeric excess of the optically active compounds were measured either from NMR chemical shift of fluorine (fluorine signals are simple and appear in uncongested part of the spectrum) of the derivatized (*Mosher's reagent*) [16] compounds or from the comparison of the magnitude of their rotations with the rotation of the respective authentic sample.

(2) Conversion of (**E**) into (**F**) has been done with CBS (Corey–Bakshi–Shibata) reagents. With catalytic amount (0.1 equivalent) of *S*-oxazaborolodine, the reaction is very slow and almost all the starting material was recovered even after 24 hr; hence, the amount was increased when the yield was radically improved.

(3) Several reagents were tried to effect debromination of (**F**) to (**D**); Na − Hg was found to be most effective.

(4) *Mitsunobu coupling* has been applied to convert (**G**) to (**H**). The coupling takes place under mild conditions along with the inversion at the site of coupling (C-5), and this effects the right stereochemical entry at C-5 (cf morphine). The inversion can be explained in the following way (Fig. 23.18).

23.3.3.2 Mechanism of Inversion in Mitsunobu Reaction

(5) Conversion of (−)-dihydroisocodeine to (−)-dihydrocodeinone has been effected by Swern oxidation. It involves oxidation of secondary alcohols to ketones and primary alcohols to aldehydes by sequential addition of oxalyl chloride (or trifluoroacetic anhydride), DMSO, and a base like Me_3N at low temperature

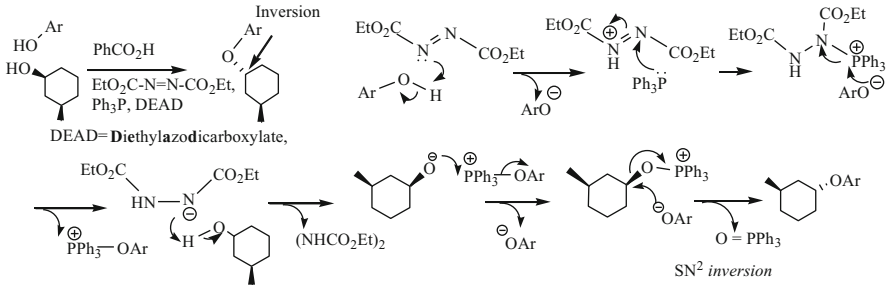


Fig. 23.18 Mitsunobu reaction and its mechanistic pathway

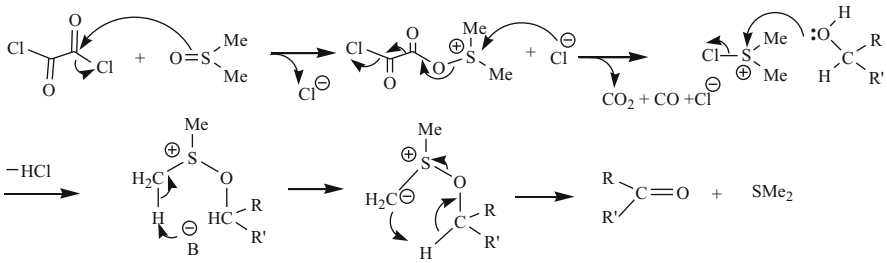


Fig. 23.19 Mechanism of Swern oxidation

(below −30 °C) in CH_2Cl_2. The reaction proceeds in the pathway shown in Fig. 23.19.

23.4 Stereochemistry. Relative and Absolute Configuration. Conformation

We are starting with the finally established stereostructures of morphine (**1**) and its 3-methyl ether codeine (**2**), which will help to immediately understand the stereochemical discussions.

Morphine (**1**) and codeine possess five chiral centers at C5, C6, C9, C13, and C14. Rapoport [17, 18] established for the first time the relative stereochemistry amongst the chiral centers. Since morphine contains five unlike asymmetric centers theoretically it is expected to have $2^5 = 32$ optical isomers and 16 (±) diastereomers. However, the ethanamine bridge involving C9 and C13 imposes rigidity by way of *cis* relationship at the two termini C9 and C13 of the bridge—either below (α-) or above (β-) the rings B and C. Hence, the number of optical isomers comes down to 16. Of all the possible optical isomers, the natural isomer (−)-morphine possessing the absolute configuration (5*R*, 6*S*, 9*R*, 13*S*, and 14*R*) is miraculously analgesic. Its enantiomer, the unnatural synthetic (+)-morphine with (5*S*, 6*R*, 9*S*, 13*R*, and 14*S*) configuration is completely devoid of analgesic activity.

23.4.1 Determination of the Relative Stereochemistry at the Chiral Centers

(1) ***Determination of the orientation (axial or equatorial) of the alcoholic OH group at C6.*** Dihydrocodeine (3-methyl ether of dihydromorphine) (Fig. 23.20) and dihydroisocodeine possess epimeric OH groups at C6, and in them ring C assumes chair conformation. Rate of oxidation of dihydrocodeine is *faster* than that of dihydroisocodeine suggesting that the OH group in the former is axial in ring

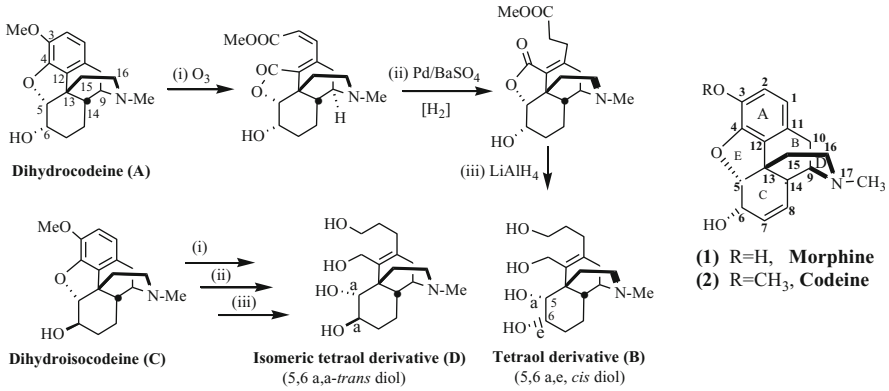


Fig. 23.20 Relative stereochemistry at C5 andC6 of (A) and (C)

C. It is a *case of steric assistance* due to the release of 1,3-*syn*-axial interactions between 6-OH and 13,12 bond and axial 8-H in the TS, when C6 is converted from the tetrahedral sp^3 hybridization in the alcohol to the trigonal sp^2 hybridization in the ketone. In the epimeric dihydroisocodeine, the OH group is thus equatorial.

The axial nature of the OH group in dihydrocodeine is also supported by the slower saponification rate of its acetate compared to that of dihydroisocodeine acetate having the acetate group equatorial. The saponification (hydrolysis) of dihydrocodeine acetate (axial acetate) is a *case of steric hindrance*.

***Conclusion*:** In codeine and hence in morphine 6-OH is pseudoaxial.

(2) *Relative stereochemistry of C5 and C6.* Dihydrocodeine (**A**) possesses a substituted catechol system (at C3 and C4), which gets cleaved upon ozonolysis (Fig. 23.20). The cleaved product when subjected to catalytic hydrogenation followed by $LiAlH_4$ reduction yields a tetraol derivative (**B**). In this tetraol the 5,6 glycol system behaves like a cyclohexane *cis*-1,2-diol (ring C), as is evident from the *faster* rate of lead tetraacetate oxidation of the tetraol (**B**) compared to that of the isomeric tetraol (**D**), obtained following the same sequence of reactions starting with dihydroisocodeine (**C**) (Fig. 23.20).

Conclusion: C5,C6-diol being *cis* in (**B**)**,** C5-O and C6-OH bonds are also *cis* in (**A**) and hence in codeine (**2**) and morphine (**1**). The 6-OH in morphine (**1**) and codeine (**2**) being pseudoaxial in ring C, the ether linkage at C5 is equatorial.

(3) *Relative Stereochemistry of the Ethanamine Bridge at C13 and C9 and C5-O Bond*

Thebaine (**3**) (Fig. 23.21), a congener morphine alkaloid, contains only three unlike asymmetric centers C5, C13, and C9. That these centers possess same relative stereochemistry as in codeine and morphine is evident from the mechanism of the following transformation reaction.

Thebaine when heated with NaOAc and Ac_2O gives a nitrogen free molecule, 3,6-dimethoxy-4-acetoxyphenanthrene- (**Q**). The mechanism involves an E2-elimination of the ethanamine bridge at C13 and opening of the oxide bridge

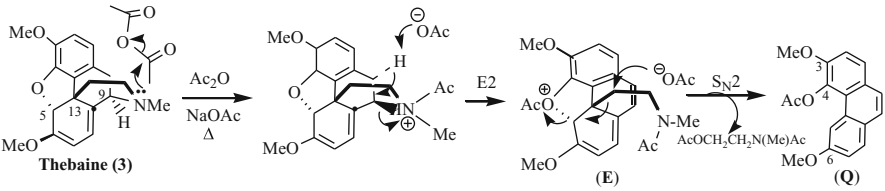


Fig. 23.21 Conversion of thebaine into 3,6-dimethoxy-4-acetoxyphenanthrene (Q)

after being acetylated in a concerted manner (*cf*. Fig. 23.10). Thebaine methiodide also gives the same phenanthrene derivative under the same condition (see Fig. 23.6) in a similar manner.

Opening of the oxide bridge of the intermediate (**E**) demands *anti* (or *trans*) relationship of C5-O bond and C13-C15 bond, a stereoelectronic requirement of an E2 elimination.

Conclusions: C5-O bond and C13-C15 bond (or the ethanamine bridge) are holding *trans* relationship to each other; thus, C5-O bond is cis to H9. In morphine 6-OH being *cis* to C5-O bond, it must also be *trans* with ethanamine bridge. The ethanamine bridge is also *trans* to C5-O bond, in thebaine, like morphine or codeine.

23.4.2 Absolute Stereochemistry

Jeger et al. [19] elegantly converted (−)-dihydrocodeinone through several steps into (−)-*cis*-2-methyl-2-carboxycyclohexyl acetic acid (**G**), which is known to have (1*S*,2*S*) absolute configuration by its formation by degradation of 17α-D-homotestane (**J**) of known absolute configuration (Fig. 23.22). In the degradation product (**G**) the chiral carbons 13 and 14 codeinone appear with preserved absolute configuration. Thus, the absolute stereochemistry of the other chiral centers of codeine or morphine is also established on the basis of their known relative stereochemistry.

The final support to the stereostructure and conformation came from the X-ray crystallographic study [20] of morphine by Dorothy Hodgkin (British Chemist, 1910–1994, NL 1964). The molecule looks like a three-dimensional T (Fig. 23.23).

Additional support came from the data obtained from the exciton chirality study [21] on morphine and change in optical rotation with polarity of solvent [22]. Bick [23] correlated the absolute stereochemistry of the only one surviving asymmetric center C9 of morphine skeleton in apomorphine (Fig. 23.26), the acid rearrangement product of morphine with L-(+)-laudanosine. In this endeavor the displacement of rotations of apomorphine and L-(+)-laudanosine have been measured separately in a series of differently polar solvents, and a relationship has been drawn with the data.

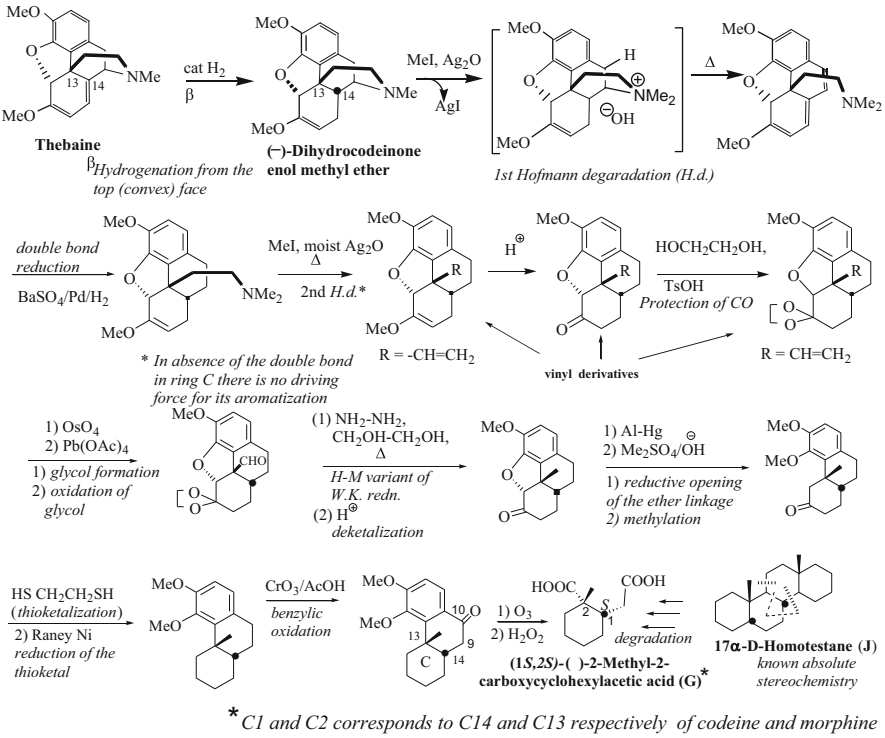


Fig. 23.22 Conversion of dihydrocodeinone enol methyl ether into a cyclohexane derivative (G) of known absolute configuration

However, it should be pointed out that the stereostructure drawn [23] for (−)-morphine has been shown later on to be that of its mirror image (+)-morphine.

23.5 Molecular Conformation of (−)-Morphine

Here, we attempt to show how a rigid molecular model of morphine can be projected in different perspectives to give different projected conformations. The morphine molecule has five dissimilar chiral centers at C5, C6, C9, C13, and C14. Thus of the $2^5 = 32$ possible stereoisomers and 16 possible diastereomers, only one stereoisomer, (−)-morphine, is produced by Nature in opium. (−)-Morphine has the absolute configuration (5*R*, 6*S*, 9*R*, 13*S* and 14*R*), as is evident from its stereoconfiguration (**1a**); this is illustrated in the Table 23.1.

A careful examination of its rigid Dreiding model held and projected in different perspectives shows different representations (**1a**)–(**1g**) (Fig. 23.23) and reveals the following features:

Table 23.1 (*R*,*S*) Specification of the chiral centers of morphine [see (1a) in Fig. 23.23]

Chiral Center	5	6	9	13	14
Sense of priority sequence (a → b → c)	O 13 6	5 HO 7	10 14 N	12 5 14	9 13 8
	acw	*cw*	*cw*	*cw*	*acw*
Viewed	from d (H)	from d (H)	away from d (H)	from d (C15)	from d (H)
(*R*,*S*)-Specification	*R*	*S*	*R*	*S*	*R*

cw = clockwise; acw = anticlockwise

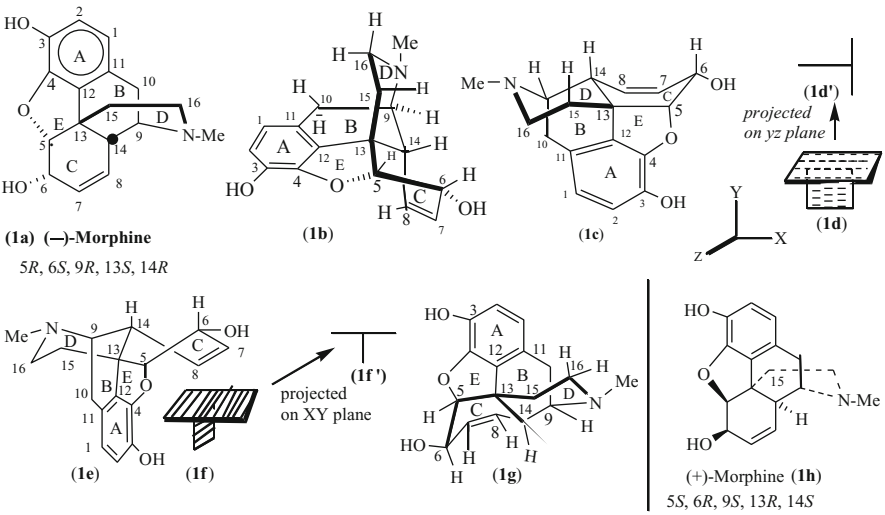


Fig. 23.23 Different representations of the molecular conformation of (−)-morphine

(i) Ring B constitutes a half chair conformation with C10, C11, C12, and C13 in the plane of ring A, and with C9 above and C14 below that plane.

(ii) *Ring D is in a rigid chair conformation* with N-Me group in the equatorial orientation, thereby avoiding its *syn*-axial interactions with axial H14 and axial H15. See (**1b**) and (**1c**).

(iii) When the molecular model is held in the way shown in (**1b**) and viewed obliquely (~20^0) from above the plane of the ring A, and from the bottom of the piperidine ring D, keeping 13-15 bond on the left side of the ring, *the ring C appears to be in a rigid half skew-boat like form* (**1b**).

(iv) If the model held in conformation (**1b**) is rotated anticlockwise to bring ring A towards the observer with OH on the right side of the ring, and the back side is tilted towards the observer and held in such a manner that one views from the top right side of the rings A, B, and E, and the front 15-16 bond comes to the lower side of the chair form of the piperidine ring D, then *ring C appears as a distorted boat form with the flagpoles H6 and H14 almost parallel* [see (**1c**)].

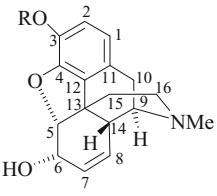
(v) When the model held to have the projection (**1c**) is rotated clockwise about 60^0 and viewed, the ring D is seen as a chair (top left) and ring C like a half skew boat (top right), while rings A, B, and E are almost eclipsed in a vertical yz plane [*cf*. (**1e**)]. In this disposition rings A, B, and E form a vertical plane, and rings C and D form the horizontal puckered plane (*cf* **1f**). Now, the conformation (**1e**), or its three-dimensional projection (**1f**), when projected on a xy plane gives an erect two-dimensional T, as in (**1f′**).

(vi) The conformer (**1c**) gives a three-dimensional projection (**1d**). In either (**1c**) or (**1d**) rings A, B, and E form its vertical portion in xy plane, and rings C and D form its horizontal portion in the xz plane. The projection of (**1c**) or (**1d**) in an yz plane gives a two-dimensional T not erect but inclined 90^0 on the right, as in (**1d′**).

(vii) If we look at the Dreiding model of (−)-morphine from the top in a slanting manner (about 50º of the rings A, B, and E, keeping C15-C16 bond towards the viewer, ring C appears like a half skew boat with C5-C13 bond in the front and ring D is seen in perfect chair with C15-C16 bond in the front (disposition **1g**).

Instead of writing (−)-morphine in a correct three-dimensional conformation (**1b**), (**1c**), (**1e**), or (**1g**), it is usually written either as (**1a**), or its up-side down form rotating it in plane 180º, or in another form obtained by rotating it in plane 90º anticlockwise, so that ring A appears on the left side.

23.6 ^{13}C NMR Spectral Data



R=H, **Morphine (1)**

R=Me, **Codeine (2)**

^{13}C NMR (d_6 DMSO):

Morphine [24]: C1-118.60, C2-116.36, C3-138.45, C4-146.30, C5-91.49, C6-66.38, C7-133.43, C8-128.5, C9-58.09, C10-20.20, C11-125.53, C12-131.04, C13-42.97, C14-40.63, C15-35.56, C16-46.05.

Codeine [25]: OCH_3 56.18, C1-119.39, C2-112.81, C3-142.12, C4-146.17, C5-91.30, C6-66.38, C7-133.32, C8-128.13, C9-58.76, C10-20.38, C11-126.71, C12-131.10, C13-42.90, C14-40.73, C15-35.80, C16-46.38.

MS: Morphine (Codeine) [26]: *m/z* 285 (299) (M^+), 256 (270) (M^+-29), 242 (256) (M^+-43), 215 (229), 200 (214), 174 (188), 162 (162), 124 (124).

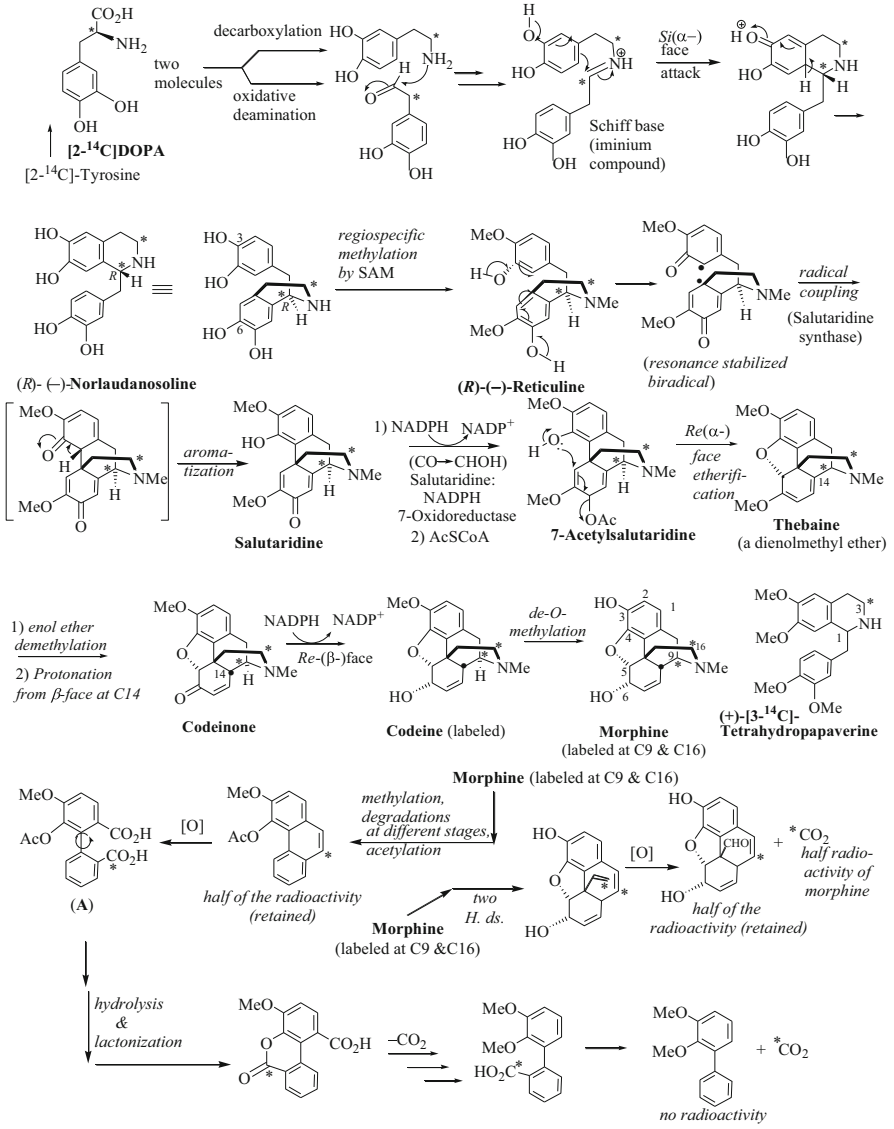
23.7 Biosynthesis of Thebaine, Codeine, and Morphine [27–31]

The crucial step in the biosynthesis of morphine alkaloids involves phenolic oxidation by a specific enzyme acting as a one-electron oxidant. The enzyme folds the substrate in such a way that only one coupled molecular product is obtained. The brilliant conception of oxidative coupling of benzylisoquinoline moiety for the biosynthesis of morphine led Robinson to arrive at the correct formulation of morphine [6, 7].

Looking at the structure of morphine, and knowing that amino acids are the sources of nitrogen in most of the alkaloids and certain part of the carbon framework of the molecule, one can retrobiosynthetically arrive at the probable precursor/s. In case of morphine alkaloids using assumed labeled precursor, it has been shown that the 1-benzylisoquinoline derivative (*R*)-(−)-*norlaudanosoline*, derived from two molecules of tyrosine/DOPA, (Fig. 23.24) is the true precursor of morphine. Norlaudanosoline is methylated regiospecifically by SAM (cf Chap. 3) to yield (*R*)-(−)-reticuline. The latter being catalyzed by an enzyme with one-electron transfer system generates a resonance stabilized biradical which by coupling followed by aromatization yields *salutaridine*. From salutaridine thebaine can be obtained by two alternative routes. Barton [29, 30] showed that in all probability the chemical conversion in vivo of salutaridine to thebaine takes place *via* its reduction products, the epimeric allyl alcohols, followed by their treatment at pH 4 at room temperature. Thebaine runs into morphine biosynthetically *via* codeinone and codeine, followed by demethylation of codeine (Fig. 23.24). During the biosynthesis of morphine from tyrosine, the chronology of hydroxylation, methylation, and demethylation (except from codeine to morphine and the biradical formation) is not known with certainty, and hence the sequence shown is speculative.

It has been shown by Battersby [27, 28, 31] that (±)-[3-^{14}C]tetrahydropapaverine (Fig. 23.24) did not get incorporated in morphine in the biosynthetic experiment, since the two phenolic OH groups at C4 and C7 are needed for radical coupling as we find in (−)-reticuline. It has been conclusively established from labeling experiments that the terminal steps involve the sequence thebaine → codeinone → codeine → morphine and that codeinone is the right intermediate (Fig. 23.24). The location of labels in morphine obtained from 2-^{14}C-tyrosine was decided by two Hofmann degradations followed by oxidation of the ethylene side chain to yield radioactive CO_2 and a phenanthrene derivative—each carrying half of the radioactivity of morphine (Fig. 23.24). Another set of degradations of morphine at various stages is carried out to obtain a substituted biphenyl decarboxylic acid (**A**) carrying half of the radioactivity of morphine. Selective decarboxylation is done by blocking CO_2H and OH of two different rings. Elimination of nonradioactive CO_2 along with the aforesaid facts established the locations of radioactivity at C9 and C16 of morphine (Fig. 23.24).

(*S*)-(+)-Reticuline (with wrong stereochemistry for morphine) is known to epimerize to (*R*)-(−)-reticuline (with right stereochemistry of morphine) in vivo. Thus, both epimers can serve as the biogenetic precursor in morphine biosynthesis.



Note: *the location of the label is thus determined in morphine biosynthesized from labeled tyrosine*

Fig. 23.24 Biosynthesis of labeled morphine *via* labeled thebaine, codeinone, and codeine, and degradations to define the location of label(s)

In that event the genesis of (R)-(−)-reticuline will happen as shown in Fig. 23.25. (*S*)-(+)-Reticuline epimerizes *via* dehydrogenation to form the iminium ion, followed by dequaternization by hydrogenation with NADPH → $NADP^+$ dependent oxidoreductase (Fig. 23.25).

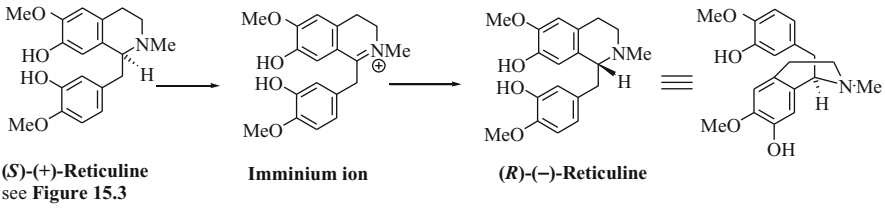


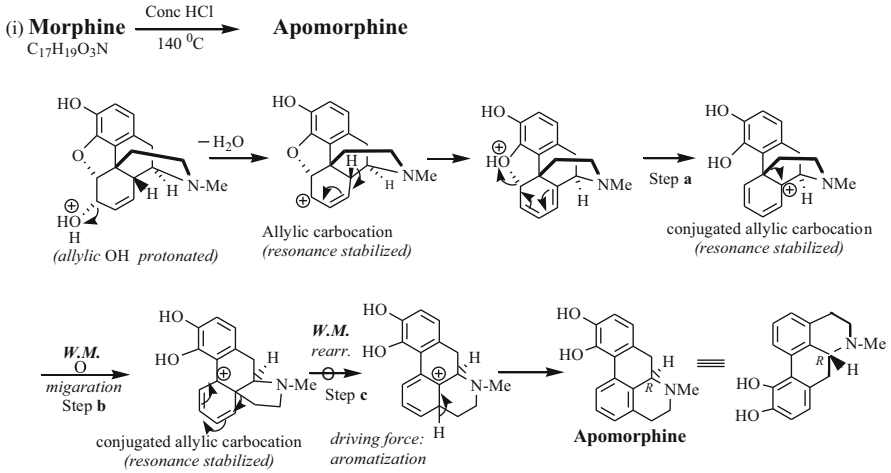
Fig. 23.25 Epimerization of (*S*)-(+)-reticuline to (*R*)-(−)-reticuline in vivo

23.8 Molecular Rearrangements. Mechanisms

Ginsburg wrote [2] that while describing the position of morphine in organic chemistry, Robinson (NL 1947) "...called it the proteus among molecules, the star performer among molecular acrobats." Thebaine, a structurally closely related congener alkaloid is even a better player on the molecular trapeze. The molecular rearrangements in these compounds in acidic medium are triggered by the protonation on the hetero atom/s present in the molecule. The protonated heteroatom-carbon bond thus becomes more polar and prone to be cleaved and helps in the generation of *t*-carbocation/allylic carbocation. The latter may be quenched in different ways, *e.g.*, stereoelectronically suitably disposed bond migration and finally proton elimination. In effect sterically strained bonds get relaxed through bond cleavage and rearrangements. The structures of all the rearranged products were established. Though in the acidic medium all the heteroatoms are protonated, for the sake of convenience, only protonation of the heteroatom involved in the heteroatom-carbon bond migration/cleavage is shown. The formation of some rearranged products [in (**i**) to (**viii**)] under the specified conditions has been rationalized mechanistically in the Figs. 23.26, 23.27, 23.28, 23.29, 23.30, 23.31, 23.32, and 23.33. Rearrangement (i) (Fig. 23.26) is shown at the top of the next page.

(ii) **Codeine** $\xrightarrow{H_3PO_4, \Delta}$ **Apocodeine** (Fig. 23.27)
On similar treatment with phosphoric acid, codeine produces apocodeine (monomethyl ether of apomorphine) (Fig. 23.27), following the same sequence of steps as in Fig. 23.26.

(iii) **Thebaine** $\xrightarrow[100\ ^\circ C]{\text{conc.HCl}}$ **Morphothebaine** (Fig. 23.28)
Thebaine upon heating with conc. HCl undergoes partial demethylation as well as skeletal rearrangement to yield morphothebaine (Fig. 23.28). Demethylation of the enol methyl ether at C6 must have taken place during acid treatment to form the 13-cation (**A**), prior to aromatization of ring



Notes :

Steps **a** and **b** may well be written in a concerted way.

Each of the steps **a**, **b** and **c** produces resonance stabilized allylic tertiary carbonium ion

Fig. 23.26 Rearrangement of morphine to apomorphine

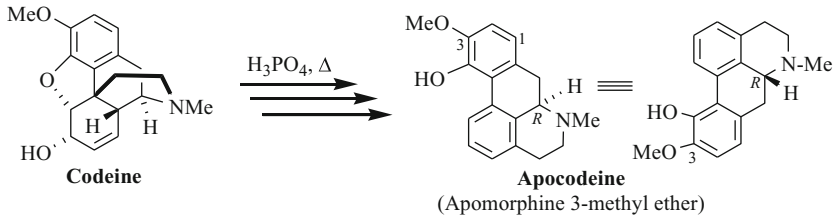
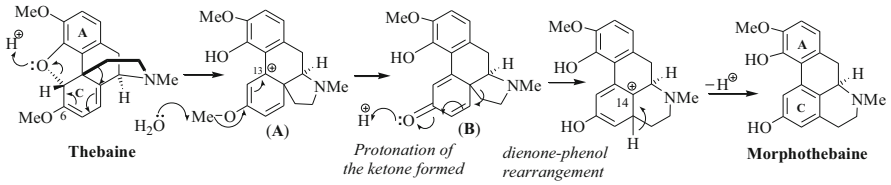


Fig. 23.27 Rearrangement of codeine to 3-methyl ether of apomorphine



Note: *Enol methyl ether (A) undergoes facile demethylation in mineral acids like HCl*

Fig. 23.28 Rearrangement of thebaine to morphothebaine

C. Unlike morphine and codeine, thebaine does not suffer loss of oxygen atom during its acid rearrangement. In the presence of conc. HCl the intermediate dienone (**B**) undergoes dienone-phenol rearrangement to form morphothebaine (Fig. 23.28).

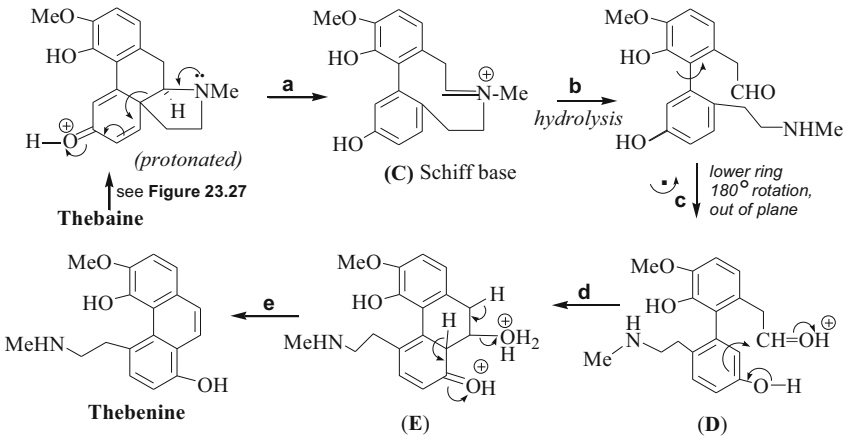


Fig. 23.29 Rearrangement of thebaine to thebenine

(iv) **Thebaine** $\xrightarrow[\text{with dil HCl (2 min)}]{\text{boiled briefly}}$ **Thebenine** (Fig. 23.29)
Plausible mechanistic sequence of steps is shown in Fig. 23.29.

(v) **Thebaine** $\xrightarrow{\text{(i) PhMgBr}}$ **Phenyldihydrothebaine** (Fig. 23.30)

(vi) **α - Codomethine** → **Acetylmethylmorphol** (Fig. 23.31)
Perhaps 6-OH is first converted to 6-OAc which then through the resonance stabilized allylic carbocation at C6 collapses by the loss of 14β-H. The product simultaneously may undergo acetylation of the ether oxygen, followed by E2 type elimination by attack of acetoxy group on C15 to form acetylmethylmorphol

(vii) **Thebaine** $C_{19}H_{21}O_3N$ $\xrightarrow[\text{dil HCl}]{\text{MeOH}}$ **Methebenine** $C_{19}H_{21}O_3N$ $\xleftarrow[\text{dil HCl}]{\text{MeOH}}$ **Codeinone** $\xrightarrow{\text{Sn/HCl}}$ **Metathebainone** (Fig. 23.32)
Under this condition thebaine is first converted [via (**A**)] to (**B**) (see Fig. 23.28), which undergoes the same sequence of reactions (**a to d**) as shown in Fig. 23.29 to form (**E**). Probably (**E**) undergoes methylation as shown in Fig. 23.32 to give methebenine.

Codeinone, when treated with Sn/HCl (Fig. 23.32), the protonated ketone induces the loss of 14-H, followed by *a W.M. rearrangement* to give a 13-cation, which collapses to form (**B**). The 7-8 double bond of the latter, which is not conjugated with the aromatic ring A, gets reduced to form metathebainone, demonstrating the formation of the intermediate (**B**), as in case of formation of morphothebaine from thebaine (Fig. 23.28). The 13-cation may also follow the path x to form the intermediate Schiff base

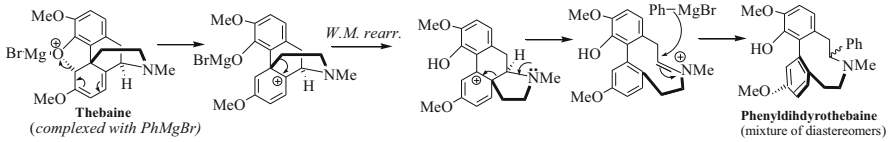


Fig. 23.30 Conversion of thebaine to phenyldihydrothebaine by Grignard reaction

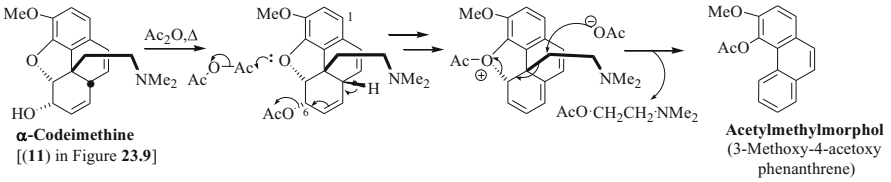


Fig. 23.31 Conversion of α-codomethine to acetylmethylmorphol

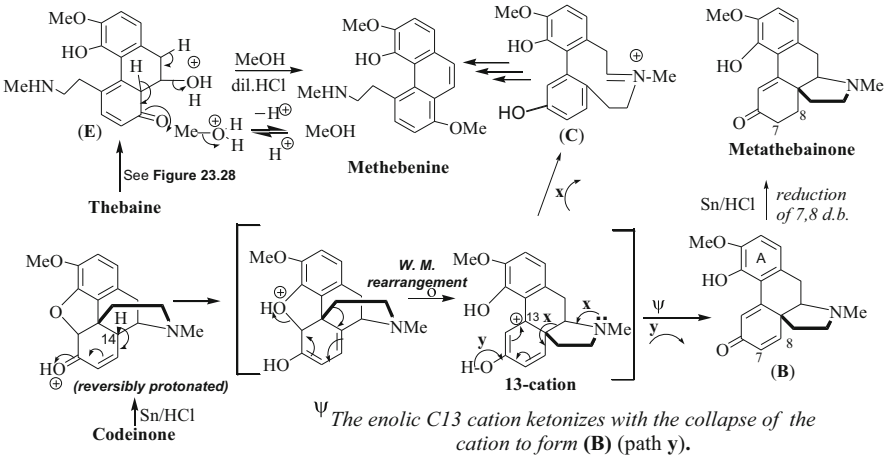
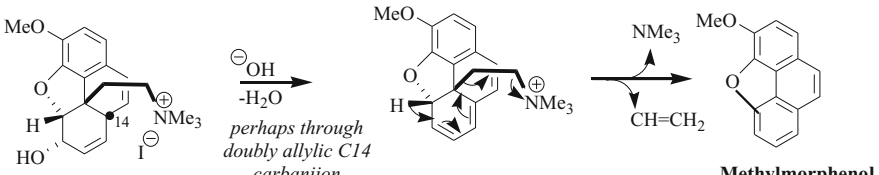


Fig. 23.32 Rearrangements of thebaine to methebenine and of codeine to methebenine and metathebainone respectively

(**C**). The latter undergoes the steps **b, c, d,** and **e,** shown in Fig. 23.29, to form thebenine. However, under the reaction condition, methylation of the phenolic OH takes place at any step after (**C**) to give the final product methebenine.

(viii) **α - Codeimethine methiodide → Methylmorphenol** (Fig. 23.33)



Note: *Perhaps dehydration takes place in the first step via the doubly allylic C14 carbanion, and then the electronic shifts could go all the way round the ring as shown, thus avoiding the simple stereochemical limitation of cis-elimination.*

Fig. 23.33 Conversion of α-codeimethine methiodide to methylmorphenol

23.9 Some Bioactive Derivatives of Thebaine Synthesized Through Diels–Alder Cyclization

23.9.1 Bridged Oripavine Derivatives

In an attempt to obtain semisynthetic drug having morphine skeleton with better analgesic property and reduced addiction liability, thebaine (**3**) has been subjected to Diels–Alder reactions using appropriate dienophiles. It has been found that bridged oripavine (=3-demethylthebaine) derivatives, obtained from Diels–Alder cyclizations of thebaine and methyl vinyl ketone, followed by conversion of the COMe group of the adducts by Grignard (RMgBr) reactions to RCMe(OH) groups and subsequent 3-demethylation (Fig. 23.34) [33, 34] confers highly increased analgesic properties. Formation of ring C cleaved products has been outlined in Fig. 23.35.

23.9.2 Hetero Diels–Alder Reaction of Thebaine. Unexpected Cleavage of C5–C6 Bond

The regioselective hetero Diels–Alder cycloaddition of thebaine with heterodienophile transient acylnitroso (RC(O)N = O) compounds (generated in situ) have been reported (Fig. 23.35) to yield 6O-14 N-oxazine derivatives. Recently [35], an unexpected cleavage of C5–C6 bond of the oxazine adducts has been observed rather than the formation of the expected codeinone derivative, when the adducts are treated with 2 equivalents of SmI_2 in THF at low temperature. This constitutes the first report of such C-ring cleaved products in morphine alkaloids (Fig. 23.35).

A number of adducts have been prepared with a series of acylnitroso dienophiles (generated in situ) and thebaine. The rigid geometry of thebaine allows the attack of the dienophile from the β-(convex) face leading to the formation of only one diastereoisomer 6βO-14βN-adduct (Fig. 23.35) in each case.

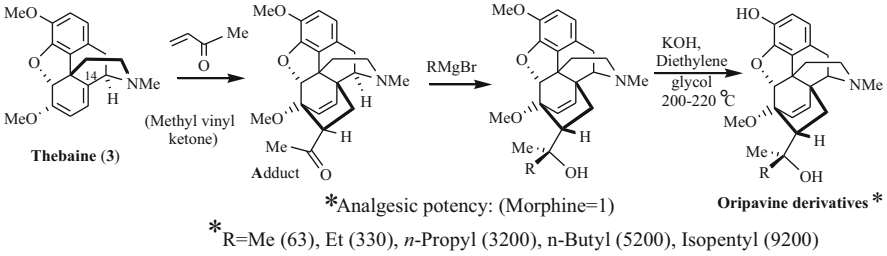


Fig. 23.34 Conversion of thebaine to oripavine derivatives

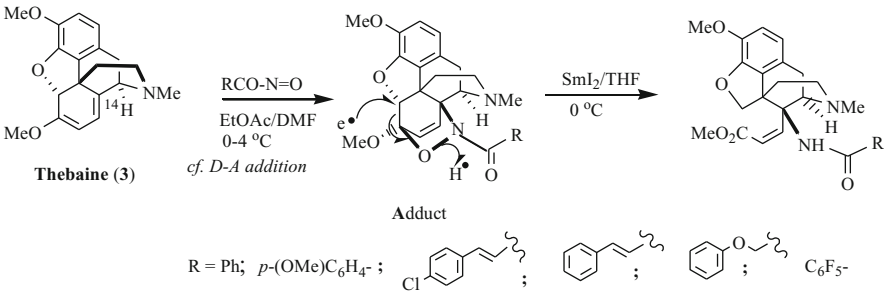


Fig. 23.35 Formation of Ring C cleaved products

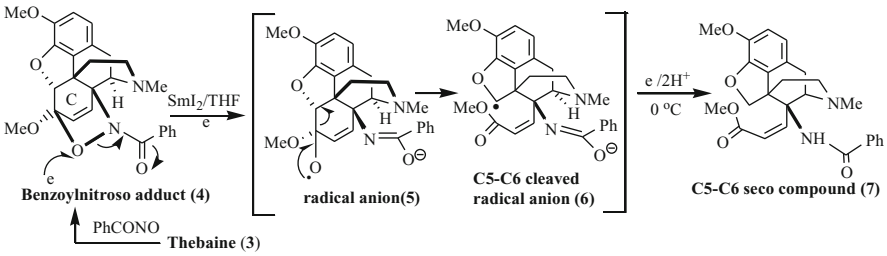


Fig. 23.36 Probable mechanism of C5-C6 bond cleavage of the benzoylnitroso adduct of thebaine

The cleavage of C5-C6 bond of the benzoylnitroso adduct (**4**) of thebaine is assumed to proceed via the radical anion (**5**), generated by the electron transfer from SmI_2, and its subsequent fragmentation to produce the other radical anion (**6**). The latter, being conformationally unable to cyclize, is reduced by a second molecule of SmI_2 followed by protonation to yield the unexpected C5-C6 seco compound (**7**) [33, 34] (Fig. 23.36). The geometry across the nitrogen–carbon bond shown here to be *E* (Fig. 23.36) is inconsequential since the amide (**7**) is the product.

23.10 Uses

See Chap. 33

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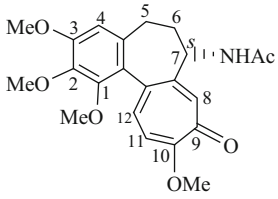
Chapter 24
Colchicine, *A Phenylethylisoquinoline Alkaloid Derived from L-Tyrosine*

24.1 Introduction, Biological Activity

Colchicine (**1**), $C_{22}H_{25}O_6N$, m.p. 157 °C (crystallized from CH_2Cl_2-Et_2O) [1, 2], $[\alpha]_D$ –143.5 (*c* 0.58, $CHCl_3$) [3, 4], containing comparatively less familiar carbocyclic rings, is an important antimitotic agent. It occurs as a poisonous compound in various plants of Liliaceae/Colchicaceae family. It is the major and the active principle of *Colchicium autumnale* (commonly known as "meadow saffron" as well as "autumn crocus" with white flowers at the leafless stalk), a common European plant. According to the conservative definition of alkaloids (Chap. 15), it may be placed in the outskirts of the alkaloid family. However, its profound biological activity and the involvement of amino acids during its in vivo biosynthesis procured for it a seat in the row of alkaloids. It is interesting to know the genesis of the name of the genus *Colchicium*. It derives its name from "Colchis," a region of east black sea, the abode of the notorious poisoner Medea (Greek mythology) [5, 6]. *Glorisa superba* (Liliiaceae) is now the commercial source of colchicine.



(a*R*, 7*S*)-(—)-Colchicine (**1**)

The plant is well known for more than 2,000 years in the treatment of pain, especially at joints and serious types of gouts. *Colchicium* extract was reported as a treatment for gout by Pedanius Dioscorides in the first century, by Alexander of Tralles (550 AD), by Avriena (eleventh century, Persia), and by Baron Anton von Störck (1763). Benjamin Franklin, himself a patient of gout, was responsible for

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bringing it to America [7, 8]. *Its anti-inflammatory and anticancer (studied later) properties are related to its ability to bind tubulin (a globular protein) to inhibit its polymerization to microtubules (thus arresting cell-division at metaphase) and to reduce the mobility of neutrophils into joints* (Chap. 33). A large number of colchicine analogues have been prepared with the objective of finding new antitumor agents. However, the general toxicity of (−)-colchicine could hardly be suppressed. Thiocolchicine [SMe in place of OMe at C10 of (**1**)] shows a comparable activity with slightly lower toxicity and enjoys medical application [5, 6].

The pure compound colchicine was isolated in 1820 by Pelletier and Caventau. The molecular formula and the accumulated data on various degradation products [9, 10] could not elaborate the exact correct structure of colchicine (relative locations of methoxyl and keto groups in the cycloheptatrienolone ring). M. J. S. Dewar in 1945 made a significant suggestion of the presence of a cycloheptatrienolone moiety (aromatic) in colchicine on the basis of his work on stipitatic acid, a fungal metabolite; he introduced the terminology "tropolone" for such cycloheptatrienolone moiety [11, 12]. This marked the beginning of tropolone chemistry (Chap. 31). S. Zeisel developed (1883–1913) a method for the estimation of the methoxyl group (Chap. 31) [13–15] while working on the structure of colchicine. The method was widely used for structural studies of natural products till the availability of NMR spectroscopy (before mid 1950s).

24.2 Structure, Stereochemistry: Absolute Configuration, Conformation, and Axial Chirality

The correct structure with cycloheptatrienolone moiety was confirmed by X-ray analysis of its crystal [16], and the absolute configuration of its only chiral center has been shown to be (*S*) by chemical correlation [17]: Colchicine is subjected to ozonolysis, followed by permanganate oxidation of the ozonolysis product to yield (–)-N-acetylglutamic acid of known structure and absolute configuration (*S*) (Fig. 24.1). During the degradation, the only chiral center C7 of colchicine remained untouched, and thus its absolute configuration has been shown to be (*S*). The sense of the priority sequence of the four different ligands around the chiral center in both is same. The chiral axis of the molecule passes through the midpoint of the C9-C10 bond, and through C12a, C1a, and C3 (Fig. 24.1). By application of CIP rules (Sect. 2.16), the axial chirality of the molecule is shown to be (*aR*) [18, 19], and hence its helicity is (*M*), as shown in the figure. The two aromatic rings are twisted out of the plane making a dihedral (interplanar) angle of ~54°. This inherent helical twist gives the molecule (*aR*) chirality, which is responsible for its binding to tubulin (Chap. 33) and its antimitosis behavior [18, 19].

Berg et al. [20] could separate the atropisomers (a*S* and a*R*) of desacetamidocolchicine and showed that the (a*R*)-atropisomer binds to tubulin.

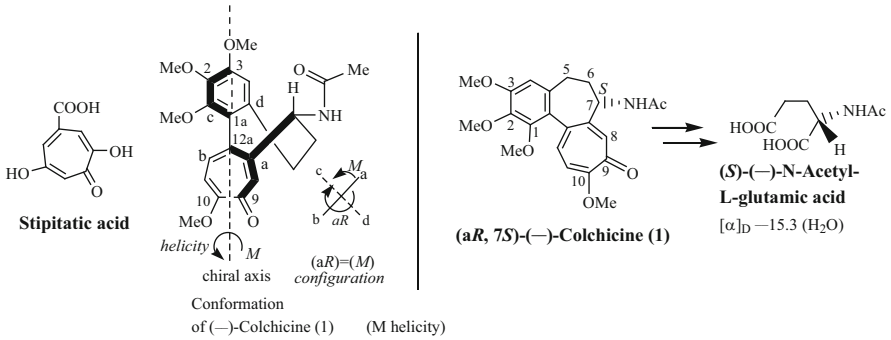
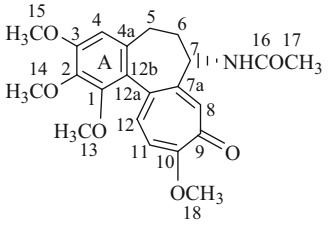


Fig. 24.1 Absolute configuration of axially chiral (–)- colchicine

24.3 Spectral Data of Colchicine [4, 21]



Colchicine (**1**)

UV: λ_{max} (EtOH): 234 (log ε 4.46), 246 (4.49), 357 (4.22) nm

IR: ν_{max} ($CHCl_3$): 3,285 (NH), 1,725 (COMe), 1,674, 1,620 (s) (conjugated CO), 1,597 (s), cm^{-1}

^{1}H NMR (360 MHz, $CDCl_3$): δ 7.79 (d, J 6.4 Hz, 1H, N*H*), 7.59 (s, *H*8), 7.34 (d, J 10.8 Hz, *H*12), 6.88 (d, J 10.8 Hz, *H*11), 6.53 (s, *H*4), 4.69–4.62 (dt, J 6.4, 12.2 Hz, 1H, *H*7), 4.01, 3.94, 3.90 and 3.65 (each s 3H, probably 10-OCH_3, 3-OCH_3, 1-OCH_3 and 2-OCH_3, respectively), 2.55–2.47 (m, 1H, $H_1$5), 2.44–2.27 (m, 2H, $H_2$6), 1.97 (s, 3H, COCH_3), 1.91–1.80 (m, 1H, probably $H_1$5).

^{13}C NMR: (90 MHz, $CDCl_3$) δ 179.5 (C9), 170.0 (C16), 164.0 (C10), 153.5 (C^a3), 152.4 (C^a7a), 151.2 (C^a1), 141.7 (C2), 136.9 (C^b12a), 135.6 (C8), 134.2 (C^b4a), 130.5 (C12), 125.6 (C12b), 112.8 (C11), 10.3 (C4), 61.6 (C13), 61.4 (C14), 56.4 (C15), 56.1 (C18), 52.7 (C7), 36.0 (C6), 29.9 (C5) and 22.8 (C7).

MS [18, 19]: *m/z* at 399 (M^+), 371 (M^+-CO), 356 (M^+-$COCH_3$), 328 (M^+-CO-$COCH_3$), 312 (base peak, M^+-CO-NH_2COCH_3), 297 (312-CH_3), 281 (312-OCH_3).

The hydrogen and carbon chemical shifts cited here are as reported [4]. Their assignments were not given earlier [4]. The assignments of some quaternary carbons having similar δ-values, e.g., C3, C7a, and C1 (a) or C12a and C4a (b), and some primary carbons, e.g., C13 and C14 or C15 and C18, are arbitrary and interchangeable.

24.4 Total Synthesis

During the last 50 years, the unusual ring pattern of colchicine and its profound biological activity attracted the attention of a large number of outstanding synthetic organic chemists including Eschenmoser (1959), van Tamelen (1959), Nakamura (1962), Woodward (1963), Scott (1965), Toromanoff (1965), Kaneko (1968), Kato (1974), Tobinga (1974), Evans (1984), Boger (1985), Banwell [(–), (1996)], and Cha [(–), (1998)], who have successfully achieved the total synthesis (publication year and chiral synthesis are indicated within brackets, the rest are of (±)-variety) of colchicine or its derivatives. These have been excellently reviewed by Graening and Schmalz [5]. A total synthesis of (–)-colchicine via a Rh-triggered cycloaddition cascade has been reported by Schmalz [22] in 2005. This new methodology allows an efficient enantioselective route to this alkaloid and its analogues. The first, the last and three other syntheses have been outlined by Hoffmann [6].

The major difficulty in most of the syntheses lies in the construction of the highly oxidized ring C in an array of 6,7,7-ring system fused in an angular fashion (cf. Phenanthrene) and the regioselective enol methylation in ring C.

24.4.1 The Pioneering Eschenmoser Synthesis

The first successful total synthesis of (±)-colchicine by Eschenmoser [1, 2] was reported in 1959 using commercially available purpurogallin as the starting material. The sequence of reactions with fairly detailed experimental conditions is delineated in Fig. 24.2. The key step is the generation of cycloheptatriene moiety through ring expansion involving a cyclopropane system (norcaradiene derivative), generated in situ.

Plausible mechanistic sequences of ***steps C* and *D*** of Fig. 24.2, not so obvious, are given in Fig. 24.3.

24.4.2 Chiral Synthesis of (–)-Colchicine

A novel total chiral synthesis of (–)-colchicine by Cha and coworkers, reported in 1998 [3] is delineated with fairly detailed experimental conditions and reagents in Fig. 24.4. The oxygen bridge in the step C was removed by treatment with TMOSTf/NEt_3 to furnish the product tropolone with correctly placed methyl ether functionality, and no isomeric tropolone was produced. Mechanisms of two interesting steps have been shown in Fig. 24.5. The synthesis of the oxazole intermediate (**I**) has been reported in 2000 by Lee and Cha [4] by a different route, starting from 2,3,4-trimethoxybenzoyl chloride.

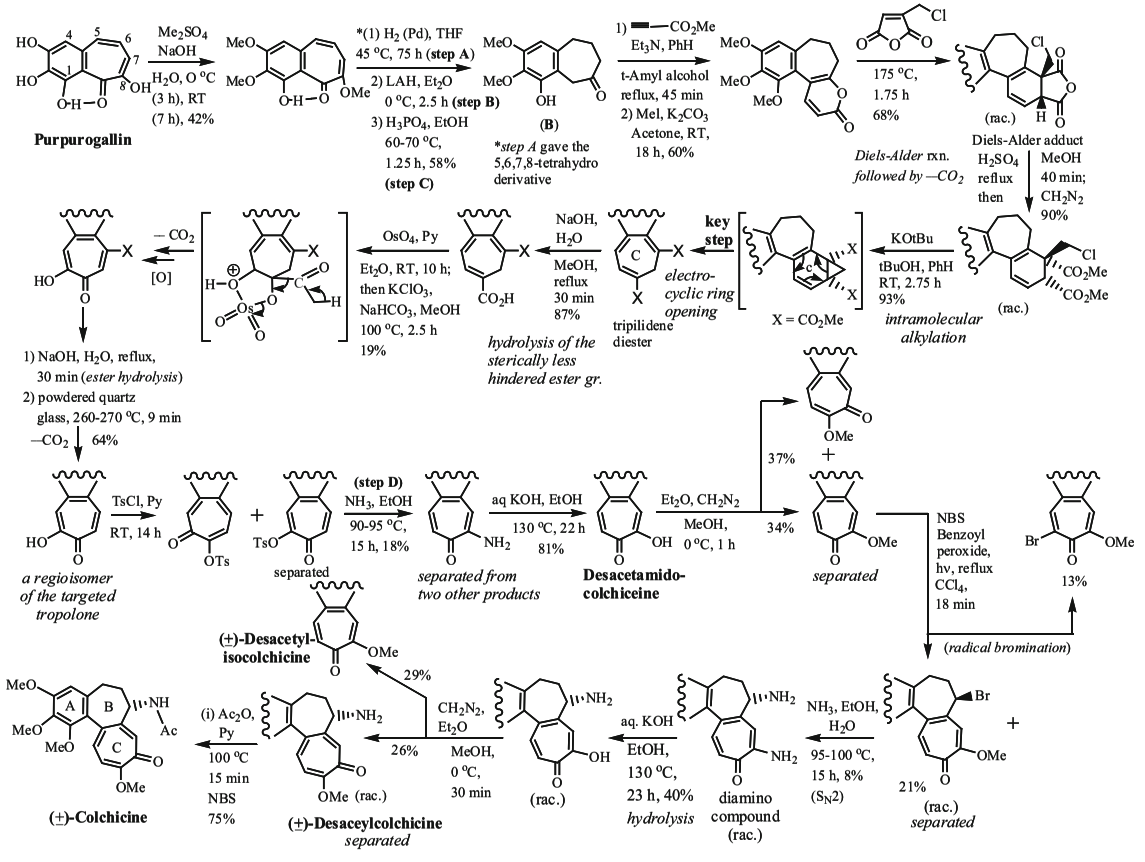


Fig. 24.2 Eschenmoser's first synthesis of (±)-colchicine [1, 2] (1959)

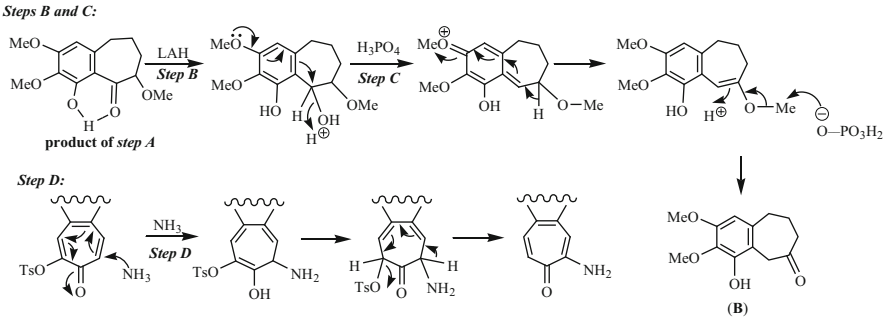


Fig. 24.3 Probable mechanisms of some steps of Fig. 24.2

A total synthesis of (–)-colchicine via a Rh-triggered cycloaddition cascade has been reported [22] in 2005. This new methodology allows an efficient enantioselective route to this alkaloid and its analogues.

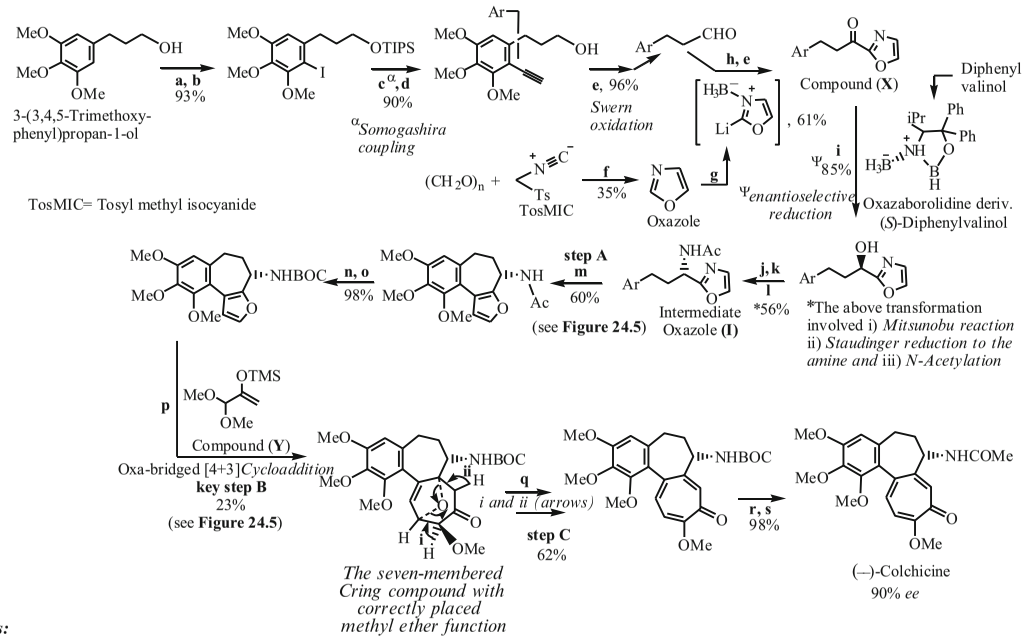
24.5 Interconversion of Colchicine, Colchiceine, and Isocolchicine

The chemistry of tropolones derivatives has been studied extensively [5, 6]. Tropolones exist in tautomeric equilibrium and behave like phenols toward aromatic substitution. The acidity (pKa = 7) of the tropolones is more than that of phenols but less than that of carboxylic acids. The carbonyl group may be considered as a part of a vinylogous carboxylic acid.

Colchicine (**1**) on treatment with 0.1 *N* HCl at 100 °C gives colchiceine (**2**), which is in tautomeric equilibrium with (**3**). Colchiceine upon treatment with CH_2N_2 gives colchicine (**1**) and isocolchicine (**4**) in equal proportion. Methylation of colchiceine thus involves the problem of regioselectivity. Isocolchicine, like colchicine (**1**) upon treatment with 0.1 *N* HCl at 100 °C gives colchiceine (Fig. 24.6).

24.6 Biosynthesis of Colchicinoids [23–26]

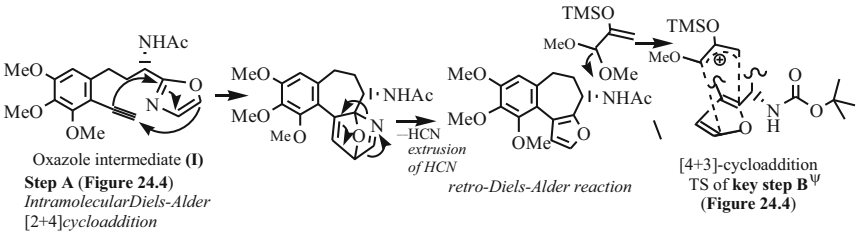
A.R. Battersby (see Appendix A) is the major contributor to the biosynthetic studies of colchicines [23–26]. On the basis of a large number of experiments with labeled precursors Battersby suggested the currently accepted proposal of the use of L-tyrosine and L-phenylalanine by plants during the biosynthesis of (–)-colchicine



Reagents and conditions:

a TIPSOTf, 2,6-Lutidine, CH_2Cl_2, 0 °C → RT, 5 h
b I_2, $Ag(CF_3CO_2)$, $NaHCO_3$, $CHCl_3$, 3h
c HC≡CTMS, $[Pd(PPh_3)_2Cl_2]$, CuI, Et_2NH, DMSO, 90 °C, 20 h
d TBAF, THF, 0 °C, → RT, 7 h
e $(COCl_2)$, DMSO, CH_2Cl_2, −78 °C, 1 h 15 min; then NEt_3, −78 → 0 °C
f TosMIC, K_2CO_3, DMSO, RT, 5 h; then KOH, $HOCH_2CH_2OH$, RT, 5 h
g BH_3.THF, THF, RT, 1 h; then t-BuLi, −78 °C, 40 min
h THF, −78 °C, 3 h; **i** BH_3.THF, (*S*)-(−)-diphenylvalinol, THF, −78 °C, 20 min, RT; then (**X**), THF, 6 h
j $(PhO)_2P(O)N_3$, PPh_3, DEAD, THF, RT, 12 h; **k** PPh_3, H_2O, THF, RT, 50 h
l Ac_2O, NEt_3, DMAP, Et_2O, RT, 7 h; **m** *o*-Dichlorobenzene, reflux, 66 h
n $(BOC)_2O$, NEt, DMAP, CH_2Cl_2, 35 °C, 10 h
o LiOH, THF, 12 h
p **Y**, TMSOTf, $EtNO_2$, −78 °C, 1 h → 60 °C, 12 h
q TMSOTf, NEt_3, CH_2Cl_2, 0 °C, 2 h
r HCl(1*M*), Et_2O, RT, 1 h
s Ac_2O, NEt_3, DMAP, Et_2O, 25°C. 10 h
TIPS = Triisopropylsilyl; TMSOTf = Trifluoromethanesulfonyl
TMS = Trimethylsilyl; TBAF = Tetra-*n*-butylammonium fluoride
BOC = *t*-Butoxycarbonyl; DIAD = Diisopropylazodicarboxylate
DMAP = 4-Dimethylaminopyridine

Fig. 24.4 Synthesis of (–)-colchicine by Cha and coworkers [3, 4]



It is an overall [4+3]-cycloaddition to the BOC-protected compound,which proceeds under the influence of the bulky amide function on the regio- and stereoselectivity of the cycloaddition shown. Thus, the stereochemical assignment was tentatively made on the expected approach of the W-shaped α-methoxy-(trimethylsilyloxy)-allyl cation, generated in situ, from the less hindered β-face of the furan in an endo-like mode, to avoid steric interactionwith the t-butyl group of BOC in the α-face (exo-like mode). (The exo-mode approach takes place when –NHAc is not blocked by BOC, producing the undesired regioisomer with β-oxa bridge.)

Fig. 24.5 Mechanisms of steps A and B of Fig. 24.4

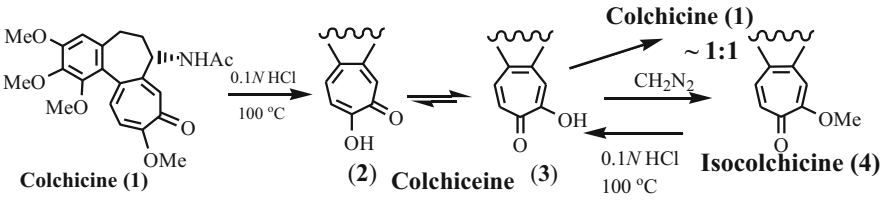


Fig. 24.6 Interconversion of colchicines (1), colchiceine (3) and isocolchicine (4)

(Fig. 24.7). In the process L-tyrosine undergoes *o*-hydroxylation and decarboxylation to yield dopamine. The latter reacts with a molecule of cinnamic acid derived from L-phenylalanine (Sect. 13.1.4) to form an amide which subsequently undergoes cyclization (Bischler-Napieralski type) to form the isoquinoline nucleus. Reduction of the olefinic double bond yields (*S*)(–)-autumnaline, an isoquinoline alkaloid occurring in *Colchicium cornigerum*. The intermediacy of this alkaloid in the biosynthetic pathway of colchicines has been experimentally supported by using labeled autumnaline and its observed incorporation. Autumnaline thus formed undergoes *para*/*para* phenolic coupling followed by methylation (by SAM) and dehydrogenation ($NADP^+$) to form an enamine. The original tyrosine part undergoes ring expansion via a cyclopropane ring and the 6,7,7-ring system with a cycloheptatrienolone moiety with four methoxyl groups is obtained. The MeNCHO group being an amide is hydrolyzed to –NHMe and then the methyl group suffers oxidative elimination to form deacetylcolchicine, which is acetylated by acetyl coenzyme A to yield (–)-colchicine (Fig. 24.7). All these steps are governed by appropriate specific enzymes. The sequence of some of the steps has not been confirmed and may be otherwise. That the seven-membered tropolone ring has originated from the ring expansion of the tyrosine derived aromatic ring has been proved by labeling the benzylic carbon of tyrosine as shown (Fig. 24.7).

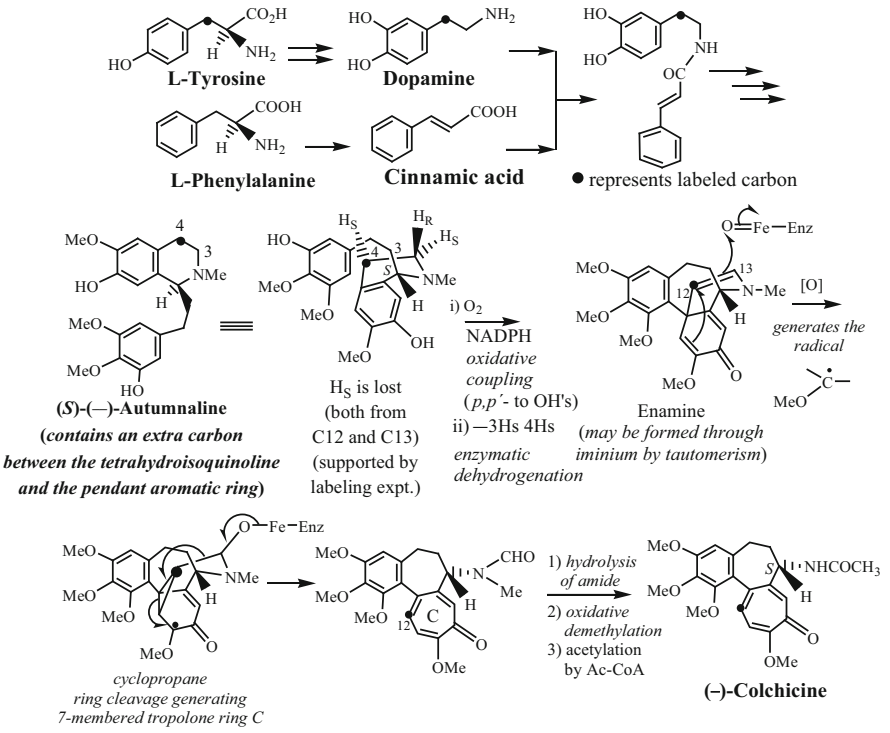


Fig. 24.7 Biosynthesis of colchicine

While studying the biosynthetic pathway of colchicine Battersby et al. synthesized some labeled colchicine related alkaloids, which are present in various *Colchicium* species, to show their intermediacy in the terminal sequence of colchicine biosynthesis (Fig. 24.8). Further the loss of H atoms from both C12 and C13 [24, 25] during the biosynthesis of colchicine has also been shown by labeling the pertinent protons. These elegant experiments unequivocally mapped the biosynthetic pathway of colchicine [22–24].

24.7 Photochemical Reactions of Colchicine

Aqueous solution of colchicine (**1**) on ultraviolet irradiation yielded three photoproducts (Fig. 24.9). The products have been characterized by several workers [27], *α-lumicolchicine* (**2**), and the diastereomers *β-lumicolchicine* (**3**) and *γ-lumicolchicine* (**4**). The photoproduct (**3**) being stereochemically favored (better steric fit) over (**4**), undergoes *photodimerization* in the presence of UV to give the dimeric product (**2**).

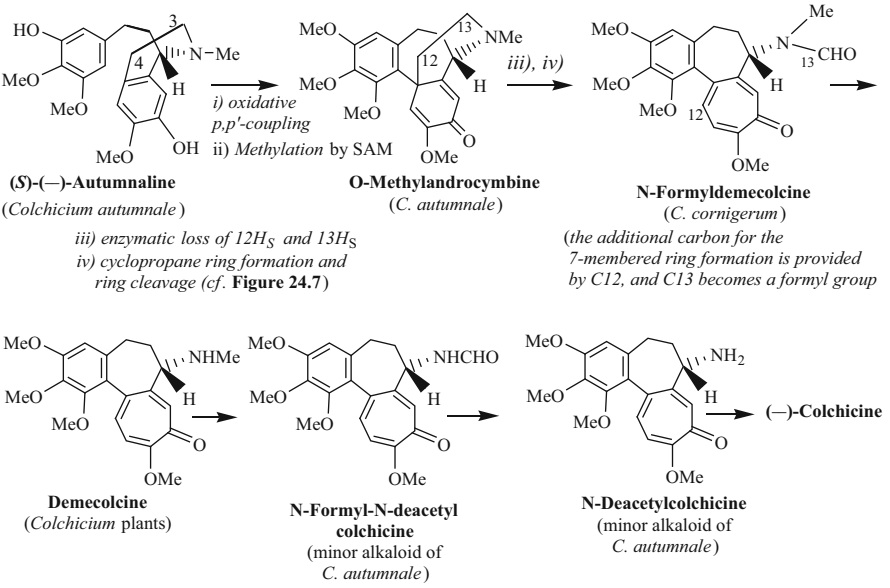


Fig. 24.8 Terminal sequence of the biosynthesis of colchicine

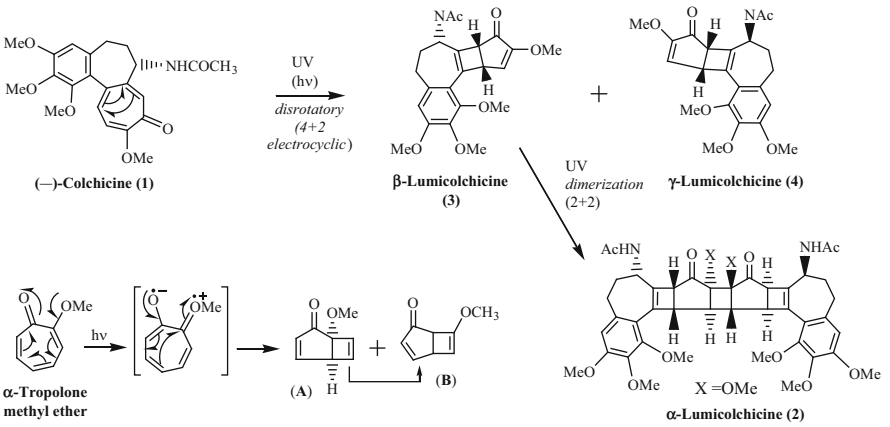


Fig. 24.9 Photoproducts of colchicine

The formation of (**A**) and (**B**) from α-tropolone methyl ether by photolysis (Fig. 24.9) serves as the first example of an electrocyclic reaction. Here the products are thermodynamically much higher in energy but are thermally stable. In the conversion of tropolone to the bicyclo system aromaticity and the conjugation between methoxy and keto groups are lost.

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Chapter 25
Quinine. *Cinchona* Alkaloids (*Tryptophan Derived Quinoline Alkaloids*)

25.1 Introduction and Historical Background

Quinine is perhaps the only natural drug that has the long tradition of saving thousand millions of malaria- infected people for more than 300 years [1]. The disease malaria immediately reminds of the *Cinchona* bark and root, for that matter, quinine, their active principle. David Livingstone (a Scottish Missionary and an Explorer, 1813–1873) fell prey several times to malaria (Italian "m*al*" "*aria*," meaning bad air) but recovered, using *Cinchona* bark preparations, though became weak due to side effects. Finally he died of malaria in 1873 at the Lake Tanganyika [1].

To the tourists in Cairo the guides still spin the yarn of the story of Tutankhamun's (Fourteenth Century BC ~1324 BC, Egyptian King of 18th dynasty circa 1550–1070 BC) untimely death at the age of 19 years as being due to a perfectly designed murder disguised in an accident in a remote place. It is an attractive story to the visitors. However, recent analysis of DNA of Tutankhamun (also spelt as Tutankhamen/Tutankhamon) showed that his death in all probability was caused by *malaria tropica*. His parents also died of malaria. These are probably the first known cases of malaria, though detected and recorded [2, 3] only in 2010.

The treatment of malaria was quite expensive, as the *Cinchona* bark, in the form of powder or tonic, had to be imported from Peru, South America. In around 1630, a physician, Juan de Vega treated Countess of *Chinchon*, Dona Francisca Henriquez de Rivera, wife of the Spanish Viceroy (1629–1669) of Peru, with the *Cinchona* bark, locally known as *quina* bark, and she recovered from the fever. Such miraculous property of a bark in Quito (a city in Ecuador) was published in the *Chronicle of St. Augustine* [1] in ≈1633.

Livingstone sensed the relationship of mosquito and malaria, and a French physician, Alphonse Laveran in Algeria found the protozoan *Plasmodium* parasite in the infected blood cells in 1880.

Ronald Ross (1857–1933, NL, 1902), while working alone at a small regional hospital at Secunderabad, India, cut open a stomach cell of a mosquito, found a

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cluster of small black pigment granules, and identified the parasite *Plasmodium crescents* that causes malaria. The parasitic nature of the disease was thus proved on 20th August (1897) morning. The next morning he wrote to his wife a poem:

This day relenting God
A wonderous thing; and God
Seeking His secret deeds
I find thy cunning seeds
I know this little thing
O Death, where is thy string?
Hath placed Within my hand
Be praised. At His Command
With tears and toiling breath
O Million – murdering Death
A myriad men will save
Thy victory, O Grave?

Ronald Ross

Ross moved to Calcutta (now Kolkata) in January 1898 and worked for 6 months under the Director General of the IMS in Calcutta. He showed that the parasites which develop inside the mosquitoes within 2 months could be transmitted by a female mosquito (*Anopheles balbacensis*) in large amounts to the victim she bites. He received the Nobel Prize in 1902 for his work done in India. Incidentally, it may be mentioned that Wagner von Jauregg Julius (1857–1940, NL 1927) discovered the therapeutic value of malaria inoculation in the treatment of syphilitic meningoencephalitis.

The word quinine is derived from *quina* which is Spanish for the bark of *Cinchona* species containing quinine. Quinine (**1**) in the pure form was first isolated from the *Cinchona* bark by Pelletier and Caventau in 1820 [4]. It is the primary alkaloid of various species of Cinchona. This compound contributed greatly to science, chemistry, and medicine as well as society and industry for over last 190 years.

European countries were also in need of this drug. It was sold under the name Jesuit's powder after the Roman Catholic missionaries who officially discovered it. Because of the religious taboo Protestants did not like to use the drug associated with papacy [1]. Many important peoples' refusal to use this drug led to their death: One of them had been Oliver Cromwell (1599–1658, English General, leader of the Parliamentary troops in the civil war, Lord Protector of England, 1653–1658).

Cinchona (Fam. Rubiaceae) species are large trees indigenous to South America. They are cultivated in many parts of the world, especially in India, Indonesia, Sri Lanka, Kenya, Tanzania, and Bolivia. Three main *Cinchona* species (together with varieties and hybrids), *viz*., *C. calisaya*, *C. ledgeriana*, and *C. succirubra* are cultivated for good alkaloid content (4–14 %) in their bark. Selected hybrids can give up to 17 % alkaloids.

Several alkaloids have been isolated from the bark of *Cinchona* species (8–12 years old)—of which quinine (the major alkaloid) and quinidine, and cinchonidine and cinchonine, represent two pairs of diastereomers (Fig. 25.9); they account for about 30–60 % of the total alkaloid content. Corresponding 9-epimers of these compounds also occur in *Cinchona* species in small amounts.

25.2 Structure of Quinine. Some Pertinent Reactions [5]

Extensive investigations for the structural elucidation of quinine laid the foundation for much of our knowledge of the chemistry of quinoline and pyridine bases. Huge data accumulated through the degradative works by many outstanding chemists of nineteenth and twentieth centuries such as Baeyer, Koenigs, Körner, von Miller, Pasteur, Rabe, and Rohde. Paul Rabe is the acknowledged master of *Cinchona* alkaloids who suggested the correct structure of quinine in 1908 [5, 6]. The correct relative and absolute stereochemistries of the four chiral centers were settled much later. The full representation of the quinine molecule is shown as (**1**) (Fig. 25.1).

Rabe's suggestion of the gross structure was based on the degradation products. Only certain reactions of quinine, which are particularly pertinent to its structure elucidation, and synthetic endeavor, will be briefly discussed here.

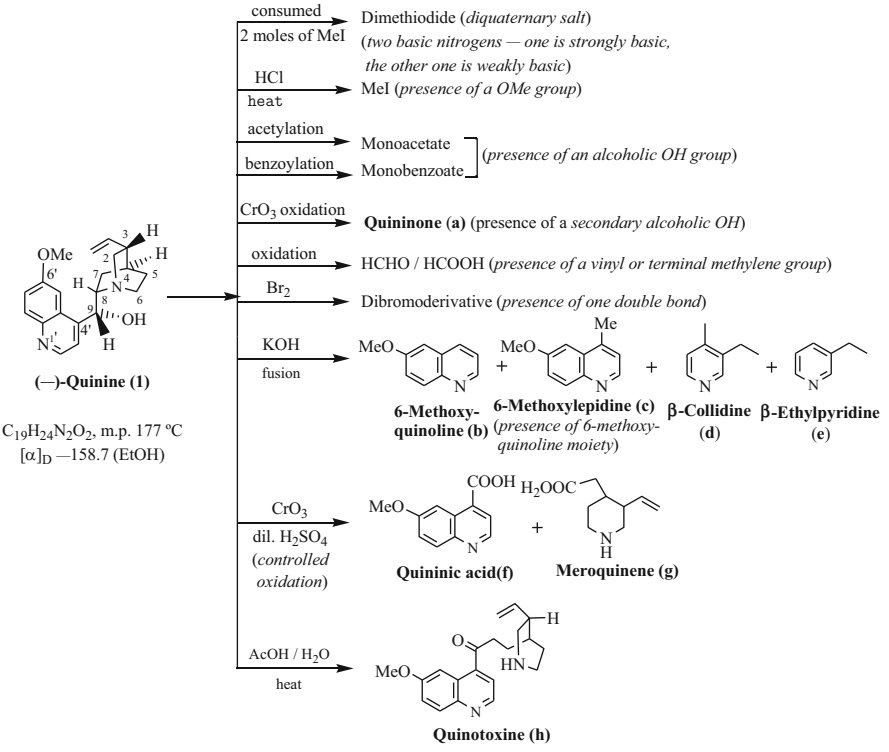


Fig. 25.1 Some reactions and degradation products of quinine [5]

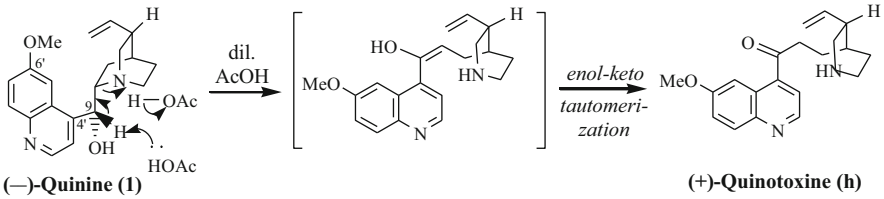


Fig. 25.2 Conversion of (−)-quinine to (+)-quinotoxine [10]

25.2.1 Formation of Quinotoxine [7–9]

Quinine upon heating with a mild acid like dil. acetic acid forms quinotoxine (**h**) (Fig. 25.1). This reaction may be rationalized as shown in Fig. 25.2. The acidity of the H9 is due to its attachment to the 4-position of the pyridine ring, since the resulting carbanion is stabilized by resonance with quinoline N. The acidic H at C9 carrying an OH group, together with the vicinal presence of the quinuclidine N atom forms an ethanolamine system, QCHOH–CH–N<, which accounts for the fragmentation of quinine (**1**) to form quinotoxine (**h**) [7]. The H9 is removed by the base, and the quinuclidine N becomes protonated to form an adequate leaving group. Thus this reaction does not take place smoothly in either strong base or strong acid; rather it takes place only in a weak acid like acetic acid, in which the cooperation of both acid and base is possible. Quinotoxine served as an important landmark in the structure elucidation of *Cinchona* alkaloids. Pasteur observed in 1853 [8, 9] that the reaction of quinine with aqueous sulfuric acid led to the formation of a new product which we now know as quinotoxine. The structures of quinine and quinotoxine were revealed much later, and this conversion can now be rationalized through the mechanism shown in Fig. 25.2. Only difference is that here H9 will be abstracted by H_2O instead of AcOH, and both N atoms will remain protonated by H_2SO_4 in all the compounds.

The companion alkaloid cinchonine was similarly converted to cinchotoxine, which differs from quinotoxine only in the absence of 6′-methoxyl function.

25.2.2 Conversion of Quininone to Ethyl Quininate and Meroquinene

Rabe in 1909 showed that quininone (**a**) upon treatment with amyl nitrite and sodium ethoxide is cleaved into two components, ethyl quininate and the oximino compound (**i**) which was readily hydrolyzed to meroquinene (**g**) (Fig. 25.3).

The ready hydrolytic cleavage of the oximino compound (**i**) to its open-chain precursor needs explanation. It presumably first forms the lactam (−OC–N<), which undergoes facile hydrolysis. Here the electrophilicity of the CO group is

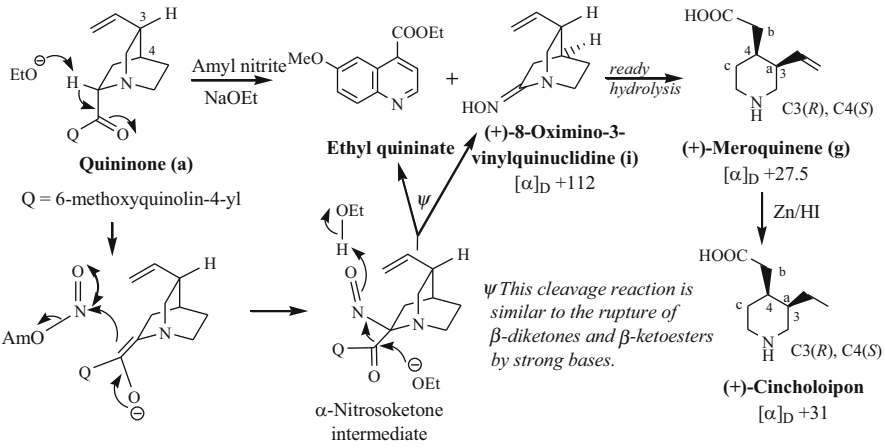


Fig. 25.3 Conversion of quininone to ethyl quininate, meroquinene, and cincholoipon

not reduced as in a normal lactam, since the bicyclic nature of the quinuclidine nucleus precludes any contribution of $^{-}OC(R)=N^{+}<$canonical form, N being at the bridgehead; this would amount to violation of Bredt's rule. Thus this lactam behaves more like a ketone than an amide.

25.2.2.1 Conversion of Quinotoxine to Quinine

Starting with quinotoxine Rabe was able to reconstruct the quinuclidine ring system in two steps, followed by reduction of the resulting quininone to get back quinine [10] (Fig. 25.4). Hypobromous acid treatment of (+)-quinotoxine gives the N-bromo derivative which upon base treatment effects the ring closure to form an equilibrium mixture of (+)-quininone and (+)-quinidinone, epimeric at the C8 asymmetric center. In fact, a freshly prepared solution of quinidinone mutarotates to form its 8-epimer quininone in equilibrium. Either (−)-quinine or (+)-quinidine when oxidized with benzophenone and potassium-t-butoxide in t-BuOH (Oppenauer oxidation) forms first (+)-quininone or (+)-quinidinone, which slowly changes to their equilibrium mixture; the lesser soluble (+)-quinidinone can be isolated from the equilibrium mixture. The ketone mixture when reduced with Al powder, EtOH, and NaOEt gave rise to four diastereomers: quininone produced quinine and epiquinine (C9 epimers), while quinidinone generated quinidine and epiquinidine (C9 epimers) (Fig. 25.4). Thus, a total synthesis of quinotoxine constitutes the formal synthesis of quinine or any of its other three diastereomers [10].

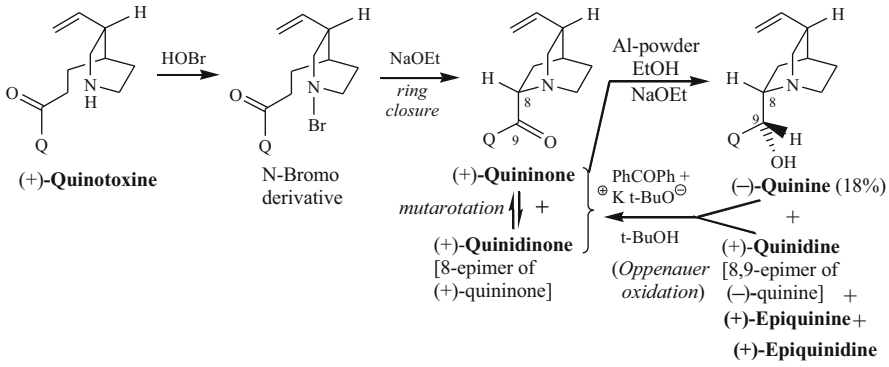


Fig. 25.4 Rabe's partial synthesis of quinine from quinotoxine [6]

25.3 Relative and Absolute Stereochemistry of *Cinchona* Alkaloids [5]

(i) ***cis-Fused bridge at N1 and C4***. Quinine and its congener alkaloids quinidine, cinchonine, and cinchonidine possess a tertiary sp^3 nitrogen each, with three different ligands and a pair of electrons in an sp^3 orbital. Since this nitrogen is a part of a bicyclic system, the bridge must be a result of *cis* fusion; hence the two chiral centers at the *cis* juncture may be treated as one chiral center.

(ii) ***Configurations at C3 and C4***. All the *Cinchona* alkaloids give the same optically identical (dextrorotatory) degradation products, (+)-quinotoxine (from quinine series) (Fig. 25.2), (+)-cinchotoxine (from cinchonine series), (+)-meroquinene, (+)-8-oximino-3-vinylquinuclidine (Fig. 25.3)—without disturbing the stereochemical integrity of the C3 and C4 centers. It is, therefore, concluded that *the absolute configurations of C3 and C4 are identical in all of them*. Thus they differ by the chirality of C8 and C9. One pair of diastereomers, quinine and quinidine possess the aromatic methoxyl group at C6′ position of the quinoline nucleus, while it is absent in the other pair of diastereomers, cinchonine and cinchonidine. Each of the four diastereomers has a 9-epimer, named accordingly—which also occur in *Cinchona* species.

(iii) ***The relative stereochemistry* (*cis*) *at C3 and C4*** was determined by Prelog [5, 11] who chemically converted (+)-cincholoipon, one of the above conversion products (see Fig. 25.3), to an optically inactive *cis*-1,2-diethylcyclohexane, through a series of steps (Fig. 25.5) without touching the chiral centers in any one of them.

(iv) ***Configuration at C8. Epimeric relationship at C8 for each diastereomeric pair***. 9-Deoxycinchonine and 9-deoxycinchonidine with no chirality at C9 are different and have different optical rotations. Since both have identical chirality at C3 and C4, they must thus differ in chirality at C8. It is thus

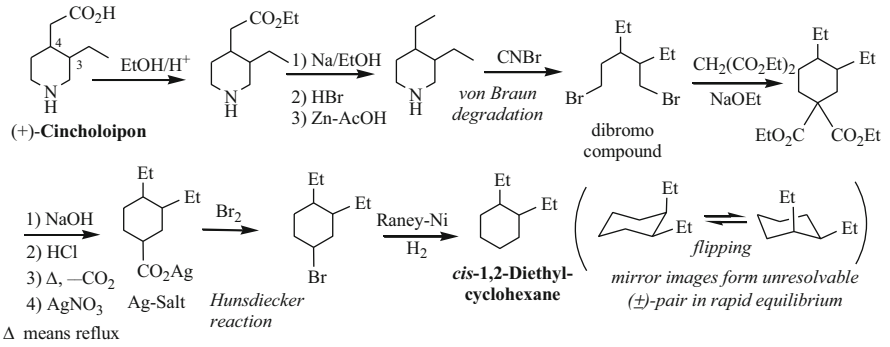


Fig. 25.5 Conversion of (+)-Cincholoipon to *cis*-1,2-diethylcyclohexane

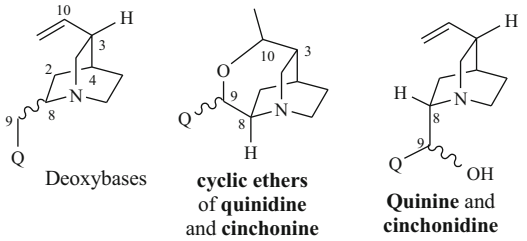


Fig. 25.6 Deoxybases and cyclic ethers of quinidine and cinchonine

concluded that cinchonine and cinchonidine must have epimeric relationship at C8. Similarly 9-deoxyquinine and 9-deoxyquinidine showing different optical rotations must be epimeric at C8. Cinchonine and quinidine are both dextrorotatory and can form cyclic ethers (Fig. 25.6). Hence in these alkaloids C9-OH and the vinyl group, more precisely, C8–C9 and C3–C10 bonds are *cis* oriented. Whereas, cinchonidine and quinine are both laevorotatory and are unable to form any such cyclic ether; hence in these alkaloids C8–C9 and C3–C10 bonds are *trans* oriented.

(v) ***Configuration at C9***. Determination of the C9-configuration of the *Cinchona* alkaloids is more difficult, since it is the only chiral center which is not a part of the rigid quinuclidine system. However, by application of the "*Rule of Optical Superposition*" the direction of rotation of this chiral center may be envisaged. The degradation products of all *Cinchona* alkaloids containing the same two chiral centers C3 and C4 are all dextrorotatory. Thus the total contribution of C3 and C4 in all the alkaloids is dextro rotation. Using this and the $[\alpha]_D$ of all the alkaloids King et al. presumed the contributions of the four chiral centers toward the final direction of rotation in the different alkaloids as shown in the Table 25.1.

Table 25.1 Contribution of the chiral centers toward the final direction of rotation

Alkaloid	$[\alpha]_D$	C3 & C4	C8	C9	Alkaloid	$[\alpha]_D$	C9
Quinine	−158	+	–	–	Epiquinine	+43	+
Quinidine	+254	+	+	+	Epiquinidine	+102	–
Cinchonidine	−111	+	–	–	Epicinchonidine	+63	+
Cinchonine	+224	+	+	+	Epicinchonine	+120	–

Table 25.2 pK_a values of ephedrine and quinine and related compounds [11]

	pK_a	pK_a difference		pK_a	pK_a difference
(−)-Ephedrine (*erythro*)	9.14		Quinine (*erythro*)	7.73	
(+)-ψ-Ephedrine (*threo*)	9.22	0.08	Epiquinine (9-epi) (*threo*)	8.44	0.71
(−)-*N*-Methylephedrine (*erythro*)	8.50		Quinidine (*erythro*)	7.95	
(+)-*N*-Methyl-ψ-ephedrine (*threo*)	8.81	0.31	Epiquinidine (9-epi) (*threo*)	8.32	0.37

(vi) ***Relative Configurations at C8 and C9***. Prelog and Häflinger [11] compared the pK_a values measured in 80 % cellosolve [12] of the *Cinchona* alkaloids with those of ephedrine, ψ-ephedrine, and their N-methyl derivatives (Fig. 22.7). The pK_a values obtained are listed in Table 25.2 (cf. Table 20.1).

The weaker bases, ephedrine and *N*-methylephedrine are the *erythro* isomers, whereas the stronger bases, ψ-ephedrine and *N*-methyl-ψ-ephedrine are the *threo* isomers. By analogy, quinine and quinidine, which are weaker than their epimers, are assigned *erythro* configuration and the 9-epimeric bases the *threo* configuration. This leads to the relative (not absolute) configurations of the *Cinchona* bases as shown in Fig. 25.7. It may be explained in the way that the conjugate acid of the *erythro* bases cannot be stabilized due to steric repulsion of the phenyl and methyl groups in case of ephedrine and of the Q (6′-methoxy-4′-quinolyl gr) and quinuclidine system in case of quinine and quinidine, whereas there is no such repulsive interactions in the conjugate acids of the *threo* bases ψ-ephedrine and epiquinine or epiquinidine. This also explains the greater pK_a differences between *threo* and *erythro* isomers in case of the *Cinchona* alkaloids (Table 25.1). The H-bonded system –HO˙˙H–NHMe– is also stabilized by the electrostatic attraction between unlike charges on the nitrogen and oxygen (partial charge) (see also Sect. 20.5).

(vii) ***The Relative Configurations at C8 and C9 from the NMR Spectral Study of the Derived Oxiranes*** [13]. The configurations at C9 of the natural *Cinchona* alkaloids, quinine, quinidine, cinchonine and cinchonidine, and their C9 epimers have been resolved [13] in the following way. They were converted to their C10–C11 dihydro-N1-benzyl derivatives by catalytic hydrogenation (Pt/H_2) followed by heating under reflux (6–8 h) with 1 equiv. benzyl chloride in EtOH. The appropriate benzyl chloride salt was stereospecifically

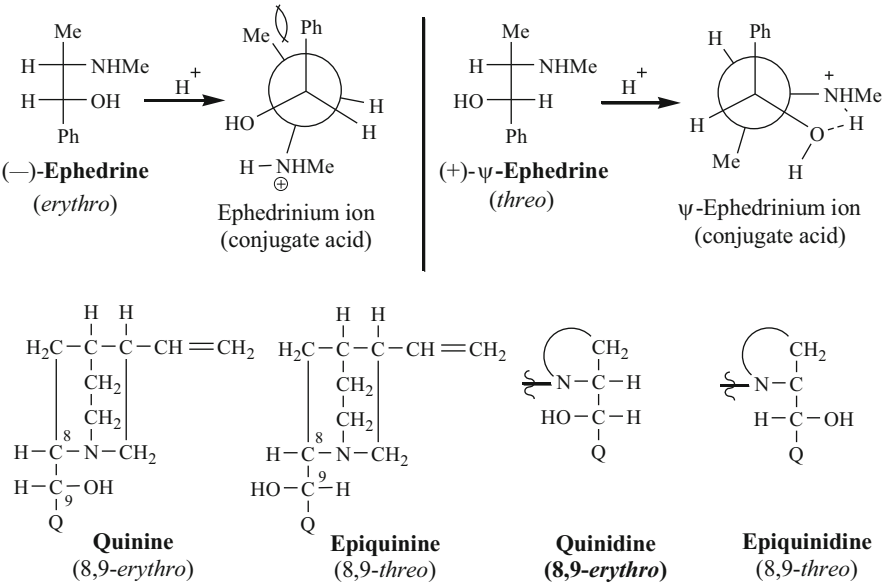


Fig. 25.7 Fischer projection formulae of (−)-ephedrine and (+)-ψ-ephedrine and quinine, epiquinine, quinidine, and epiquinidine (relative configurations) [11]

converted to the respective conformationally rigid oxirane by boiling with freshly prepared K-t-OBu (three to sixfold excess by wt.) in anhydrous *t*-BuOH. The product was purified by repeated chromatography.

The configurations of the oxiranes (Fig. 25.8) were determined by ^{1}H NMR spectroscopy. The oxirane derivative is formed in a stereospecific manner involving retention of configuration at C9 and inversion at C8, from which the ammonium function is expelled (Fig. 25.8). It is unequivocally established that the natural *Cinchona* alkaloids are entirely of the *erythro* configuration at C8 and C9 and that the epi-bases are *threo*. The same conclusion was derived in (vi) of this section.

(viii) ***Absolute Configuration*** [5]. In determining the absolute configuration of C3, Prelog converted the dibromo compound (Fig. 25.5) into (−)-3-methyl-4-ethylhexane; the latter was synthesized from (*R*)-(−)-ethylmethylacetic acid of known absolute configuration (Fig. 25.9) (correlated with D-glyceraldehyde).

Therefore, the absolute configuration of C3 was determined as *R*, and all the chiral centers of the *Cinchona* alkaloids with respect to C3 can be expressed, as shown in Fig. 25.10. All the four epicompounds have the same absolute configuration at C3, C4, and C8 as the corresponding parent compound and the opposite absolute configuration at C9.

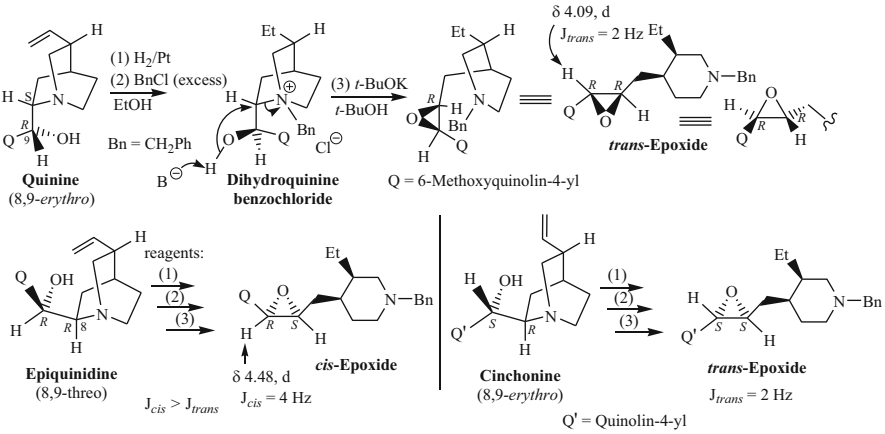
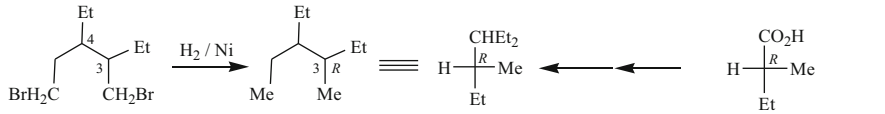
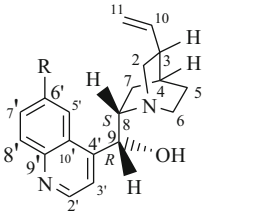


Fig. 25.8 The configurations at C8 and C9 of *Cinchona* alkaloids from the ^{1}H NMR signals of the prepared corresponding oxiranes

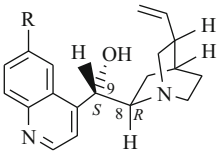


(from **Figure 25.5**)*
(**numbering corresponds to cincholoipon*)

Fig. 25.9 Absolute configuration of C3 of *Cinchona* alkaloids [11, 12] by chemical correlation



R=OMe, (—)-**Quinine**
R=H, (—)-**Cinchonidine**



R=H, (+)-**Cinchonine**
R=OMe, (+)-**Quinidine**

Absolute configuration

	C3	C4	C8	C9
(—)-**Quinine**	*R*	*S*	*S*	*R*
(—)-**Cinchonidine**	*R*	*S*	*S*	*R*
(+)-**Cinchonine**	*R*	*S*	*R*	*S*
(+)-**Quinidine**	*R*	*S*	*R*	*S*

Note: *Epibases are epimeric at C9 of the corresponding alkaloids.*

Fig. 25.10 Absolute configuration of the *Cinchona* alkaloids

25.4 Synthesis

The profound beneficial antimalarial properties and structural beauty of quinine with four stereochemical centers interested a number of outstanding synthetic organic chemists to achieve its total synthesis [14–16].

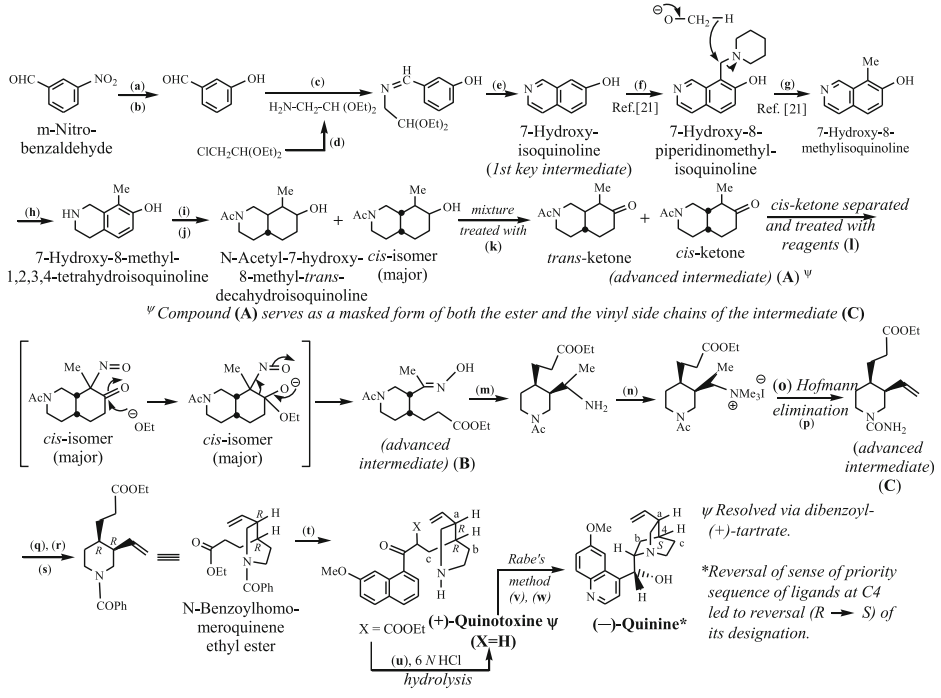
Woodward and Doering [17–19] reported the first total synthesis of quinine. They selected (+)-quinotoxine as the target intermediate, obtained earlier by Pasteur [8] (1853) and also later by Rabe [6] on treatment of quinine with dilute acid (Fig. 25.2). Rabe subsequently converted (1918) quinotoxine to quinine (Fig. 25.4) [10].

25.4.1 Woodward–Doering Synthesis of Quinine [17–19]

Apart from quinotoxine Woodward also considered other degradation products of quinine, e.g., meroquinene as a guide to select an intermediate that could serve as the precursor for quinuclidine moiety of the molecule and ethyl quininate for isoquinoline part in his synthetic planning for quinine. During non-asymmetric synthesis each time a chiral center is generated, it is accompanied by its enantiomeric molecule. However, as per convention, the structures with right configurations matching the natural (−)-quinine have been drawn as delineated in Fig. 25.11. The (+)-enantiomer separated from synthetic (±)-quinotoxine, via the dibenzoyl tartrate, has been subjected to Rabe's sequence of three reactions [10] to convert it into (−)-quinine.

Regarding the validity of the last three steps in this synthesis (Rabe's conversion of quinotoxine to quinine, hence Woodward's work on this part) *some controversial statements* appeared in print [20], since Rabe did not furnish adequate experimental details of this conversion in his 1918 paper [10]. Further in his later papers he reported such reactions with data for other *Cinchona* alkaloids, but did not mention of quinine any more. Jeffrey I. Seeman has researched on this issue extensively and went through a number of published and unpublished works on this conversion. He published his findings in 2007 [20] in detail, in which he wrote on the basis of his findings, "I conclude that Rabe and Kindler did convert (+)-quinotoxine into (−)-quinine in 1918. There has never been any question that Woodward and Doering completed a total synthesis of (±)-homomeroquinene and (+)-quinotoxine in 1944. I, therefore, conclude that the Woodward-Doering/Rabe-Kindler claim of the total synthesis of quinine is valid. The overriding goal of this historical review is to set the record straight" [20].

Subsequently in 2008, Aaron C. Smith and Robert M. Williams [22] corroborated Jeffrey's findings. They searched for the nature of aluminum powder of Rabe and found that aluminium powder when contaminated with some Al^{III} compounds gave much amount of isolable quinine from quinotoxine while the fresh one furnished only a trace amount. This observation provides experimental affirmation of Woodward–Doering's formal total synthesis of quinine. Smith and William [22]



Reaction Conditions:

(a) Pure $SnCl_2$, conc. HCl, 0 °C → 100 °C, quenched by ice; (**b**) $NaNO_2$, conc. HCl, 4-5°, boiling H_2O; (**c**) steam bath, 90 min; (**d**) cooled, MeOH, dry NH_3; (**e**) H_2SO_4, 0 -3 °C, 12 h, RT 36 h; (**f**) piperidine, 95% EtOH, 35% aq HCHO, Δ, 6 h; (**g**) NaOMe, 220 °C (hydride transfer from methoxide); (**h**) H_2, AcOH, PtO_2, Py; (**i**) Ac_2O, MeOH; (**j**) H_2, Raney Ni, EtOH, 3000 lb, psi, 160°C; (**k**) glacial AcOH, CrO_3 in dil AcOH, 2-3 h (ice-bath) → RT → 50 °C; (**l**) NaOEt, EtOH, EtONO; (**m**) H_2, PtO_2, AcOH; (**n**) MeI (excess), EtOH, K_2CO_3; (**o**) 60% NaOH, 140 °C, (**p**) KCNO (neutral aq. solution); (**q**) dil HCl; (**r**) HCl, EtOH; (**s**) PhCOCl, K_2CO_3; (**t**) ethyl quininate (excess), dry NaOEt; (**u**) 6 *N* HCl; (**v**) NaBr, NaOH (*quininone formation*); (**w**) Al power, NaOEt, EtOH

Fig. 25.11 Woodward and Doering's formal total synthesis of quinine [17–19] (1944, 1945)

inferred from their studies "that the Rabe-Kindler aluminium-powder reduction may be reviewed as an early 'activated' progenitor of the Meerwein-Ponndorf Verley (MPV) reduction." Woodward also used MPV reagent for the reduction of quininone to quinine in 30 % yield [19]. The entire finding serves as a cautionary tale of not commenting on any issue without proper verification of its validity.

25.4.2 *The First Stereoselective Total Synthesis of Quinine by Stork [23]*

Stork reported [23] the first chiral total synthesis of (−)-quinine in 2001. (−)-Deoxyquinine has been selected as the immediate intermediate to which Hoffmann–La Roche oxygenation protocol [24, 25] has been applied to yield (−)-quinine. Stork's retrosynthetic analysis for deoxyquinine and the chiral synthesis of the azoaldehyde (**B**) are delineated in Figs. 25.12 and 25.13, respectively.

Stork's stereoselective total synthesis of quinine (1) from 6-methoxy-4-methylquinoline (**A**) and the azoaldehyde (**B**) has been delineated in Fig. **25.14**, wherein the reaction conditions of all steps and rationales of some intriguing steps have also been indicated.

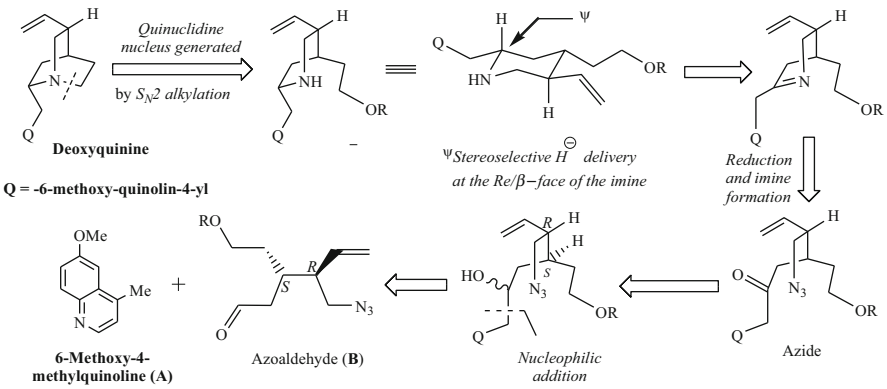
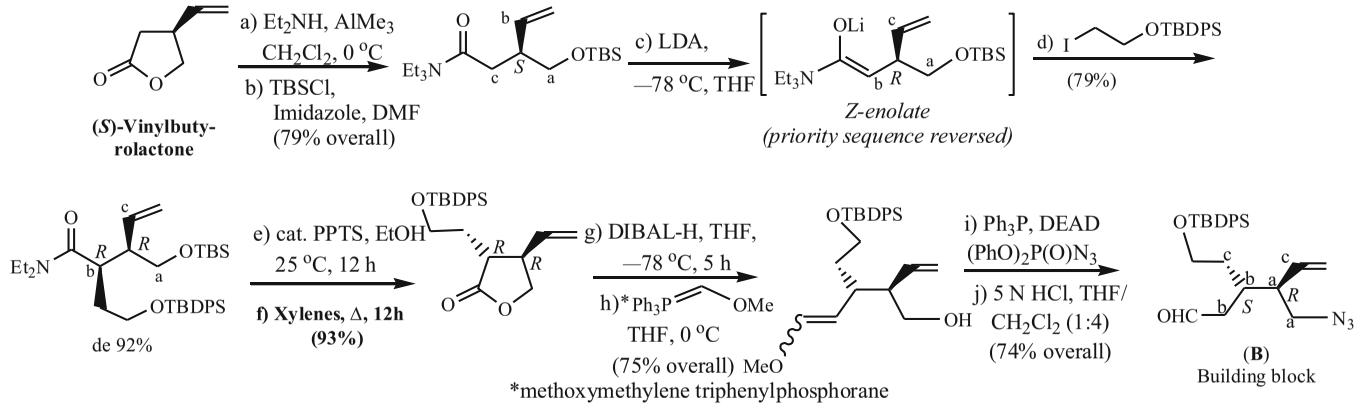


Fig. 25.12 Stork's retrosynthetic analysis of deoxyquinine



Changes/reactions occurring

a) *opening of the ring and amide formation*
b) *silylation of the CH_2OH*
c) *Z-enolate formation*
d) *electrophilic approach from the less bulky vinyl side (β -)*
e) *selective lysis of the TBS gr*
f) *lactonization*
g) *controlled reduction to the lactol*
h) *Wittig one-C homologation of the open chain ol-al equil. form*
i) *azide formation (Mitsunobu-type protocol)*
j) *ready hydrolysis to aldehyde*

TBS	*t*-Butyldimethylsilyl
TBDPS	*t*-Butyldiphenylsilyl
PPTS	Pyridinium *p*-toluene sulphonate
DEAD	Diethylazodicarboxylate

Fig. 25.13 Stork's chiral synthesis of the building block (**B**) [23]

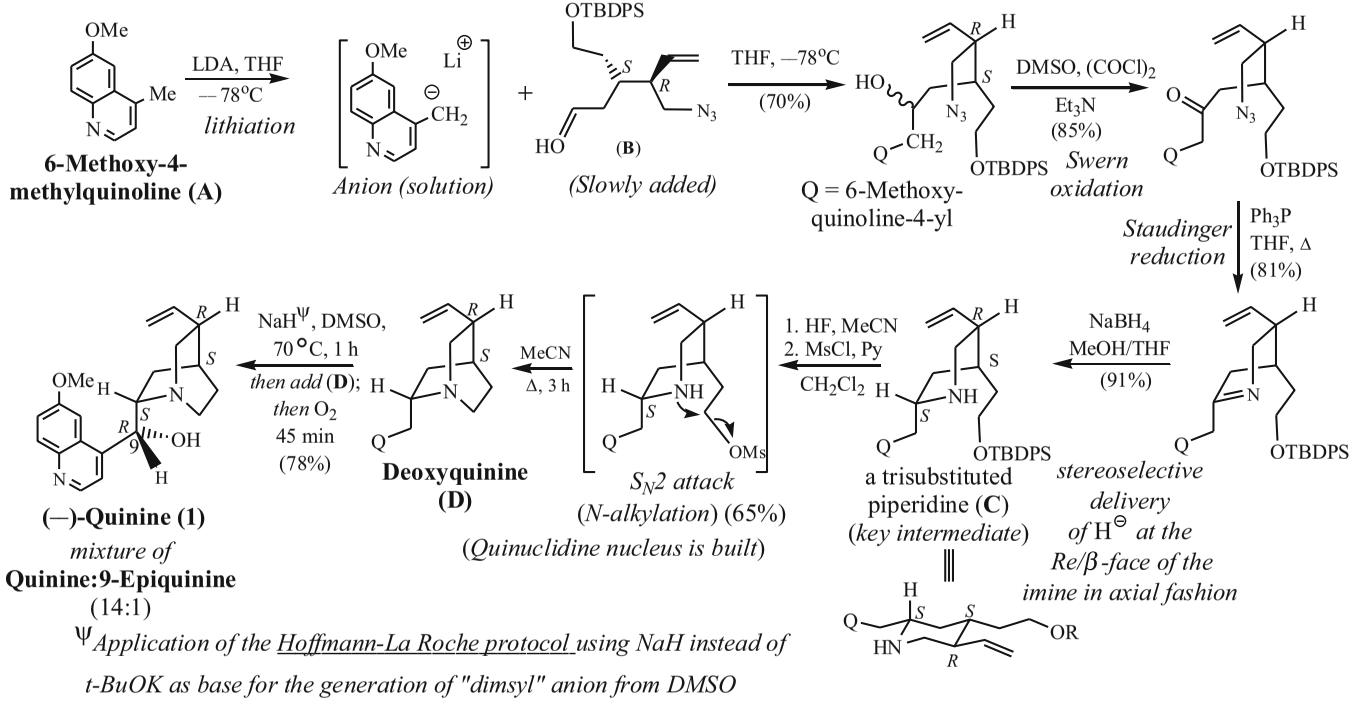


Fig. 25.14 Stork's synthesis of (−)-quinine (1) from the building blocks (A) and (B) via the key intermediates (C) and (D) (deoxyquinine) [23]

25.4.3 Other Syntheses of Quinine, Quinidine

The objectives of the synthesis of quinine and other *Cinchona* alkaloids were manifold: (a) to make sufficient quantities of quinine for pharmacological applications, (b) to use them as a platform for developing unique synthetic strategies especially for stereoselective synthesis, and (c) to view the quinoline and quinuclidine systems as excellent probing bases for new methodology.

Quite a number of stalwart synthetic organic chemists have achieved the total synthesis of quinine and quinidine, *e.g.*, by Milan Uskokovic et al. [24, 25] in 1970; Marshall Gates et al. [26] in 1970; catalytic asymmetric total synthesis by Jacobsen et al. [27] in 2004; and a concise stereocontrolled formal synthesis of (±)-quinine and (±)-7-hydroxyquinine by Peter Webber et al. [28] in 2008.

25.5 ^{13}C NMR [29] and Mass Spectrometry [30] of Cinchona Alkaloids

Chemical shifts of all the carbons as observed in the ^{13}C NMR spectra of quinine and quinidine (see Fig. 25.10 for numbering of carbons) [29] are recorded in Table 25.3 for comparison. It is observed that ^{13}C chemical shifts of C2 and C6 can be used to distinguish between quinine and quinidine derivatives. It has also been observed that δ C4′ provides a means of distinguishing between *erythro* and *threo* compounds (quinine and epiquinine, or quinidine and epiquinidine derivatives, respectively, see Fig. 25.7). Thus δ C4′ values in quinine and epiquinine are 148.3 and 144.26 ppm, respectively, and in quinidine and epiquinidine are 148.2 and 144.6 ppm, respectively.

Characteristic Features of the Mass Spectra [30] Quasimolecular ion $(M+1)^+$ is invariably more abundant relatively in the chemical ionization (CI) mode (using methane as the reactant gas) than is the molecular ion in the EI mode. The complimentary nature of CI (using methane as the reactant gas) and EI mass spectra is very clear in case of quinine. In case of the EI mass spectrum the fission of the C8–C9 bond forms the base peak at *m/z* 136 (the quinuclidine moiety, $C_9H_{14}N$), whereas the molecular ion peak at *m/z* 324 is only 0.5 % (relative intensity). In the CI mass spectrum of quinine the base peak is the protonated $(M+1)^+$ ion, while the *m/z* 136 peak is only 30 %.

Table 25.3 ^{13}C NMR in $CDCl_3$ of quinine and quinidine (δ-values are approx. at first decimal place)

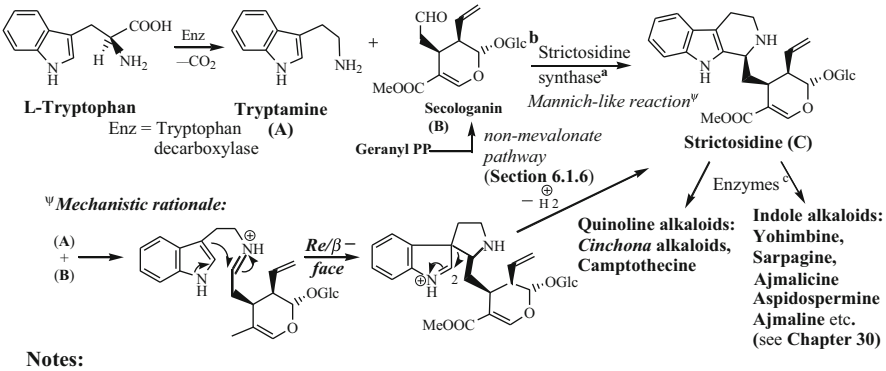
	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
Quinine	**56.9**	39.8	27.7	27.5	**43.0**	21.4	59.8	71.5	141.7	114.1
Quinidine	**49.8**	39.9	28.1	26.2	**49.4**	20.8	59.8	71.5	140.5	114.2
	C2′	C3′	C4′	C5′	C6′	C7′	C8′	C9′	C10′	OCH_3
Quinine	147.0	121.1	**148.3**	101.4	157.4	118.3	130.9	126.4	143.7	55.4
Quinidine	147.6	121.1	**148.2**	101.2	157.3	118.2	130.9	126.3	143.6	55.3

25.6 Biosynthesis

Quinine and other congener alkaloids ***quinidine***, ***cinchonine***, and ***cinchonidine*** are all derived from an intermediate alkaloid ***strictosidine*** [31], formed out of *tryptophan* and *secologanin* (Sect. 6.1.6). Strictosidine was first isolated from *Rhazya stricta* (Fam. Apocynaceae) by George Smith at Manchester in 1968 as an amorphous substance (yield 0.01 %). Its involvement as an intermediate in the biosynthetic pathway of monoterpene indole alkaloids has been demonstrated by biosynthetic experiments. It is an amazingly versatile biosynthetic key intermediate for a large number of monoterpene indole alkaloids with dramatically diverse skeletal patterns. The genesis of so many complex patterns of alkaloids through the rearrangement of strictosidine serves as an awesome example of Nature's synthetic strategy for complex secondary metabolites.

The biosynthesis of strictosidine is outlined in Fig. 25.15. The occurrence of some indole alkaloids, ***cinchonamine***, ***quinamine***, ***cinchophylline***, etc., in some *Cinchona* (*C. calisaya*) and *Remijia* (*R. purdicana*) species supports the involvement of this monoterpene indole alkaloid in the biosynthesis of *Cinchona* alkaloids having quinoline heterocycle moiety in their molecules.

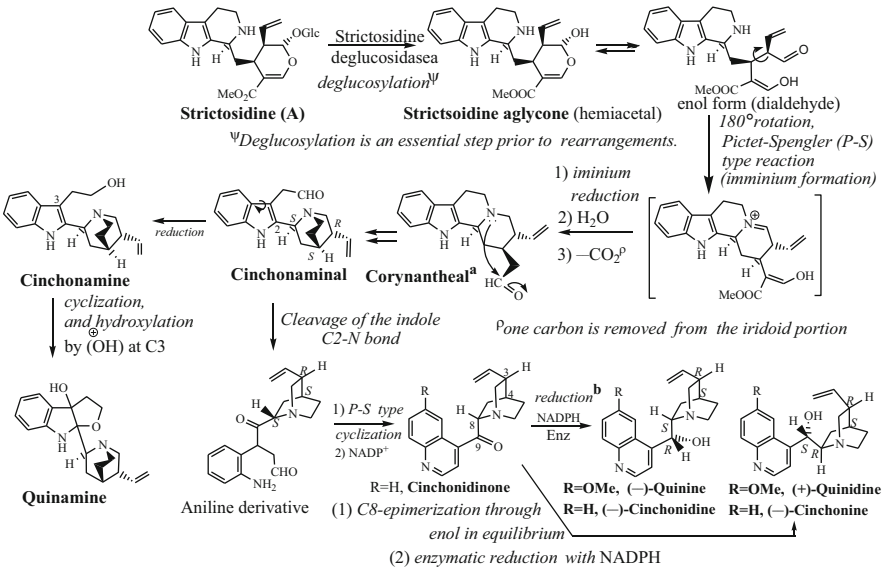
In the proposed biosynthesis of quinine and its congeners [31] (Fig. 25.16), the obligatory precursor, *strictosidine* undergoes deglucosylation by the enzyme strictosidine β-D-glucosidase, deblocking the reactive hemiacetal. The hemiacetal opens up to the enol form of a dialdehyde. The latter forms a harman compound *corynantheal* by Pictet–Spengler type cyclization between the aldehyde and the secondary amine, followed by reduction, hydrolysis, and decarboxylation. Label experiments with corynantheal and its incorporation to quinine support decarbomethoxylation at an earlier stage. Opening of the indole ring, generation of an aniline derivative, and another Pictet–Spengler type cyclization yield the alkaloidal ketones, cinchonidinone and quinidinone. Isomerization of C8–H through enol at equilibrium and stereospecific reduction yield quinine, quinidine, cinchonine, and cinchonidine. Strictosidine synthase tolerates a variety of substituents in the aromatic nucleus. This may account for the methoxyl group of quinine and quinidine.



Notes:

[a] *This enzyme can tolerate a variety of substitution on the benzene ring of tryptophan.*
[b] *Glucose serves as the protecting group of the reactive species.*
[c] *With different sets of enzymes rearrangement to different skeletons occur.*

Fig. 25.15 Biosynthesis of strictosidine (**C**), the key precursor of terpene indole and terpene quinoline alkaloids



Note: [a] *Hydroxylation and methylation takes place at some stage after formation of corynantheal*
[b] *regio- and stereospecific reduction.*

Fig. 25.16 Proposed biosynthesis of *Cinchona* alkaloids and some indole alkaloids

However, since strictosidine and corynantheal are incorporated in quinine and quinidine, hydroxylation and methylation must have taken place at a later stage. The aromatic ring hydroxylation and subsequent methylation of the hydroxyl group are common phenomena in various biosynthesis pathways.

It is interesting to note that the indole alkaloids *corynantheal* and *cinchonaminal* are formed as the precursors in the biogenetic pathway to *Cinchona* alkaloids. *Cinchonaminal* upon reduction gives the indole alkaloid *cinchonamine* which upon cyclization and electrophilic attack by $^{+}$OH gives rise to another indole alkaloid *quinamine*.

25.7 Uses

See Chaps. 32 and 33.

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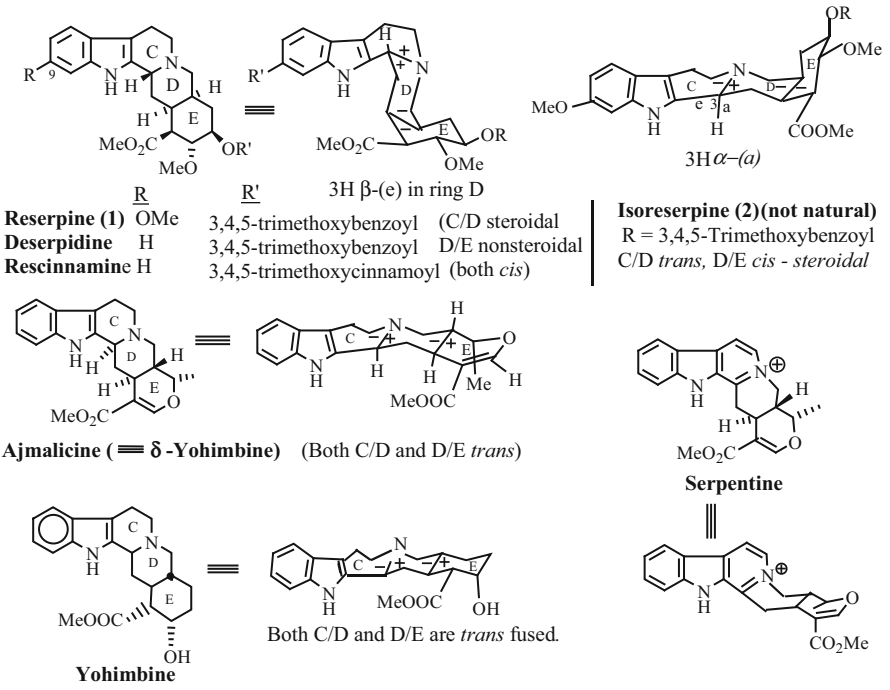
Chapter 26
Reserpine

26.1 Introduction. Occurrence

Various *Rauwolfia* (*Rauvolfia*) species (Apocynaceae) have been used for centuries as folk medicine in the treatment of hypertension, mental disorder, and other ailments. *Rauwolfia serpentina* ("Snake root," "sarpagandha"), a small shrub, grows in India, Pakistan, Burma, and Thailand. The decoction prepared from its rhizomes and leaves has been used as an antihypertensive agent and as a tranquilizer. *Rauwolfia serpentina* is rich in indole alkaloids. Stereostructures and conformations of some representative indole alkaloids of *R. serpentina* are shown in Fig. 26.1. The presence of the sedative principle of this plant in its "oleoresin fraction" was first reported in 1947 [1]. Reserpine, an active principle, first isolated in pure and crystalline state in 1952 from *Rauwolfia serpentina* [1], has been shown to have antihypertensive and sedative properties and to have the gross structure shown in Fig. 26.1 [2–4]. Since its isolation reserpine remained an important alkaloid for many years because of its usefulness in the treatment of hypertension and metal disorders. However, its prolonged use causes severe side effects, and it is now hardly prescribed as a drug (Chap. 33). Reserpine has also been isolated from the roots of *Rauwolfia densiflora* Benth (0.025 %) along with the major alkaloid ajmaline (0.25 %) [5] in early 1955. Its occurrence has been reported during 1955–1959 from nearly forty more *Rauwolfia* species [5–12] and also from other Apocynaceae plants, *e.g.*, *Vinca rosea*, *Alstonia constricta*, *Tonduzia longifolia*, *and Vallesia dichotoma* [6].

S.K. Talapatra and B. Talapatra, *Chemistry of Plant Natural Products*,
DOI 10.1007/978-3-642-45410-3_26,



Notes:

1) *– and + signs signify torsion angle signs at the bonds in the rings* (*see* Sections **2.3.1 and 2.12.1.1**).
2) *– /+ Indicatestrans fusion* **(2.15.1.2),** *+/+ cis steroidand – / – cis nonsteroid conformations* **(2.15.1.3).**
3) *The conformations of the C/D/E rings of the molecules shown (with correct stereochemistry) have also been verified by Dreiding models*

Fig. 26.1 Stereostructures and conformations of some pentacyclic indole alkaloids of *Rauwolfia serpentina*

26.2 Gross Structure [1–4, 7, 8]

Reserpine, $C_{33}H_{40}O_9N_2$, m.p., 286–288 ^{0}C $[\alpha]_D^{24}$-118.9 ($CHCl_3$) [1] on hydrolysis furnished reserpic acid (isolated as its methyl ester), trimethoxybenzoic acid (trimethylgallic acid), and methanol. Reserpine could be regenerated from methyl reserpate and trimethoxylbenzoyl chloride in presence of pyridine suggesting that reserpine is an ester of reserpic acid having a hydroxyl group with trimethoxybenzoic acid and that during hydrolysis reserpic acid part does not undergo structural change [2]. Hence the structure determination (Fig. 26.2) of reserpic acid established the structure of reserpine [1–4]. Degradations of reserpine and reserpic acid are delineated in Fig. 26.2.

Hence the reactions in Fig. 26.2 suggest that reserpic acid must have the yohimbine skeleton (orientation of the pentacyclic system), but does not give any stereochemical information.

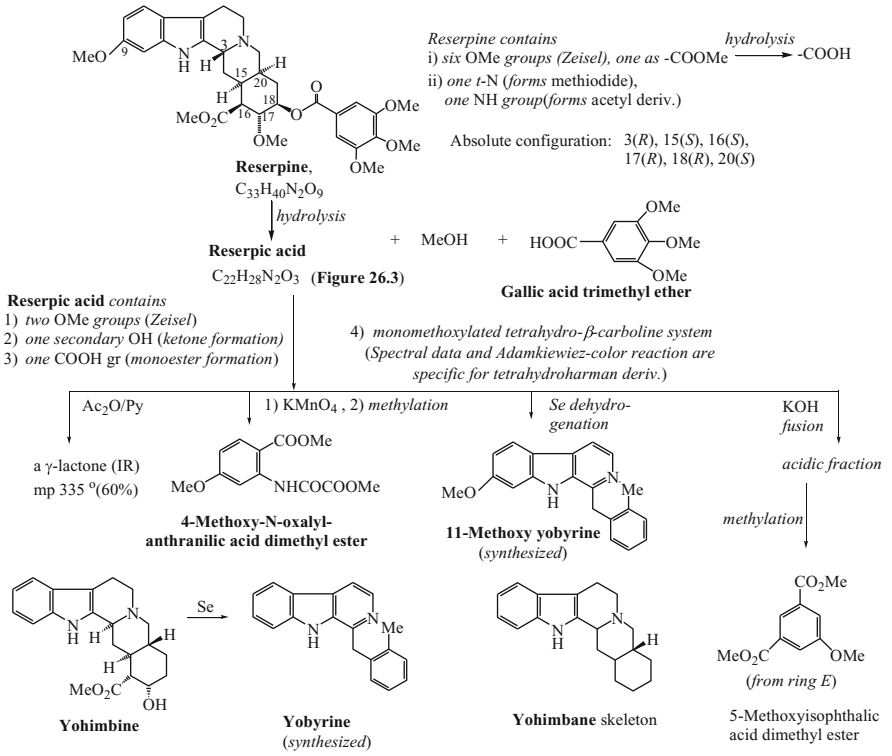


Fig. 26.2 Degradative reactions of reserpine and reserpic acid leading to their structures (connectivities of the constituent atoms)

26.3 Stereochemistry [9–11]. Conformation

Reserpine reveals the presence of the following stereochemical features in its molecule.

(i) Six chiral centers at C3, C15, C16, C17, C18, and C20. Except at C3, all the five contiguous chiral centers are present in the core of a cyclohexane ring (ring E).

(ii) ***Stereochemistry of C3***: C3–H is β and equatorial to ring D; axial attachment of the indole to piperidine ring D renders the stereochemical arrangement less stable at C3 in reserpine, compared to the thermodynamically more stable C3 epimer, isoreserpine (see the conformation in Fig. 26.1), and the compounds of this series.

(iii) Rings C/D and D/E are *cis* fused. Its conformation maintaining the correct absolute configuration of the ring junctions shows that C/D and D/E possess steroidal and nonsteroidal conformations with torsion angles at the ring junctions +/+ and −/−, respectively (Fig. 26.2) (cf. Chap. 2).

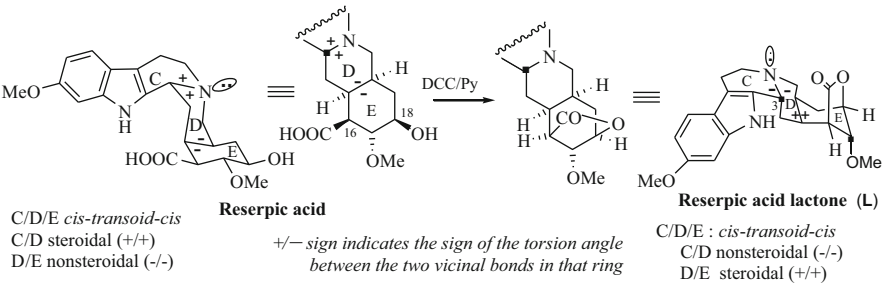


Fig. 26.3 Formation of reserpic acid lactone from reserpic acid

(iv) C18 bearing OH in reserpic acid was shown to have (*R*) configuration by use of Prelog's rule (Chap. 2).

(v) Reserpic acid (Fig. 26.2) upon treatment with DCC forms a γ-lactone, named reserpic acid lactone (**L**); the C/D/E (*cis-transoid-cis*) undergoes ring inversion to change the 16-COOH (e) and 18-OH (e) to axial orientations prior to γ-lactone formation (Fig. 26.3). Here, the inversion of the torsion angle signs at the C/D and D/E ring junctions, as a result of flipping of both cis fused systems, is noteworthy. Thus the *cis*-orientation of 16-COOH and 18-OH (both β- and equatorial) in reserpic acid has been established. Since C18 bears (*R*)-configuration (OH is β-), COOH group at C16 must be β-, and hence C16 will have (*S*)-configuration. The relative configurations of the chiral centers have been established as shown in (**1**). Thus the absolute configurations of the chiral centers are as follows: 3(*R*), 15(*S*), 16(*S*), 17(*R*), 18(*R*), and 20 (*S*). In isoreserpine, the absolute configuration of C3 changes to (*S*), and the indole moiety is equatorially attached to the piperidine ring D (Fig. 26.1).

(vi) The facts that C18–OH and C17–OMe are anti to each other, and that C/D and D/E rings are *cis* fused are evident from the formation of the products (**B**) and (**C**) (Fig. 26.4) upon refluxing of methyl reserpate-C18-tosylate (**A**) with collidine. The formations of (**B**) and (**C**) are rationalized in terms of the conformation of (**A**) and the intermediates in Fig. 26.4.

26.4 Synthesis of Reserpine

Reserpine is the most complex and prominent member of the yohimbine group of indole alkaloids. It contains five contiguous stereogenic centers embedded in a *cis*-fused perhydroisoquinoline ring system (D/E). Synthesis of (±)-reserpine by Woodward in 1956 [13–16] still remains a masterpiece in organic synthesis. The major problem in reserpine synthesis lies in the regiochemstry and stereochemistry of its chiral centers and their correct incorporation during its synthesis. As already

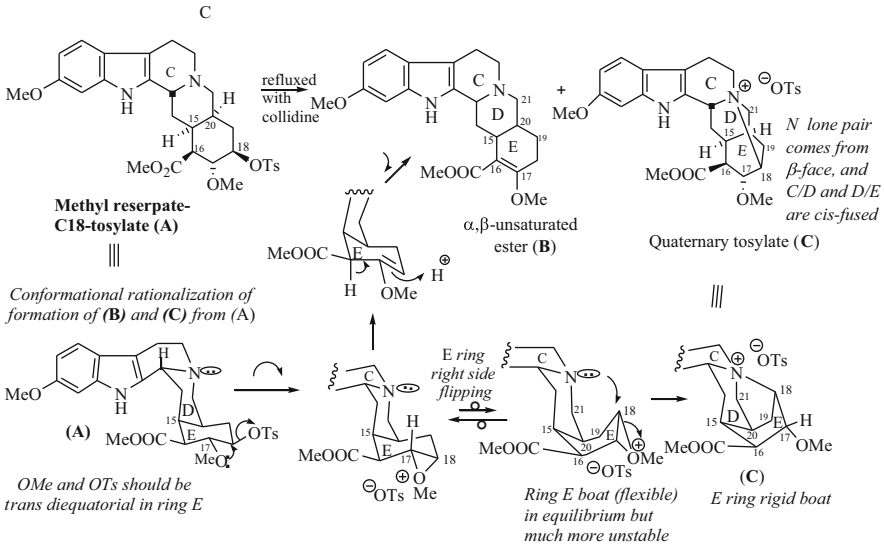


Fig. 26.4 Conformational rationalization of the formation of (**B**) and (**C**) from (**A**)

stated C3 in reserpine carries *an axial attachment of the indole moiety to the piperidine ring* (D), *and thus it is less stable compared to its C3-epimer* (*iso*-series) (conformation shown in Fig. 26.1). Woodward adopted a novel strategy for the conversion of the more stable isoreserpine series into the less stable reserpine series.

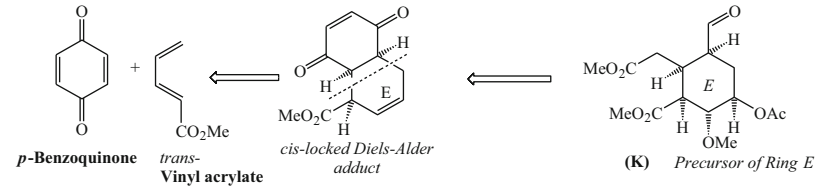
26.4.1 Woodward's Total Synthesis of Reserpine

Woodward's brilliant synthetic strategy for the first total synthesis [13–16] of (±)-reserpine in 1956 *within a year of its full characterization* [5–12] is shown in the retrosynthetic format in Fig. 26.5 in two parts, (**a**) and (**b**). The following portrayal of the total synthesis of (±)-reserpine [13–15] is mainly based on its description by Woodward [15] and Nicolaou [16].

Woodward's Synthesis of (±)-Reserpine [13–16] (Figs. 26.6–26.13) Diels–Alder reaction is stereospecific and the *endo* adduct is formed via the *endo* TS when the electron withdrawing group is in *cis* relationship in the dienophile (*p*-benzoquinone) and the substituents of the diene are *trans* (vinyl acrylic ester) (Fig. 26.6).

Both the carbonyls of (**A**) (Fig. 26.6) are reduced by the attack of hydride from the same less hindered convex face as the bridge head hydrogens are located (Fig. 26.7). It was known that the unhindered CO at C5 on reduction would give

a *Retrosynthetic analysis of the key intermediate* **(K)** - a highly functionalized cyclohexane derivative serving as the precursor of ring E of reserpine – containing five chiral centers with right relative stereochemistry.



b Participation of **(K)** in the synthesis of reserpine

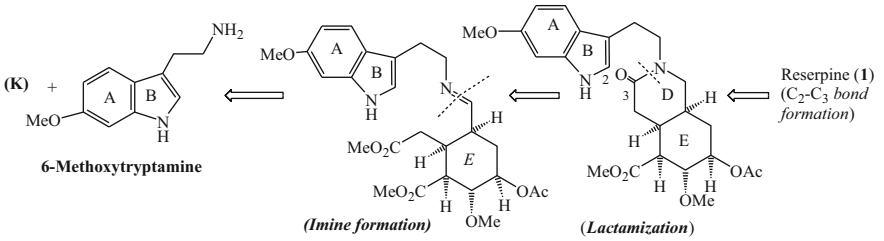
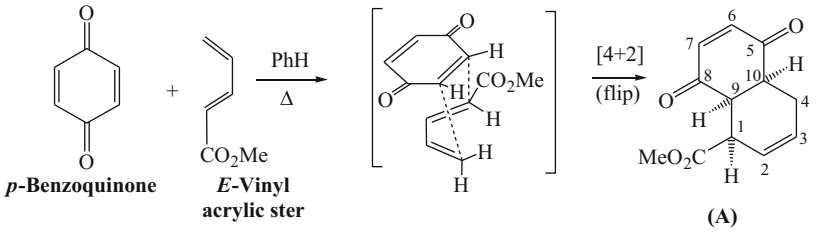


Fig. 26.5 Retrosynthetic analysis of (±)-reserpine



a cis-locked decalone system containing three out of 5 chiral centers; two more required could be generated out of $\Delta^{2,3}$.

Fig. 26.6 Formation of the *cis*-locked Diels–Alder adduct

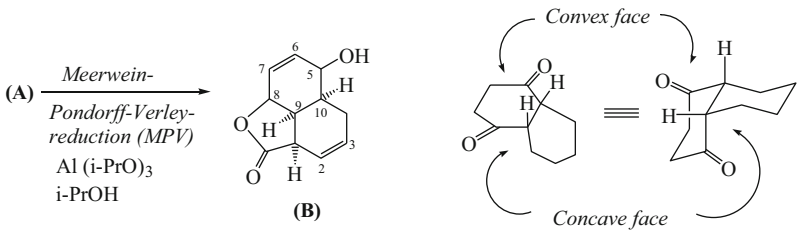


Fig. 26.7 Formation of a lactone

an equatorial alcohol, while the hindered CO at C8 would give an axial alcohol as the major product (see conformation of the cis-decalin system in Fig. 26.7). Thus the folded book-like conformation of cis-decalin dictates the course of the MPV reduction of the carbonyl groups. Consequently, a lactone (**B**) was formed through suitably disposed C8–OH (a) and C1–COOMe (e) groups (Fig. 26.7).

Related models' study showed that $\Delta^{6,7}$ is markedly resistant to bromination at room temperature (rt). On treatment with bromine, compound (**B**) gives a bromoether derivative (Fig. 26.8), the formation of which can be explained by intramolecular nucleophile attack of C5–OH on the bromonium ion formed (between C2 and C3) from the less unhindered convex face prior to the attack by external bromide nucleophile. Sodium methoxide treatment generated a *cis* fused lactone (**C**) with an α-methoxy group. The methoxylactone may be formed by the Michael addition of $MeO^{\ominus}$ on the briefly generated α,β-unsaturated lactone (Fig. 26.8).

The C6–C7 double bond of (**C**), though resistant to bromination with bromine at rt, underwent *trans* diaxial bromohydrin formation (with aqueous NBS in presence of H_2SO_4 at 80 °C), which is then oxidized to a bromoketone (Fig. 26.9). The latter on treatment with metallic Zn in glacial acetic acid causes (i) reductive opening of a lactonic ring to release a COOH group at C1 and (ii) the reductive cleavage of oxide bridge to generate an OH group at C3 to form (**D**). Thus C5–O is smoothly transferred to C3–O with right relative stereochemistry and location for the ring E. Subsequent periodate cleavage of (**D**) followed by methylation yields the ring E

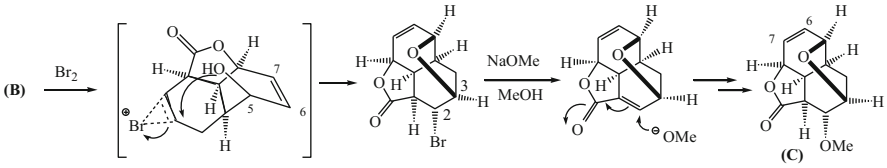


Fig. 26.8 Formation of the methoxyether (**C**)

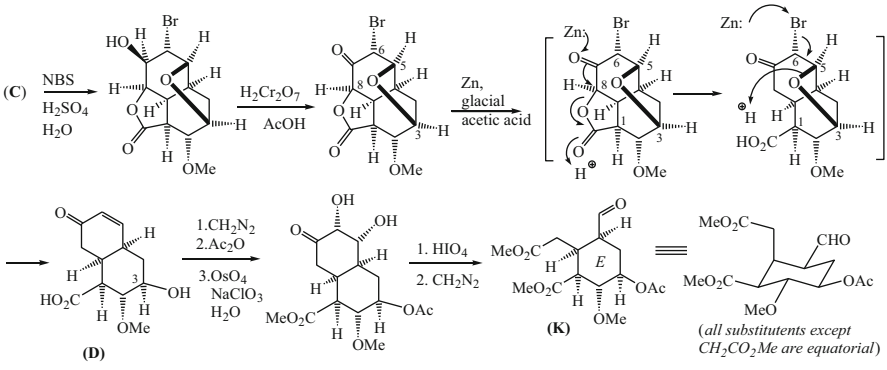


Fig. 26.9 Formation of the key intermediate (**K**) (ring E)

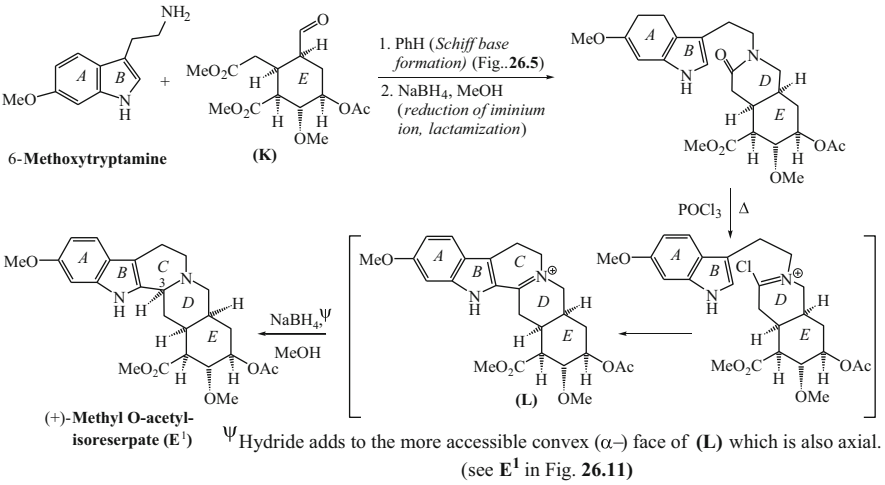
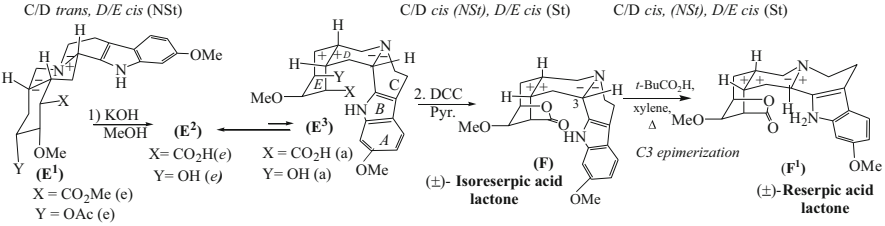


Fig. 26.10 Synthesis of (±)-methyl-O-acetyl-isoreserpate



Note: *The above conformations have been written upside down [15] for convenience, reversing the order reversing the order of the numbered carbons, and a-,β- orientations, but keeping the e,a orientations unaltered.*

Fig. 26.11 Unfavorable conformer is locked as a lactone, followed by C3 epimerization

precursor (**K**) having five contiguous chiral centers with correct relative configuration (*one enantiomer is shown*) (Fig. 26.9).

Following the sequence of reactions, delineated in Fig. 26.10, (±)-methyl *O*-acetyl-isoreserpate, (**E**$^{\mathbf{1}}$), a methyl reserpate stereoisomer (epimeric at C3) is formed.

The compound (**E**$^{\mathbf{1}}$) is thermodynamically more stable, the indole moiety being locked by equatorial orientation to the piperidine moiety (*cf.* (**2**) in Fig. 26.1). The less stable (axially oriented indole moity) C3 epimer being the part of the natural reserpine (**1**) has been obtained from the more stable iso-series via epimerization at C3. The tactic used for such epimerization is perhaps one of the most beautiful pieces of Woodward's imagination that finds way into the laboratory (Fig. 26.11). The ester (**E**$^{\mathbf{1}}$) has been hydrolyzed with methanolic potash, to a hydroxy acid (**E**$^{\mathbf{2}}$).

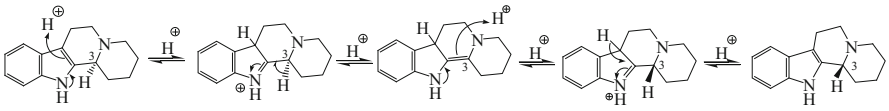


Fig. 26.12 Mechanism of epimerization at C3

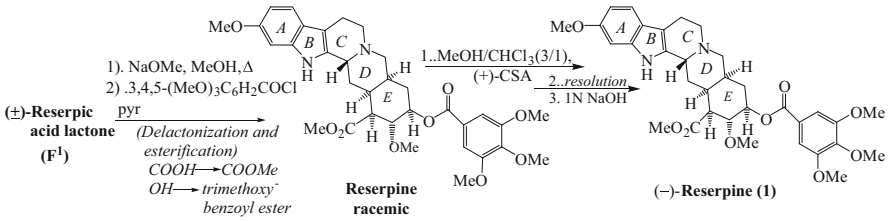


Fig. 26.13 Final steps of reserpine synthesis: delactonization and esterifications

The C/D ring junction of (**E**^{2}) undergoes *trans* to *cis* fusion through inversion of N to give its very unfavorable conformer (**E**^{3}). This event also effects the inversion of *cis* fused D/E rings from the nonsteroid form (**E**^{2}) to the steroid form (**E**^{3}) and enforces all three substituents of ring E in axial orientations. This most unfavorable conformation is irreversibly locked in the form of a 1,3 diaxial lactone (**F**) using DCC in pyridine. Treatment of (**F**) with pivalic acid in refluxing xylene leads to quantitative epimerization at C3 to form reserpic acid lactone (**F**^{1}) (keeping the lactone moiety untouched) that may be rationalized through a presumed cascade of events (Fig. 26.12).

Cleavage of the lactone ring in (**F**^{1}) with methoxide ion gives methyl reserpate in which all three substituents immediately attain the much more stable equatorial orientations. This event becomes possible through the steps, *viz*, inversion of nitrogen to convert the C/D junction from *trans* back to *cis* (steroid form), accompanied by inversion of D/E *cis* ring junction from steroid to nonsteroid form, as present in reserpine (**1**) (Fig. 26.1). Subsequent acylation of the OH group with 3,4,5-trimethoxybenzoyl chloride in pyridine forms racemic reserpine. High crystallinity and low solubility of (−)-reserpine (+)-camphorsulfonate in methanol allowed ready resolution of (±)-reserpine (Fig. 26.13).

Figures 26.6–26.13 taken together portray the elegant synthesis of reserpine by Woodward.

In conclusion we may quote Nicolaou [16] who paid a tribute while analyzing and describing the total synthesis of reserpine ". . .one of Woodward's most brilliant achievements, and perhaps one of the most remarkable total syntheses of all time. The strategy is brilliant and the tactics even more spectacular. Memorable highlights include the demonstration of the Diels–Alder reaction as an efficient method to construct highly functionalized six-membered rings, the use of a variety of substrate-stereocontrolled reactions by which the various stereocenters can be introduced around the six-membered E ring, and, finally, the ingenious maneuver that enforced the adoption of an unfavorable conformation, thus setting the stage of a facile epimerization.

The Woodward total synthesis of reserpine is an inspirational accomplishment that will, no doubt, remain a classic in the history of total synthesis."

26.4.2 Stork's Synthesis of (±)-Reserpine and (−)-Reserpine

The most important one is the chirality at C3. Hence in some of the reported syntheses, reserpine is always accompanied by the undesired but more stable C3-epimer (3-isoreserpine).

The challenging problem in the total synthesis of reserpine is the direct introduction of the right stereochemistry at C3 with the indole moiety attached axially to the ring D, as in reserpine series. Gilbert Stork and his coworkers in their regiospecific and stereoselective synthesis [17, 18] of the natural (−)-reserpine, planned for a suitable iminium ion (**A**) (Fig. 26.14), the terminal key precursor. The latter could be made to undergo the kinetic ring closure (kinetic nucleophilic "*perpendicular chair*" axial addition of the indole ring) leading to the desired less stable configuration at C3 of reserpine. The indole moiety thus becomes axial to the piperidine ring. Stork's enantioselective synthesis in 1989, achieved 33 years after the synthesis of (±)-reserpine by Woodward in 1956, is delineated in Fig. 26.15.

Synthesis of the Key Precursor Molecule (B). Synthetic Strategy and Tactics
The iminium ion precursor (**A**) carrying rings A and B and rings D and E was generated from (**C**) under suitable experimental condition. This was subjected to a kinetic ring closure (Fig. 26.16) to form reserpic acid, which on acylation with 3,4,5-trimethoxybenzoyl chloride yielded (±)-reserpine. The left-hand side of the molecule (**A**) could be obtained from 6-methoxytryptamine.The ideal precursor of the right-hand part of the molecule would be a cyclohexane moiety (*ring E precursor*) with five contiguous chiral centers carrying substituents as in (**B**). In this molecule C15 and C20 should have chemical moieties capable of reacting with tryptamine $-NH_2$ group forming a piperidine ring (ring D) with the right ring juncture geometry. The retrosynthetic analysis of (**B**), the key precursor, is given in Fig. 26.15. Compound (**B**) has been synthesized by Stork by the following route and also by two other routes, which he discussed in detail in 2005 [18].

Retrosynthetic analysis of (**B**) [17, 18] (Fig. 26.15).

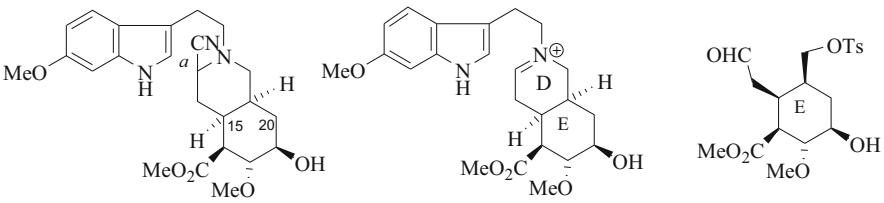


Fig. 26.14 The key precursors (A) and (B)

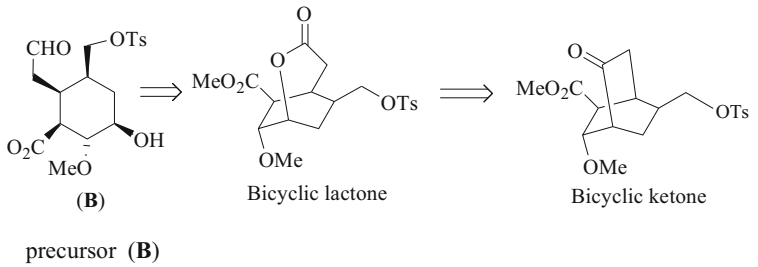


Fig. 26.15 Retrosynthesis of the key precursor (B)

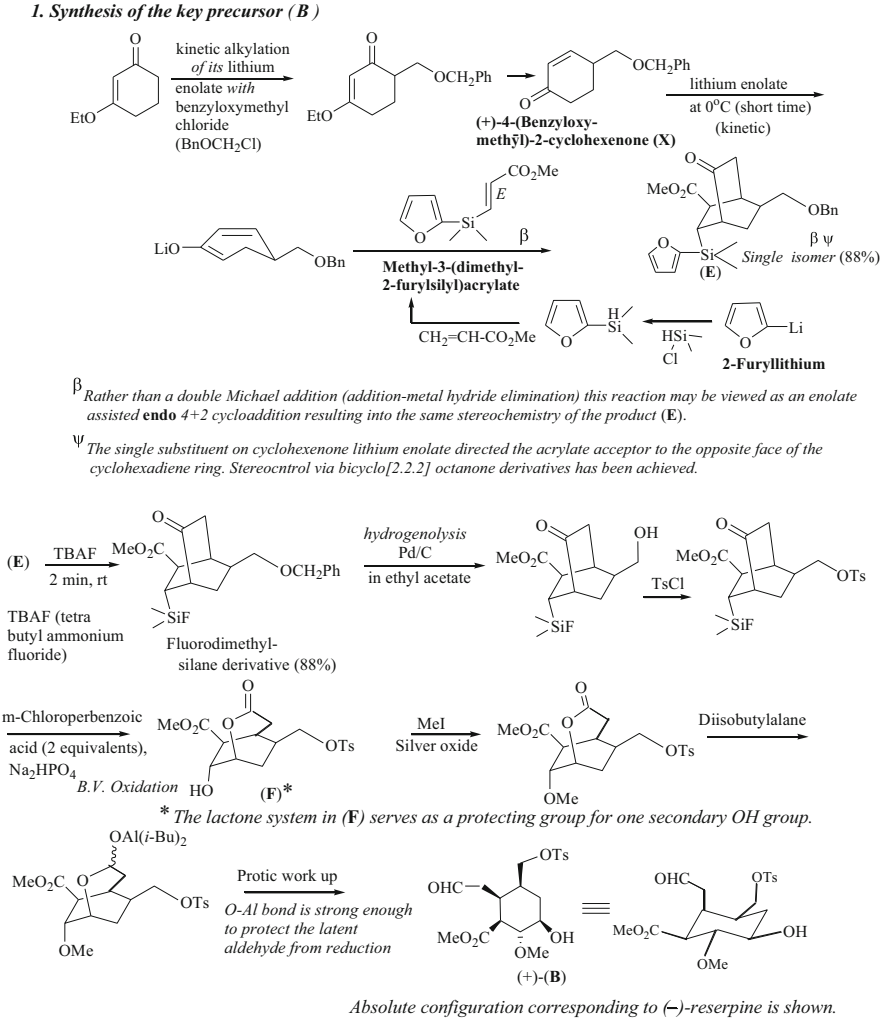
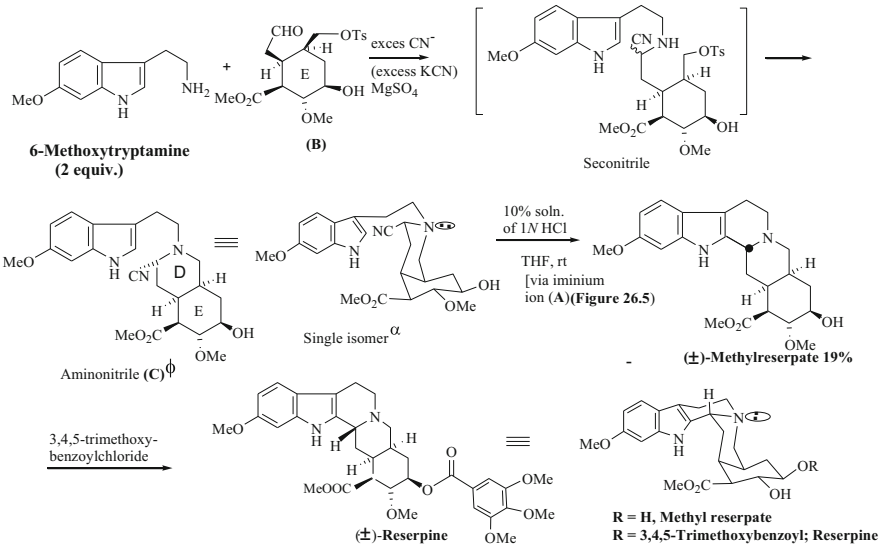


Fig. 26.16 (continued)



α *newly introduced CN group is axial; however, its configuration is irrelevant since it will be eliminated during the formation of iminium ion (A)*

ϕ *In the aminonitrile (C) CN group is axial as evident from the NMR and X-ray studies of a related cyanopiperidine. During the formation of iminium ion (A) upon refluxing with acetonitrile the CN group is eliminated and the iminium ion* (**A**) *is formed, but perhaps CN group remains as a tight ion pair between iminium and cyanide ions, and CN group being axial might block the addition of the indole to the intermediate iminium (A) ion in the "chair-axial" mode, and instead leads to a "boat-axial entry to form some methyl reserpate. However, upon treatment with 1N HCl the cyanide ion escapes, and the tight ion pair formation is avoided. The free iminium ion is then available for axial addition of the indole to the iminopiperidine ring, generating the right streochemistry at C3 of reserpic acid [17,18].*

Fig. 26.16 Synthesis of (±)-reserpine (Stork, 1989)

3. For the synthesis of natural (-)-reserpine, the optically active form of **X**, i.e., (4*S*)-4-(benzyloxymethyl)-2-cyclohexanone, $[\alpha]_D^{26}$ −109.4 (MeOH) from (1*S*)-3-cyclohexane carboxylic acid [7] has been synthesized (Figure **26.9**). Starting from (−)-**X** Stork duplicated the steps already described for the synthesis of (±)-reserpine to synthesize (−)-reserpine in just 10 steps.

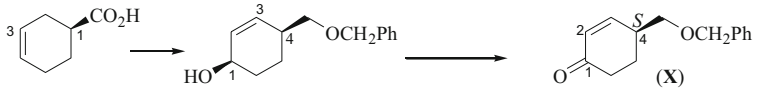


Fig. 26.17 Synthesis of (−)-(X)

Even 50 years after its first synthesis reserpine has still been and will probably continue to be a very attractive target for its construction. Several total and formal syntheses, following imaginative and independent routes, have been accomplished by Pearlman [20], Wender [21, 22], Martin [23, 24], Fraser-Reid [25, 26], Liao [27], Hanessian [28], Mehta [29], and Sparks [30, 31]. The closely related deserpidine

(9-desmethoxyreserpine) (Fig. 26.1) has been synthesized by Szantay [32], Naito [33], and Baxter [34]. The key feature of all the syntheses constitutes the construction in different ways of the ring E (of reserpine), a cyclohexane moiety wherein five of the six stereogenic centers and much of the functionality reside.

26.4.3 A Formal Synthesis of (±)-Reserpine by Mehta [29]

Mehta et al. [29] executed the synthesis of Woodward's ring-E intermediate (**A**), in eighteen straightforward steps, as delineated in Fig. 26.18, following a *hydrindane*

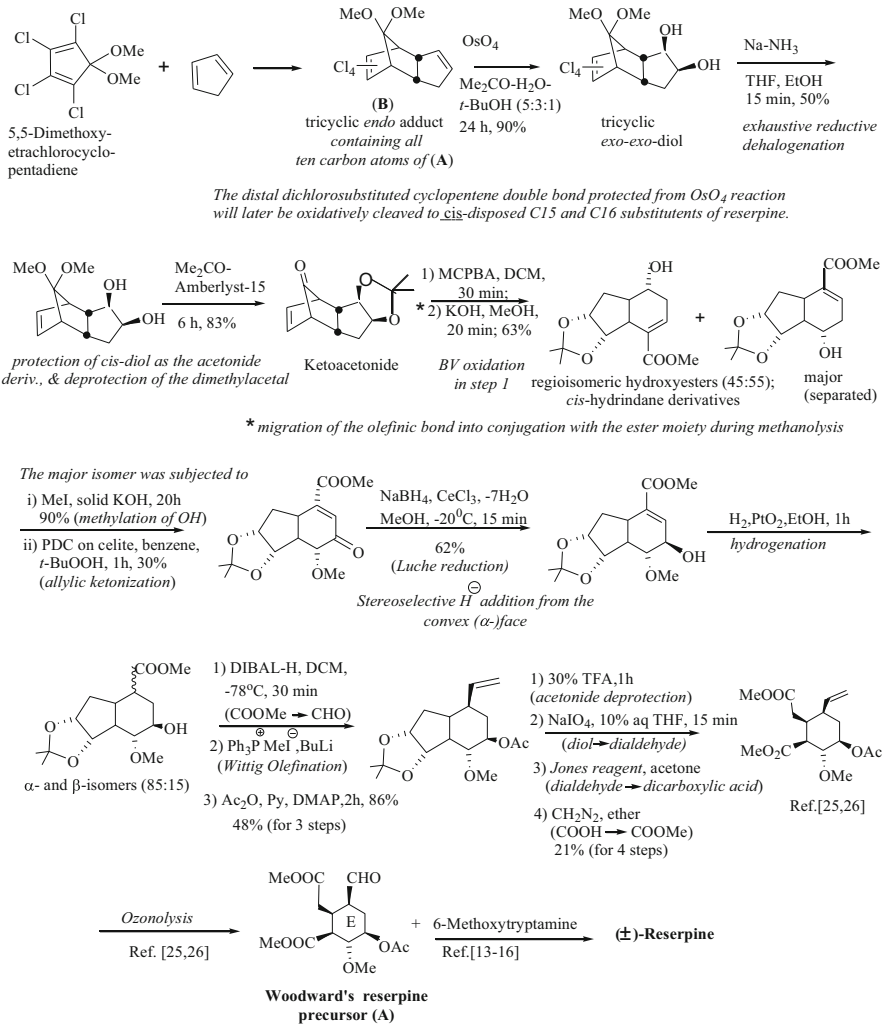


Fig. 26.18 A formal synthesis of reserpine (Mehta) [29] (2000)

approach, which in a formal sense constitutes a new synthesis of (±)-reserpine. Two abundantly available C5 building blocks, cyclopentadiene and 5,5-dimethoxytetrachloro-cyclopentadiene were used as starting materials, which readily entered into Diels–Alder reaction to furnish the tricyclic *endo*-adduct (**B**).

26.5 Biological Activities and Uses

The biological activities of reserpine and its medicinal value have been discussed in Chap. 33.

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Chapter 27
Strychnine, *an Alkaloid with Heptacyclic Dense Molecular Scaffold*

27.1 Introduction. Structure

About strychnine, a monoterpene dihydroindole alkaloid, it has been said, "For its molecular size it is the most complex substance known." [1, 2] Its structural complexity is reflected in its IUPAC nomenclature which is a very long and complicated one. It is a highly poisonous substance and the fatal dose is very low. Strychnine is such a notorious poison that when Pelletier and Caventou who isolated it in 1818 [3] from the fruits of *Strychnos ignatii* (Loganiaceae) wanted to name it Vauqueline after the name of a brilliant scientist Vauquelin with whom Pelletier was associated for some time, the officers of the Académie des Sciences at Paris rejected the naming [1, 2] because the Academy did not want to associate the name of an esteemed chemist with such a deadly poisonous compound. Strychnine has been used as a lethal agent in many murder stories. In Sir Arthur Conan Doyle's (1859–1930, British Physician, Novelist and Detective story writer) Holmes mystery "The sign of four" Dr. Watson suggested the lethal agent to be …. "…. a powerful vegetable alkaloid—some strychnine like substance which would produce titanus" [4]. Strychnine occurs in other S*trychnos species* and *Strychnos-nux-vomica* is a well-known source.

The structural work of strychnine, $C_{21}H_{22}N_2O_2$, colorless crystals, mp. 270–273, $[\alpha]_D$ −139, took more than 60 years and through the brilliant work of Robert Robinson, Hermann Leuchs, and many other brilliant minds the complicated heptacyclic structural scaffold of strychnine has been constructed and the structure has been finally settled in 1947. The chemistry of strychnine has been reviewed by G. F. Smith [5] and X-ray crystallographic studies [6] supported the structure derived from chemical studies. At a later date the absolute configuration of the natural (−)-strychnine was settled by X-ray crystallographic analysis [7, 8], as shown in Fig. 27.1.

In strychnine ($C_{21}H_{22}N_2O_2$) all the atoms excepting the hydrogens and one oxygen are present as part of the main scaffold and are densely located in seven rings. There are altogether six chiral centers (7*R*, 8*S*, 12*S*, 13*R*, 14*R*, 16*S*,

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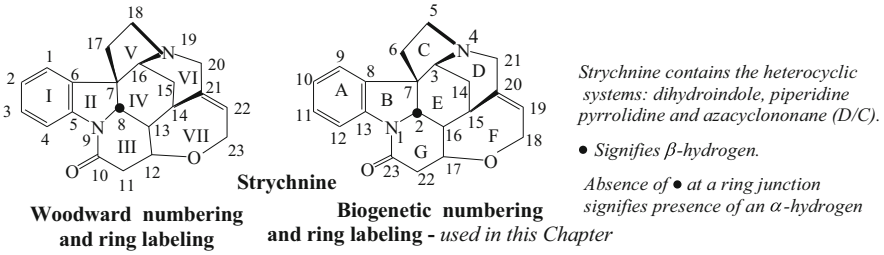


Fig. 27.1 Structure and nomenclatures of strychnine

Woodward numbering used in *Chemical Abstracts)* of which five chiral centers save C12 are in the core of the cyclohexane ring IV. Thus its structure elucidation and synthesis were equally challenging. According to the biogenetic numbering the six chiral centers possess 2*S*, 3*S*, 7*R*, 15*R*, 16*R*, and 17*S* configurations.

27.2 Synthesis

The first synthesis of (±)-strychnine has been achieved by Woodward in 1954 [1], which remains a pioneering achievement to this day in the field of synthetic organic chemistry. The landmark paper [1] begins with an exclamation "Strychnine!"—an unusual passionate expression in a scientific writing – perhaps the expression of an excitement and joy of the conquest of a formidable structure. The entire paper offers a world of chemistry and the historical introduction is indeed a delight to read. A number of groups since then attempted for the same goal and it took nearly 40 years (1992) to repeat the same feat [9]. Subsequently a number of synthesis by Stork (1992), Magnus (1992), Overman (1993), Kuehne (1993, 1998), Rawal (1994), Bonjoch/Bosch (1996, 1999) appeared in the literature, and an elegant review [10] on the synthesis of strychnine till 2000 has been published. An enantioselective synthesis of (−)-strychnine using asymmetric catalytic Michael addition and tandem cyclization has been reported in 2002 [11]. An enantioselective synthesis of (−)-strychnine by Kuehne and Xu [12, 13] is outlined in Figs. 27.2 and 27.3, and a stereocontrolled short synthesis of (±)-strychnine by Rawal et al. [14, 15] is briefly delineated in Figs. 27.4 and 27.5.

27.2.1 Enantioselective Synthesis of (−)-Strychnine

Kuehne et al. used adequately functionalized pyrrolocarbazole derivatives as intermediates for developing general and versatile strategies for the synthesis of alkaloids with aspidospermatan and strychnan skeletons. They published papers on

strychnine synthesis in 1993 [12] and in 1998 [13]. In an enantioselective synthesis of (−)-strychnine, their efforts [13] have been directed to the synthesis of (−)-Wieland-Gumlich aldehyde which was then converted to (−)-strychnine, by reported methods [9, 16].

27.2.2 *Retrosynthetic Analysis of Kuehne's Synthesis*

The pentacyclic precursor was disconnected at the N_4–C_{21} bond leading to functionalized hexahydropyrrolo[2,3-*d*]-carbazole intermediate. The piperidine ring was constructed by intramolecular electrophilic alkylation of N_4. The ABCE-tetracyclic system was then assembled based on a new condensation, a [3,3]-sigmatropic rearrangement, a tandem process between a tryptamine derivative and an α,β-unsaturated aldehyde. During the construction of E and C-ring bonds between C15–C16 and C3–C7 are created. The retrosynthetic analysis [10] and total synthesis of (−)-strychnine are delineated in Figs. 27.2 and 27.3, respectively. This constitutes a short and efficient enantioselective total synthesis of (−)-strychnine in an overall 5.3 % yield.

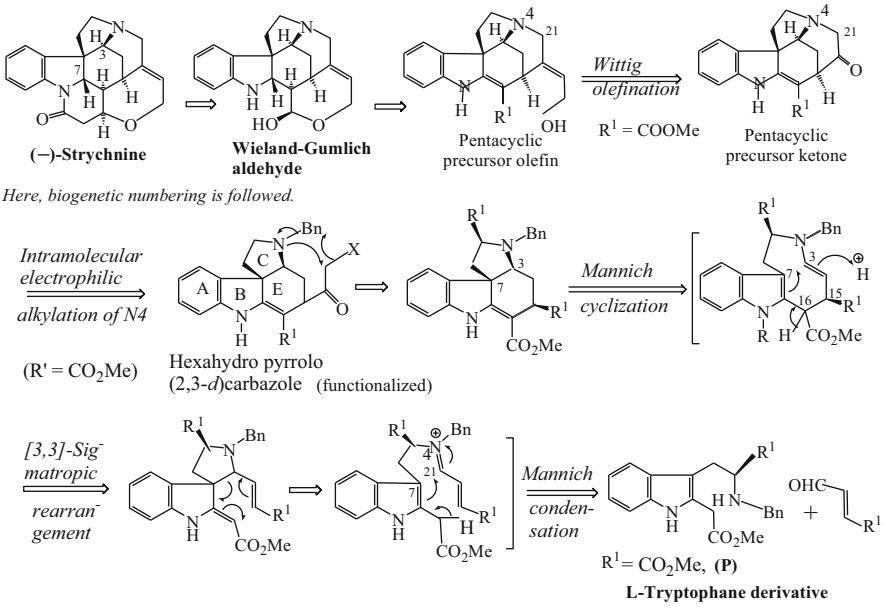


Fig. 27.2 Kuehne's retrosynthetic analysis of strychnine [10]

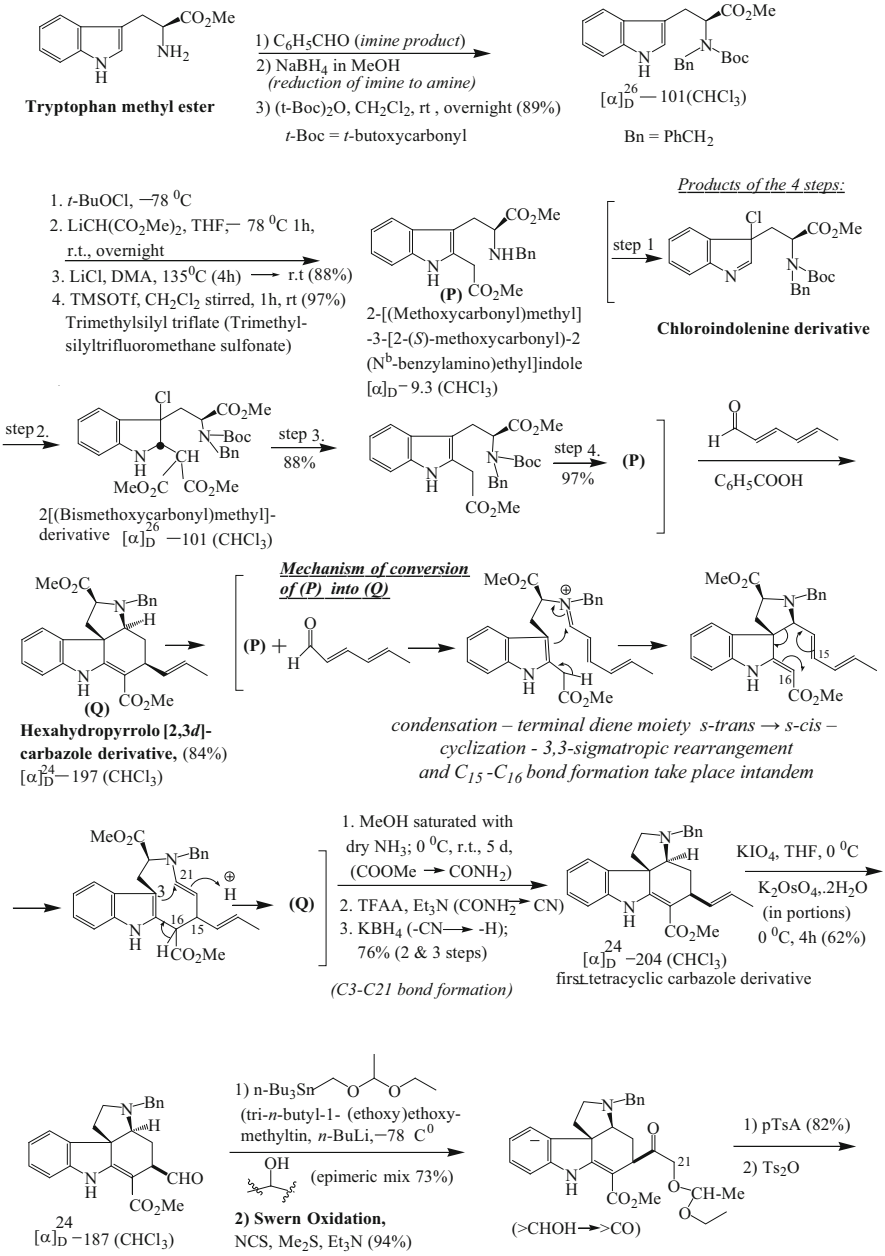
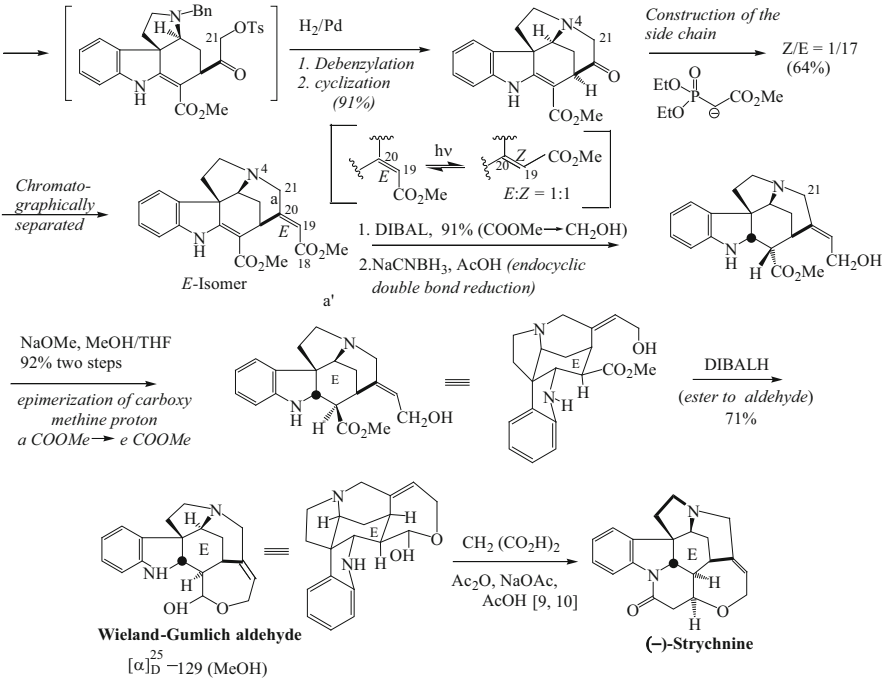


Fig. 27.3 (continued)



For the conformation of strychnine see the last str. of Fig 27.6

Fig. 27.3 Enantioselective synthesis of (−)-strychnine by Kuehne and Xu [13]

27.2.3 *Enantioselective Synthesis of (−)-Strychnine by Kuehne and Xu [13]*

Kuehne developed two synthetic routes to strychnine. The first one involves racemic series via isostrychnine [12]. The second route, directed to the Wieland–Gumlich aldehyde, constitutes a short and efficient enantioselective total synthesis of strychnine, involving 14 steps from the tryptophane derivative (**P**), which is delineated in Fig. 27.3.

27.2.4 *Stereoselective Synthesis by Rawal [14, 15]*

Rawal et al. has synthesized strychnine by a short highly stereocontrolled route that involves the efficient conversion of commercially available

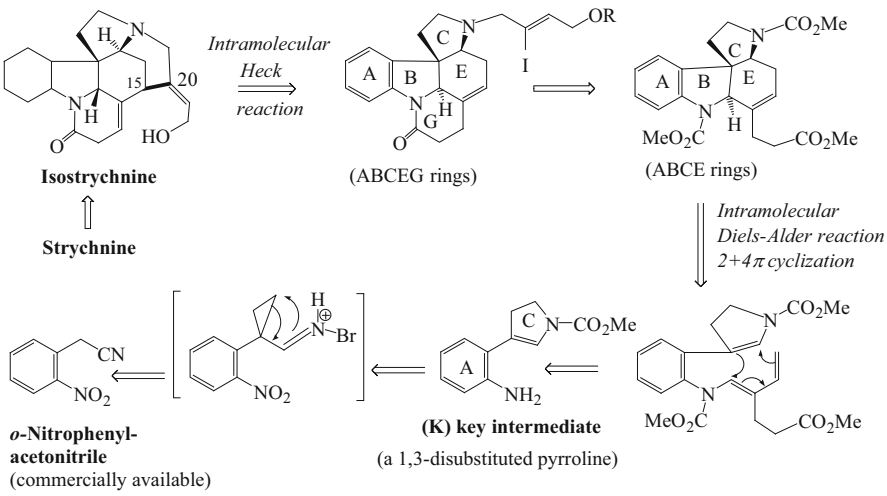


Fig. 27.4 Retrosynthetic analysis of isostrychnine

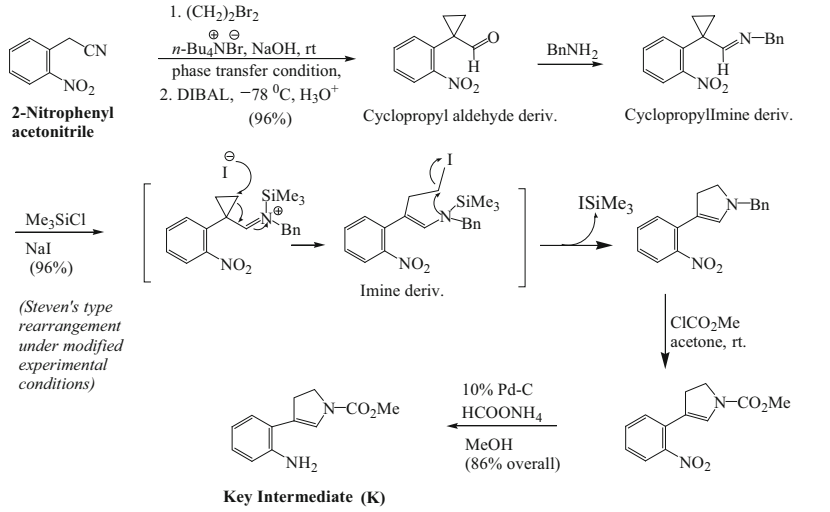
2-nitrophenylacetonitrile (**J**) to the key intermediate (**K**) [14], and its transformation into isostrychnine [15], a known synthetic precursor of strychnine. The retrosynthetic analysis [10] of Rawal's synthesis of isostrychine and its total synthesis [14, 15] are delineated in Figs. 27.4 and 27.5, respectively.

The cyclopropyl aldehyde derivative is formed under a phase transfer condition (Fig. 27.5) and the COOH group generated by the hydrolysis of the CN group has been selectively reduced to aldehyde with DIBAL under careful experimental condition, followed by an acidic quench. The cyclopropylimine derivative undergoes rearrangement as cyclopropyl iminium ion cyclization in tandem and the requaternized nitrogen is again dequaternized by the removal of $SiMe_3$ group as Me_3SiI; the probable mechanism is shown. The remaining two steps leading to the 2-substituted aniline derivative are conventional.

The triene (**L**) is conformationally favorably disposed to undergo intramolecular 2 + 4π cyclization from the exo face, adding B, C, E rings to the existing ring A and thus yielding a tetracyclic intermediate (**M**) in 99 % yield. The $N^b(N^4)$ is suitably alkylated, which with $Pb(OAc)_2$ under phase transfer catalytic condition generates ring D and is converted to isostrychnine. The latter upon treatment with KOH [1,12] undergoes base mediated cyclization to yield strychnine, but in a low yield because of an unfortunate equilibrium.

1st part of the Rawal's synthesis:

Synthesis of the key intermediate (K): **(**it could be prepared on large scale, upto 25 g with ease [14])



2nd part of the synthesis:

Synthesis of isostrychnine from the key intermediate; this constitutes a formal synthesis of strychnine.

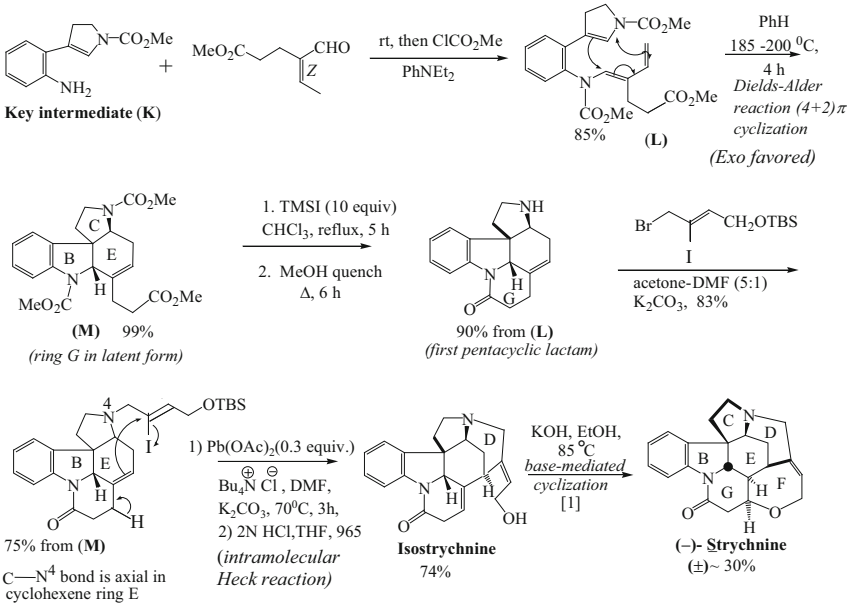
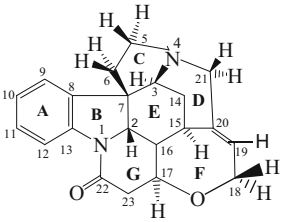


Fig. 27.5 Total synthesis of (±)-strychnine by Rawal [15]

27.3 Spectral Data of Strychnine [12, 14, 17]

^{1}H NMR in $CDCl_3$ at 220 MHz [17]: H2 3.85, H3 3.92, H5α 3.19, H5β 2.86, H6α 1.87, H9 7.15, H10 7.08, H11 7.23, H12 8.09, H14α 1.43, H14β 2.34, H15 3.13, H16 1.25, H17 4.27, H18α 4.05, H18β 4.13, H19 5.88, H21α 3.69, H21β 2.71.



Strychnine

^{13}C NMR in $CDCl_3$, δ-values in ppm down field from Me_4Si [δ(Me_4Si = δ($CDCl_3$ +76.9 ppm] : [17] C2 59.9*, C3 59.8*, C5 50.1, C6 42.6, C7 51.7, C8 132.4, C9 121.9, C10 123.8, C11 128.1, C12 115.8, C13 141.8, C14 26.7, C15 31.4, C16 48.0, C17 77.3, C18 64.3, C19 126.8, C20 140.2, C21 52.4, C22 168.8, C23 42.2. (cf. [12])

UV (EtOH) [12] : λ_{max} 288, 286, 254, 206 nm.

IR (KBr) [14] υ_{max} 2,492, 2,895, 2,886, 2,813, 1,666, 1,596, 1,473, 1,453, 1,387, 1,281, 1,187, 1,142, 1,103, 1,047, 760 cm^{-1}.

^{1}H NMR ($CDCl_3$) δ: [12] 8.10 (d, J = 8 Hz, 1H), 7.27 (m, 1H), 7.16 (d, J = 6 Hz, 1H), 7.09 (*t,* 7 Hz, 1H), 5.89 (*t,* J = 7 Hz, 1H), 4.28 (m, 1H), 4.12 (dd J =7,14 Hz, 1H), 4.06 (dd, J = 6, 14 Hz, 1H), 3.94 (*s*, 1H), 3.86 (d, J = 11 Hz, 1H), 3.70 (d, J = 15 Hz, 1H), 3.15 (m, 3H), 2.87 (dd, J = 10, 18 Hz, H), 2.72 (d, J = 15 Hz, 1H), 2.67 (dd, J = 4, 17 Hz 1H), 2.36 (m, 1H), 1.87 (m, 2H), 1.45 (d, J = 14 Hz 1 H), 1.27 (m, 1H).

MS [12]: *m/z* (relativel intensity) 335 (20), 334 (M^+, 91), 183 (7), 167 (18), 161 (20), 149 (17), 143 (22), 134 (21), 130 (29), 120 (40), 111 (31), 107 (39).

27.4 Biosynthesis. Molecular Conformation

Strictosidine is the obligatory biosynthetic precursor of various monoterpene indole alkaloids [10, 18] (see Chap. 25). Enzymatic deglucosylation activates the precursor to participate in various monoterpene indole alkaloids biosynthesis. It is biogenetically converted to 4,21-dehydrogeissoschizine, which probably undergoes N^b–C10 double bond reduction prior to bond migration (Fig. 27.6) leading to the formation of preakuammicine skeleton in which ABCDE ring systems of strychnine are present. Subsequent enzymatic biochemical processes probably forms Wieland–Gumlich aldehyde or its equivalent which finally forms (−)-strychnine via prestrychnine (Fig. 27.6).

The *Molecular conformation* (perspective formula) of strychnine, the model of which when viewed from a particular direction revealed the chair/chair forms of the *trans* fused rings G/E (cf. *trans*-decalin, Chap. 2) and the envelope form of the dihydroindole ring B, is shown at the end of Fig. 27.6.

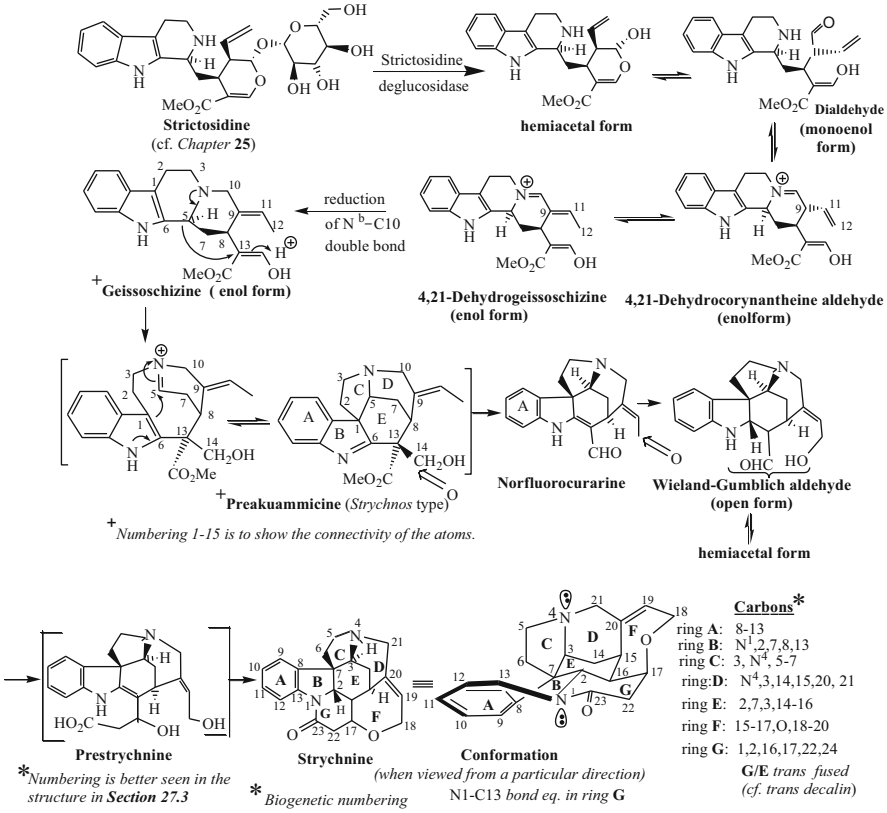


Fig. 27.6 Biosynthetic route of strychnine [10, 18]

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Further Reading

Stefan Berger and Dieter Sicker, Strychnine in *Classics in Spectroscopy*, Wiley-VCH, **2009,** 103-128.

Chapter 28
Dimeric Indole Alkaloids. Vinblastine (Vincaleukoblastine), Vincristine (Leurocristine), and Their Derivatives

The Valuable Anticancer Drugs and Other Congener Dimeric Indole Alkaloids of the Vinblastine Group

28.1 Introduction. Structures

The dimeric indole alkaloid **vinblastine** (**vincaleukoblastine**) (**VLB**), serendipitously discovered from Madagascan periwinkle (*Catharanthus roseus syn. Vinca rosea*, Apocynaceae) [1], exhibits remarkable cancer-fighting activity and contributed immensely to dispel the sufferings of human beings caused by certain types of cancer (see Chap. 33). Subsequent discovery of a few more dimeric indole alkaloids **leurocristine** (**LCR**) (also known as **vincristine**), **leurosine**, and **leurosidine** (**vinrosidine**) from the same plant (Fig. 28.1) and the study of their anticancer activities add another name vincristine to the list of important cancer-fighting drugs. The molecular structures of vincristine and vinblastine with some stereochemical reservations have been proposed from the studies of their spectral properties, degradations, characterizations of their degradation products, and on biogenetic grounds [2–4].

The stereostructure with absolute configuration of vincristine (**2**) has been established [5] from the X-ray diffraction study of single crystals of leurocristine methiodide $(C_{47}H_{59}O_{10}N_4)^+1^-$. The stereostructure of vinblastine (**1**) is known from its chemical relationship with vincristine which is des-*N*(a)-methyl-*N*(a)-formyl vinblastine. They constitute the first examples of indole–indoline alkaloids in which indole moiety is attached through C–C bond to the aromatic ring of the dihydroindole part of the molecule.

28.2 Semisynthetic Anticancer Drugs from Vinblastine

Some derivatives of vinblastine (Fig. 28.1) have been prepared and tested for their oncolytic properties and found to be promising. **Vindesine (5)** is used for lymphoid leukemia in children. **Vinorelbine (6)** has been approved by USFDA and is

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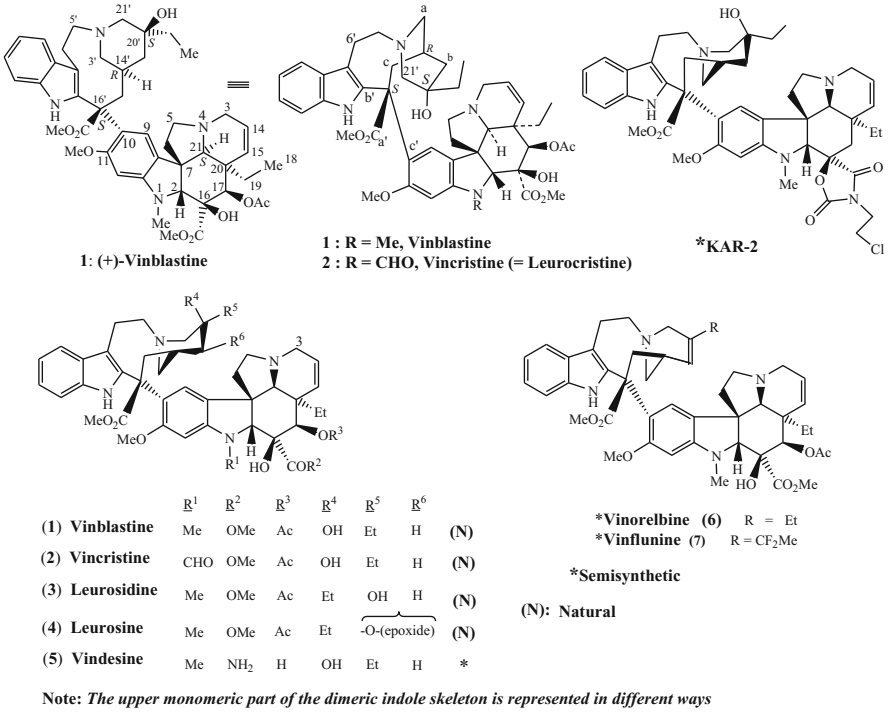

Fig. 28.1 Natural and semisynthetic dimeric indole alkaloids

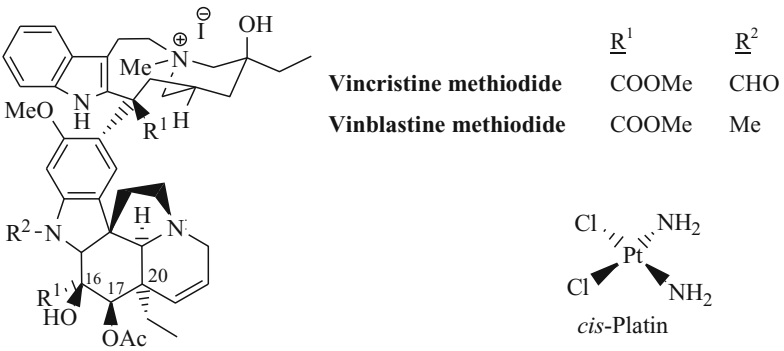

Fig. 28.2 Structures derived from X-ray diffraction study

used for breast cancer and non-small-cell lung cancer as a single agent or in combination with cis-platin (Fig. 28.2). It is orally active and possesses broader anticancer activity. **Vinflunine (7)** and **KAR-2** (Fig. 28.1) are now in advanced clinical trials.

28.3 Synthesis

Following the disclosure of the structure of **vinblastine** its four different syntheses appeared in the literature within three decades. These syntheses cannot be termed as the total synthesis, since (–)-vindoline, a natural alkaloid and also a constituent of *Vinca rosea*, serves as the source of the lower half of vinblastine in these syntheses. The C16 of catharanthine (the source of the upper half) is labeled C16′ in vinblastine group of dimeric indole alkaloids (cf. Fig. 28.1). The first total synthesis of (+)-vinblastine has been achieved by Tohru Fukuyama [1] in 2002 with the correct stereochemistry (*S*) at C16′ (Fig. 28.1), based on oxidative coupling, modifications of standard synthetic transformations with a string of inventive synthetic strategies to overcome the challenges the molecular structure of vinblastine throws. The synthesis has been thoroughly described by Fukuyama [1].

Since vinblastine and vincristine are two highly demanding and priced drugs in cancer therapy, their adequate supply to the users is needed. Unfortunately they occur in very low yields and their isolation from their sources, *Catharanthus* species, a home for nearly hundred indole alkaloids, is a difficult time-consuming task. Thus they become the most expensive drugs in the pharmaceutical market for cancer therapy.

Both the monomeric alkaloids (–)-vindoline and catharanthine (Fig. 28.3) are the major components of the aerial parts of various *Catharanthus* species. Biogenetic concept suggests that vinblastine and for that matter, vinblastine group of dimeric indole alkaloids could be generated in vitro by the nucleophilic attack of (–)-vindoline to an activated catharanthine molecule. A number of distinguished groups were involved in such endeavors. However, they arrived at anhydrovinblastine with wrong stereochemistry (*R*) at 16′ which is a pharmacologically inactive molecule. Potier and his group [6] envisaged that this stereochemical problem could be overcome using **modified Polonovski reaction**.

With this idea in mind, $>N^{+}_{b}$-OTFA derivative of catharanthine has been subjected to Polonovosky fragmentation when C16–C21 seco-catharanthene N_b iminium species with activated C16 site is formed. It is then allowed to react with

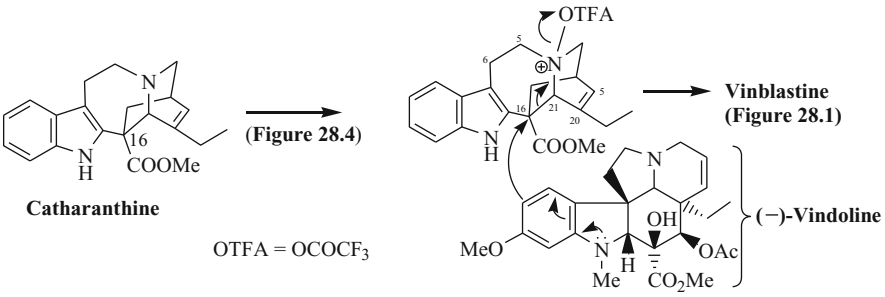

Fig. 28.3 Dimerization of two indole monomeric alkaloids to form vinblastine

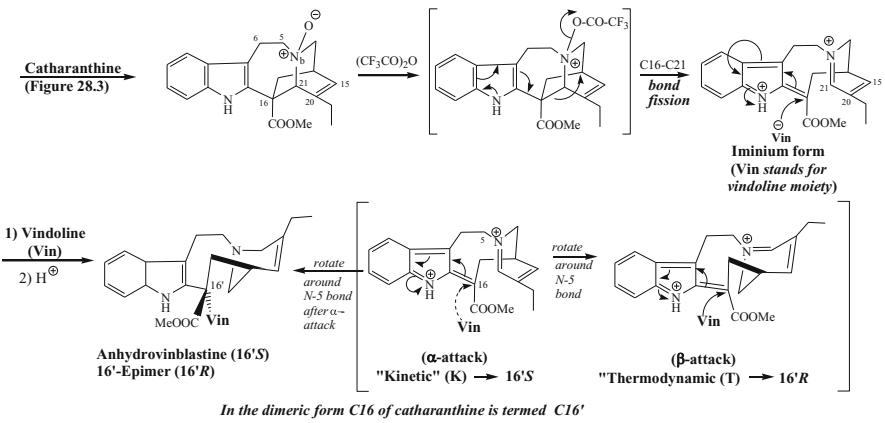

Fig. 28.4 Polonovski fragmentation of catharanthine N_b-OTFA, activation of C16, and nucleophilic coupling with vindoline at electrophilic C16 (kinetically generated species) of C16–C21 seco-catharanthine

(–)-vindoline at low temperature, –50 °C. Catharanthine > N^+_b-OTFA undergoes Polonovski fragmentation with the rupture of C_{16}–C_{21} bond, being antiparallel to $>N_b^+$-OTFA bond (leaving group), and the iminium ion thus formed remains conjugated with C5–C20 double bond. The combination of both steric and electronic factors favors the fragmentation of C16–C21 bond over C5–C6 bond of catharanthine. The stereochemical feature of this coupling at C16 (catharanthine part) leading to C16′*S* and C16′*R* of the dimer may be rationalized as delineated in Fig. 28.4.

The kinetic species (**K**) formed at low temperature with frozen conformation will allow the nucleophile (–)-vindoline to approach C16 of C16,C21-secocantharanthine N_b iminium ion from α-axial face producing the natural C16 (*S*)-stereochemistry. Upon warming, the species (**K**) is equilibrated with thermodynamically stable species (**T**) which couples with vindoline to give C16(*R*)-stereochemistry (C16 to **Vin** β-bond formation) (Fig. 28.4). The anhydrovinblastine thus formed from the species (**K**) is subsequently functionalized to yield (+)-vinblastine (Fig. 28.5).

The N-oxide of the hydrogenation product of anhydrovinblastin on treatment with Ac_2O followed by distillation in vacuo to remove the excess reagent gave a nonisolable enamine (**E**). The latter on treatment with OsO_4 followed by $NaBH_4$ reduction gave Leurosidine (30 %), while on treatment with thallium triacetate followed by $NaBH_4$ reduction yielded vinblastine (30 %) (Fig. 28.5). The contrasting behaviors of the two oxidants were explained as follows. The preferential attack of the bulky OsO_4 from the less hindered α-face on (**E**) yielded an intermediate carbinolamine (**CA**) leading after borohydride reduction to lurosidine. With thallium acetate, however, the typical axial β-attack (stereoelectronic requirement) on the enamine (**E**) takes place which on $NaBH_4$ reduction yielded (+)-vinblastine.

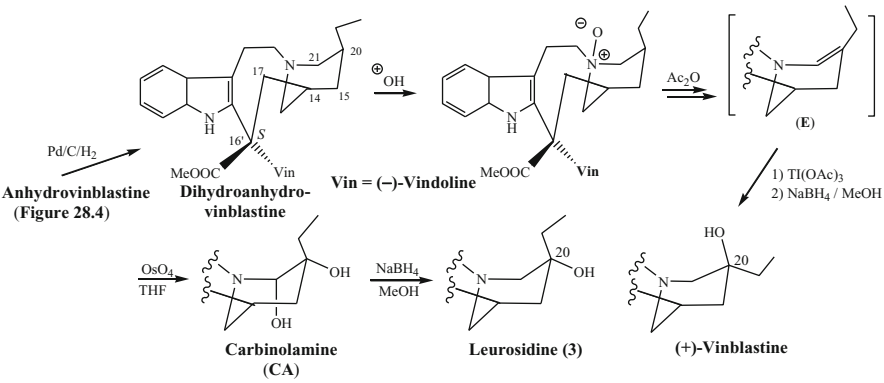

Fig. 28.5 Conversion of anhydrovinblastine to vinblastine and leurosidine

This biomimetic synthesis of (+)-vinblastine via coupling between modified catharanthene N-oxide and (−)-vindoline using Polonovsky–Potier reaction has been considered to be an outstanding achievement in the 1970s. Following this success some new methods of coupling for the preparation of vinblastine group of alkaloids have been developed [7].

28.4 Shortening of Carbon Bridge Between Indole and N_b

The carbon bridge between indole (β-position) and N_b nitrogen of vinblastine and other derivatives has been reduced by one carbon [6] and the products of the nor-vinblastine series showed interesting antitumor activity (Fig. 28.6).

The *mechanism of action of vincristine and vinblastine with tubulin* in showing anticancer activity has been discussed in Chap. 33.

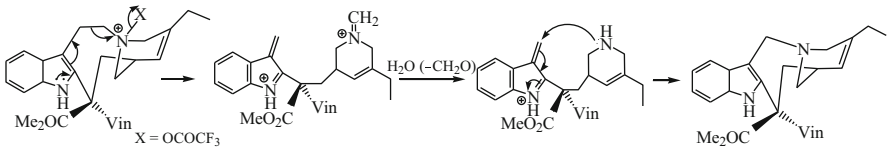

Fig. 28.6 Shortening of carbon bridge between indole (β-position) and N_b

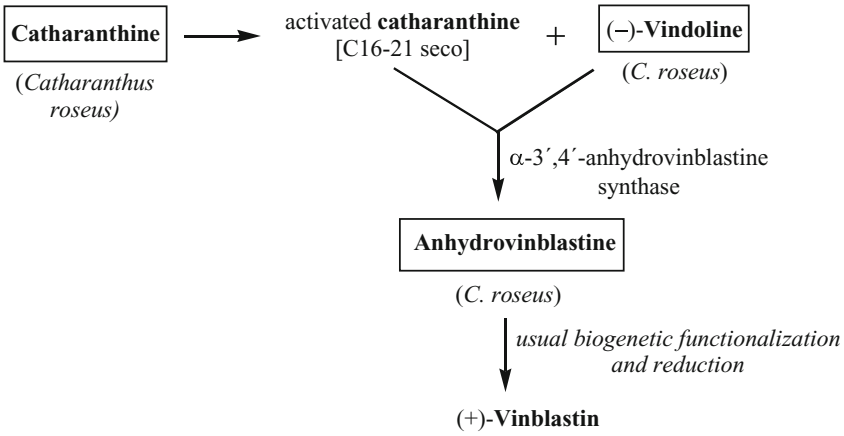

Fig. 28.7 Biosynthesis of (+)-vinblastine (an outline)

28.5 Biosynthesis of the Vinblastine-Type Alkaloids

These clinically important vinblastine group of bisindole alkaloids could well be formed in nature from its two obvious precursors present in the same plant, *viz*., catharanthine and vindoline. Anhydrovinblastin is the immediate biogenetic precursor of these dimeric indole alkaloids. In fact, Guéritte, Scott and Lee have isolated anhydrovinblastine from *C. roseus* plants [8]. The biosynthesis of vinblastine may be outlined as in Figs. 28.7 and 28.8. Each step is catalyzed by a specific enzyme.

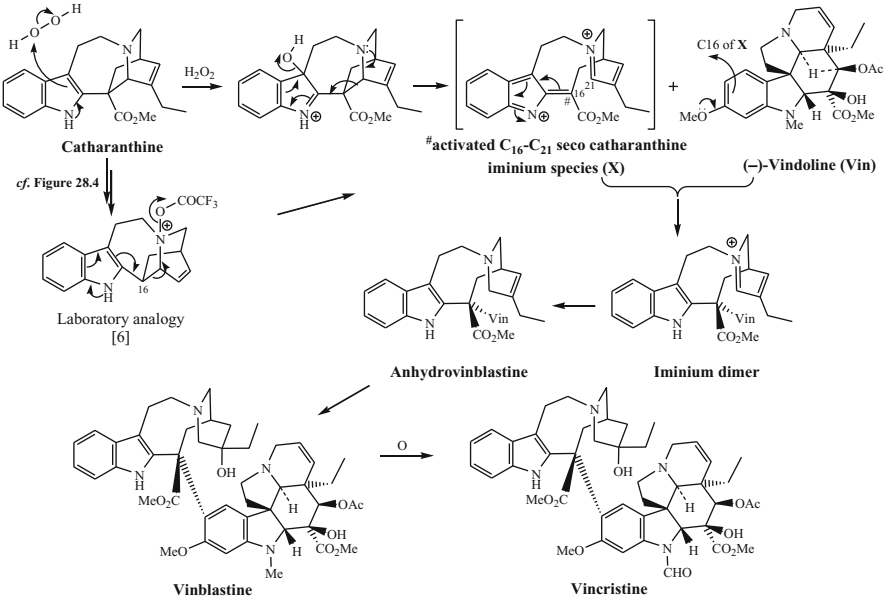

Fig. 28.8 Biosynthesis of vinblastine and vincristine

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Chapter 29
Camptothecin, A Novel Pyrrolo[3,4-b] quinoline Alkaloid: Derived by Modification of an Indole System

29.1 Introduction

Camptothecin, a high-melting, pale yellow crystalline (needles) alkaloid, m.p. 264–267 °C [1] (275–277 °C, [2]), $[\alpha]_D$ +31.3 ($CHCl_3$–MeOH, 8:2), has been isolated from the wood and bark of a Chinese tree, *Camptotheca acuminata* (Nyssaceae) [1, 3]. Later this compound has also been isolated from *Mappia foetida*, *Ophiorrhiza mungos* (Rubiaceae) [4], *Ervatamia heyneana* (Apocynaceae) [5], and Merrilliodendron *megacarpum* (Icacinaceae) [6]. Its structure (**1**) has been derived through derivatization, spectral properties, and finally by X-ray analysis [1] of its iodoacetate derivative (**2**) (Fig. 29.1). In addition to camptothecin these plants also elaborate a number of oxygenated analogues of camptothecin, *viz*., its 9-methoxy-, 9-hydroxy-, 10-hydroxy-, 10-methoxy-, 11-hydroxy-, and 18-hydroxy derivatives [5, 6].

However, Na*thapodytes nimmoniana* (Icacinaceae), a tree, has been reported to elaborate highest ever reported level of camptothecin [7]. An endophytic fungus has been isolated from *N. nimmoniana* and identified. It has been shown to elaborate camptothecin like its hosts [7].

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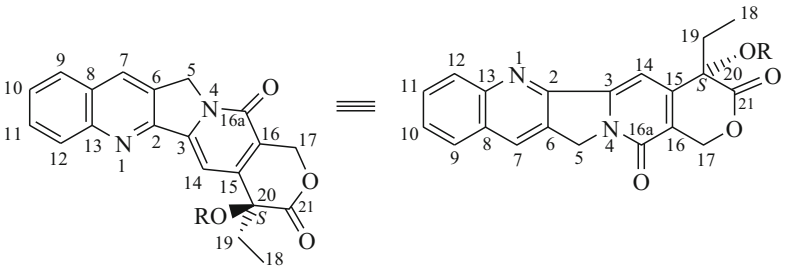

(**1**) (+)- **Camptothecin**, R = H, $C_{20}H_{16}N_2O_4$ (M^+ at *m/z* 348.1117, calcd. 348.1111) [1]
(**2**) **Camptothecin iodoacetate**, R = $COCH_2I$

Fig. 29.1 Structures and biogenetic numbering of compounds (**1**) and (**2**)

29.2 Spectral Data [1]

- **UV**: λ_{max} 220 (ε 37, 320), 254 (29, 230), 290 (4,980) and 370 nm (19, 900)
- **IR**: ν_{max} 3,440 (OH), 1,760–1,745 (lactone >=O), 1,660 (lactam C=O), 1,610, 1,585 cm^{-1} (aromatic).
- **^{1}H NMR** (CD_3SOCD_3, 60 MHz, δ), 0.91 (3H, t, CH_3–CH_2–), 1.90 (2H, m, >C(OH)–CH_2–CH_3), 5.45 (2H, $ArCH_2$–O), 5.28 (2H, $ArCH_2$–N<).
- **^{13}C NMR** (DMSO-d_6) [8], C2 156.8, C3 145.4[a], C5 50.2, C6 129.7, C7 131.4[b], C8 127.9, C9 128.4, C10 127.5, C11 129.0, C12 130.2[b], C13 149.9[a], C14 96.7, C15 147.9[a], C16 119.0, C17 65.4, C18 7.8, C19 30.6, C20 72.4

Lactam and lactone carbonyl carbon shifts are missing.
The assignments a or b are interchangeable

29.3 Synthesis of *dl*-Camptothecin [2, 9]

Danishefsky et al. synthesized *dl*-camptothecin [2] using a new pyridine synthesis developed by them [9]. The synthesis is delineated in Fig. 29.2. It is not difficult for an experienced synthetic organic chemist to visualize the suitable starting synthons by conceiving the retrosynthetic steps backwards starting from the target molecule.

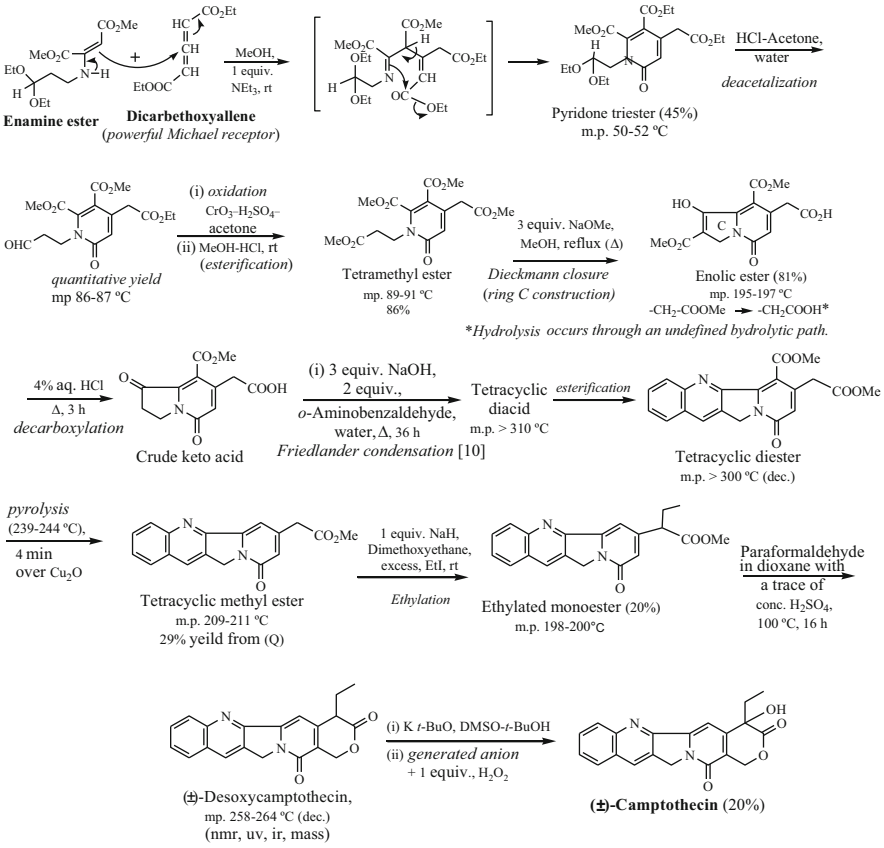

Fig. 29.2 Synthesis of (±)-camptothecin (Danishefsky [9])

29.4 Biosynthesis of Camptothecin [4–6, 8, 11]

Strictosidine (**A**) (biosynthesis: Fig. 25.14) has been proven to be the key biogenetic precursor of various monoterpene indole alkaloids [11]; in their biosynthetic pathway prior to rearrangement strictosidine undergoes deglucosylation. However, the biosynthesis of camptothecin is unique, and the rearrangement is, unlike the biosynthesis of *Cinchona* alkaloids (see Fig. 25.15), not initiated by deglucosylation of strictosidine—also a biogenetic precursor of camptothecin and *Cinchona* alkaloids. Here, the glucosyl group is assumed to stay perhaps as a protecting group of the hemiacetal moiety till the penultimate step and is hydrolyzed in the ultimate step to effect oxidation at C20 (Fig. 29.3) leading to camptothecin (**1**). A lactam (**B**) (strictosamide), the penultimate biosynthetic precursor of camptothecin is formed from strictosidine (**A**) by 180° rotation of its 14–15 bond followed by intramolecular cyclization by condensation of its amino and

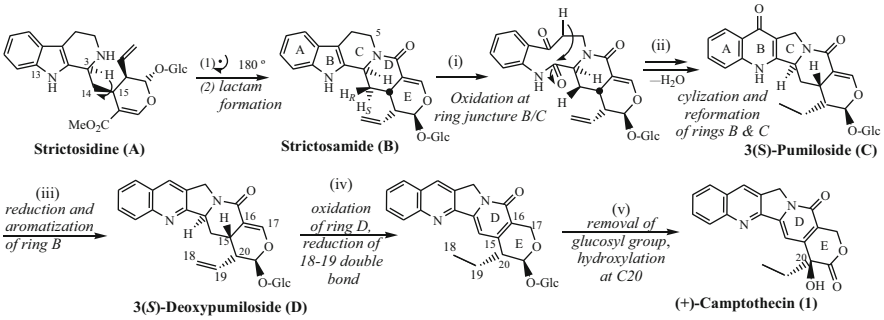

Fig. 29.3 Biosynthesis of camptothecin from its key biogenetic precursor strictosidine

carbomethoxy groups (Fig. 29.3). The participation of (**B**) in the biosynthesis of camptothecin in vivo (*Camptotheca acuminata* apical cuttings) has been validated by label experiments [4], in which the stereo- and regiospecific incorporation of [5-^{13}C] and [14-^{2}H]-strictosamide into camptothecin is observed with ^{13}C and ^{2}H NMR spectroscopic analysis. Conversion of strictosamide to camptothecin is considered to have taken place via five basic transformations [4]: (i) ring BC oxidation (ii) recyclization (when ring B is expanded to six membered, while ring C is contracted to five membered), (iii) reduction and aromatization of ring B, (iv) isomerization of 16–17 double bond to 15–16, followed by ring D oxidation, (v) removal of glucosyl group prior to hydroxylation at C20. 3(*S*)-Pumiloside (**C**) and 3(*S*)-deoxypumiloside (**D**), the two potential intermediates in the biosynthetic path, though logically supported because of their co-occurrence with camptothecin in *C. acuminate* and *Ophiorrhiza pumila,* do not have enough experimental support.

29.5 Uses

Its medicinal uses have been discussed in Chap. 33.

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3. Monroe E. Wall, Camptothecin and Taxol: Discovery to Clinic, *Med. Res. Rev.,* **1998**, *18*, 299-314.

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10. Andrew Streitweiser, Jr. and Clayton H. Heathcock, A General Method of Quinoline Preparation Using *o*-Aminobenzaldehyde and a Ketone in *Introduction to Organic Chemistry*, Second Edition, Macmillan Publishing Co., Inc., New York, **1981**, p. 1100.
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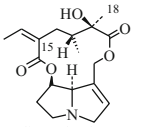
Chapter 30
Some More Alkaloids Having Diverse Skeletal Patterns

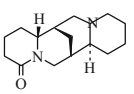
30.1 Introduction

Alkaloids are structurally most diversified natural products. They offer simple (e.g., in Chaps. 16–22) to very complex structural patterns (e.g., in Chaps. 27 and 28). Their biological activities claim a special attention for drug discovery (Chap. 33). Some representative members of a few different classes of alkaloids have been included and discussed in Chaps. 15–29. To illustrate further structural diversifications several examples of alkaloids (Fig. 30.1) not included in earlier Chapters are cited in this chapter with references. The stereostructures of these alkaloids have been settled in the usual way by suitable combinations of the following: degradation, spectral analysis, synthesis, chemical correlations, applications of some rules (e.g., Prelog's rule, Bredt's rule, etc.), and occasionally by ORD studies and X-ray crystallography. A plethora of alkaloids with diversified structural patterns belonging to different classes appearing in the literature during 1960s [1] and till 1988 [2] have been compiled with some leading references concerning their isolation/structure/stereochemistry/synthesis. Quite a number of review articles on the uses of heterocycles in the synthesis of alkaloids have collectively appeared in an edited book in 2011 [3]. They display wide structural variations of alkaloids.

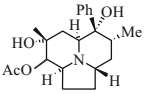
S.K. Talapatra and B. Talapatra, *Chemistry of Plant Natural Products*,
DOI 10.1007/978-3-642-45410-3_30,



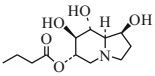
Senecionine
(*Brachyglottis repasda*)
(Pyrrolizidine alkaloid)
(various *Senecio* species, Asteraceae), and Leguminosae, Boraginaceae, Orchidaceae, Scrophulariaceae etc. plants [4-6]



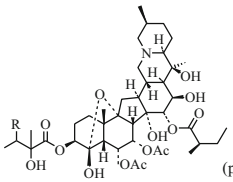
(+)-Lupanine
(Quinolizidine alkaloid)
(various *Lupinus* species, Leguminosae) [7,8]



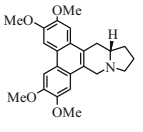
Crepidine
(*Dendrobium crepidatum*, Orchidaceae) [9-11]



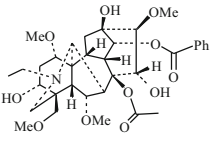
Celgosivir
(Indolizidine alkaloid)
(Leguminosae plants) [12]



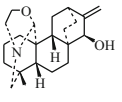
R = H, **Protoveratrine A**
R = OH, **Protoveratrine B**
(*Solanum* alkaloids) [13,14]
Veratrum album, V. viride, highly oxygenated alkaloid, medicinally used against hypertension



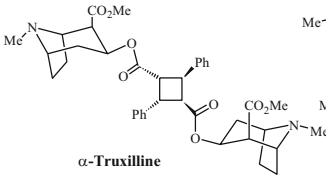
Tylophorine
(pyrroloisoquinoline alkaloid)
(*Tylophora asthmatica*, other *Tylophora* species, *Cynanchum vincetoxicum,* Asclepiadaceae), (*Ficus septica*, Moraceae) [15-18]



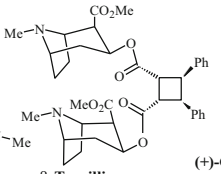
(+)-Aconitine
(Diterpene alkaloid)
(*Aconitum nepelles*, Ranunculaceae), various other *Aconitum* species [19-21]



(–)-Atisine
(Diterpene alkaloid)
(*Delphinium accumulata, Aconitum heterophyllum*, Ranunculaceae) [21-23]

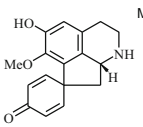


α-Truxilline

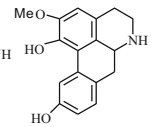


β-Truxilline

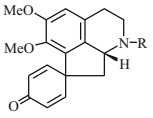
(*Erythroxylum coca,* Erythroxylaceae) [24,25,26]
(Cycloaddition products of cinnamoyl ester of methylecgonine, Chapter, **19)**



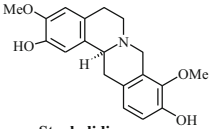
(+)-Crotonosine
(Proaporphine alkaloid)
(*Croton linearis*, Euphorbiaceae) [27]



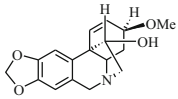
Sparsiflorine
(*Croton sparsiflorus*, Euphorbiaceae) [28]



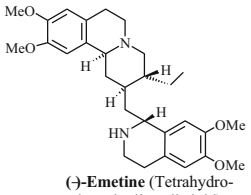
R = H, **Stepharine**
R = Me, **Pronuciferine** [29]



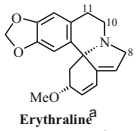
Stepholidine
(Stephania glabra [29], Menispermaceae)



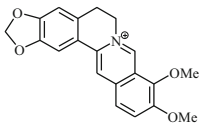
(+)-Haemanthamine
(*Haemanthus puniceus*, Amaryllidaceae), daffodils (*Narcissus pseudonarcissus*), [30]



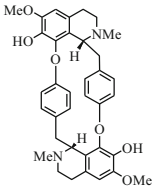
(-)-Emetine (Tetrahydro-isoquinoline alkaloid)
(*Alangium larmarckii*, Alangeaceae) [31,32]



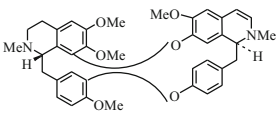
Erythraline[a]
Crystamidine[b] (=8-Oxo-10,11-dehydroerythraline)
([a]*Erythrina indica,*
[b]E. *crystagalli*,
Leguminosae) [33-35]



Berberine
(various *Berberis* species, Berberidaceae) [36]

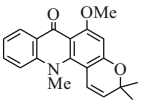


(+)-(*R,R*)-(Isochondrodendrine)
(*Chondrodendron tomentosum and* other *Chondrodendron* Species, prevalent in the Menispermaceae, Annonaceae, Umbelliferae plants [37-39]

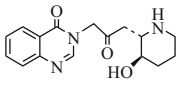


(+)-Tetrandrine
(*Cocculus sarmentosa, Cyclea barbata*, Menispermaceae) [40-42]

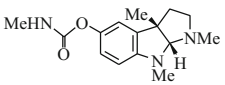
Fig. 30.1 (continued)



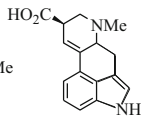
Acronycine
(*Acronychia baueri* (*Bauerella baueri*), *Melicope leptococca*, Rutaceae) [43,44]



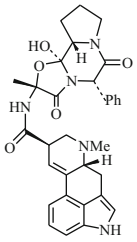
(+)-Febrifugine
(roots and leaves of Chinese drug *Dichroa febrifuga, Hydrangea* species, Hydrangeaceae) [45-49]



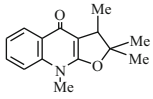
Physostigmine (Eserine)
Main alkaloid from calabar beans (*Physostigma venenosum*, Legeominosae) [50,51]



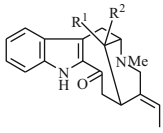
(+)-Lysergic acid
(Basic fragment derived from Ergot alkaloids), metabolite of the fungus *Claviceps purpurea* [52-54]



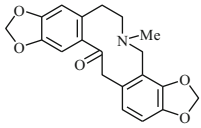
Ergotamine
Ergot (*Claviceps purpurea*) [55-57]



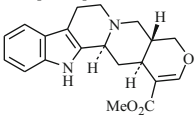
Spectabiline
(*Lemonia spectabilis*, Rutaceae) [58,59]



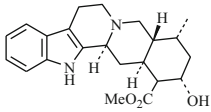
Affininine, $R^1 = CH_2OH$, $R^2 = H$
Affinisine, $R^1 = H$, $R^2 = CH_2OH$
(*Peschiera affinis*) [60]



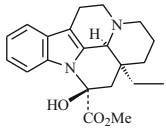
Protopine
(*Argemone bocconia, Meconopsis, chelidonium,* species and various Papaveraceae plants) [61]



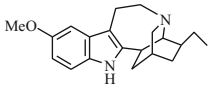
(-)-Ajmalicine
(Rauwolfia serpentina and other Apocynaceae plants, Apocynaceae) [62-64]



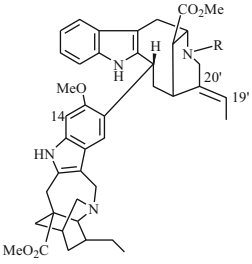
(+) Yohimbine
(*Rauwolfia, Vinca, Alchorea* species, Apocynaceae) [65-67]



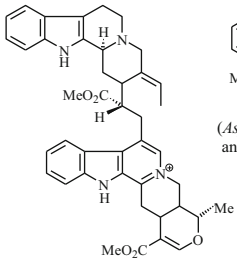
(+)-Vincamine
(*Vinca minor, Vinca major,* Apocynaceae) [68,69]



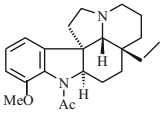
(-)-Ibogaine
(*Tabernanthe iboga,* Apocynaceae) [70-72]



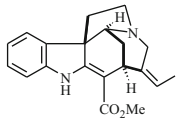
^{1}R = Me, **Condoduramine**
(*Condopharyngia durissima, Gabunia odoratissima,* Apocynaceae)
R = H, **Gabunine**
[73-75]



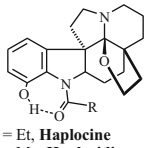
Serpentinine
(*Rauwolfia serpentina* and other *Rauwolfia* species, Apocynaceae) [76]



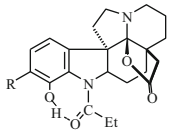
(-)-Aspidospermine
(*Aspidosperma quebracho-blanco* and other *Aspidosperma* species, Apocynaceae, [77-80]



(+)-Akuammicine
(*Alstonia scholaris, Vinca* Species, *Picralima nitida, P. kleineana , locally known as akuamma)*
[81-83]



R = Et, **Haplocine**
R = Me, **Haplocidine**
(*Haplophyton cimicidum,* Apocynaceae) [84,85]



R = H, **Cimicine**
R = OMe, **Cimicidine**
H. cimicidum [86,87]

Fig. 30.1 (continued)

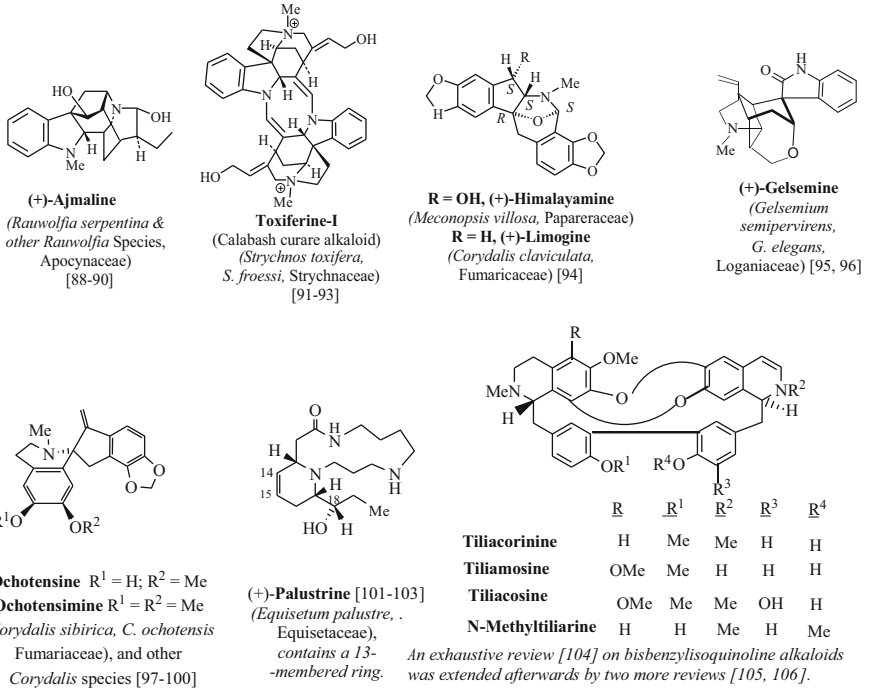


Fig. 30.1 Several alkaloids of diverse skeletal patterns (other than those included in earlier chapters)

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Chapter 31
Important Outcomes of Chemical Studies on Natural Products

31.1 Introduction

The intellectual ferment of chemical enquiry began with the man's ability to notice, observe, and reflect on things around him and with his interest and appreciation for beautiful colors, flavors, fragrance, taste, and medicinal and curative properties of plants. Thus from the dawn of civilization Nature has been appearing to be an enigma to human beings because of their innate curiosity.

If one reviews the chemical literature published during late eighteenth to late twentieth centuries, one finds the science of isolation, purification methods, structure, stereochemistry, synthesis, and biogenesis of organic compounds—mainly from plant sources. Exploration of marine flora and fauna is comparatively new. Some of the familiar plant isolates are morphine, quinine, strychnine, nicotine, atropine, colchicine, reserpine, vinblastine, vincristine, camptothecin/e, cholesterol, β-amyrin, abietic acid, taxol, caryophyllene, longifolene, santonin, geraniol, camphor, etc.—the basic updated tenets of chemistry, stereochemistry, synthesis, and biosynthesis of which have been discussed in the earlier chapters.

And a considerable part of organic chemistry has evolved from the study of the chemistry of natural products mainly during the last two centuries, though the art of brewing was known about 7000 years ago. The field of natural products chemistry is highly esteemed for being an ever-expanding and ever-giving area of research. It extends its useful hands to different planes of interactions, *viz.,* highly imaginative and elegant syntheses including stereoselective ones, the concepts of ecofriendly synthesis with maximum atom economy, green chemistry, concepts of symmetry and asymmetry of molecules, and medicinal chemistry—the research on the latter is related to man's immediate need, extending to many fundamental concepts and even laser chemistry.

In this context we may quote some relevant comments of a few outstanding organic chemists.

> The impact of all accumulated knowledge about natural compounds on pure and applied chemistry can hardly be overestimated. . .The imagination and creativity of synthetic

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> chemists was always stimulated either by usefulness, or by unusual structural and stereochemical subtleties. These features mobilized a great army of chemists who devote their knowledge of chemical reactivity, their combinatorial talents and their experimental skill in order to synthesize natural compounds with particularly challenging structures. Some of them successfully used the biomimetic methods in imitation of biogenesis. . .The fundamental concepts of stereochemistry—configuration and conformation—were conceived in the course of studies of carbohydrates, amino acids, terpenoids and related compounds. Carbonium ion chemistry, for many years a favorite topic of physical organic chemists, was discovered and to a great part investigated with bicyclic terpenes. . .Stereoelectronic effects, such as preservation of molecular orbital symmetry and anomeric effect, were uncovered in course of studies on natural products. The variety of constitutions and geometry of natural products and their analogues make them also very suitable for studies of relationship between structure and reactivity.
> V. Prelog [1]

> . . . and lure of unknown structures has in the past yielded a huge dividend of unsought fact, which has been of major importance in building organic chemistry as a science.
> R. B. Woodward [2]

> In the beginning, the isolation of chemicals from natural sources provided an unceasing stimulus to the creation and development of science.
> W. E. Doering [3]

An amazing critical review entitled "*Constructing Molecular complexity and Diversity. Total synthesis of Natural Products of Biological and Medicinal Importance*" by Nicolaou et al. [4] containing the structures and the colorful highlights of more than 75 selected natural products synthesized in his lab since 1980 and the structures of more than 100 natural products synthesized in other labs since 2000 including many complex ones and their discussions is the greatest document/archive of this field.

While writing about the ". . .newly acquired power of total synthesis to reach higher levels of molecular complexity and a wider range of structural diversity" provided by Nature, Nicolaou et al. made the following important comments in this critical review [4], "Of particular significance in most of these projects was the broadening of the total synthesis endeavor to include in its scope the discovery and development of new synthetic strategies and technologies as well as the design, synthesis, and biological investigation of novel analogues of the target molecules. Figure 45 summarizes the inspirational features of nature's molecules for total synthesis and the discoveries and inventions often derived from such challenging but rewarding endeavors. Not to be forgotten or underestimated are the unique educational and training opportunities provided to students through such demanding research programs." *Figure 45 of the article* [4] *is reproduced here as Figure* 31.1 *of this Chapter with the permission of the Royal Society of Chemistry*.

Until mid-twentieth century (pre-advanced chromatographic techniques) minor components could not be isolated. Moreover, the structural investigation work required extensive application of degradations, conversions (especially to establish stereochemistry), rearrangements, etc., and finally synthesis. *These studies required hard work of many years by pioneer chemists of those days; their vision and intellectual exertion laid the foundation of much of our present-day knowledge of chemistry*.

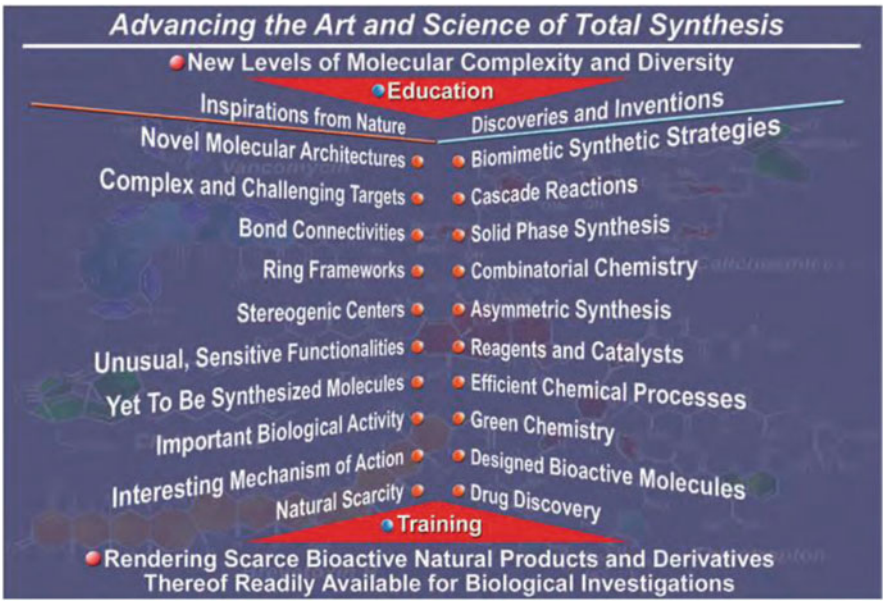


Fig. 31.1 Advancing the art and science of total synthesis through inspirations from nature (1976–2001)

Only a few important outcomes of the natural products chemistry work are included in the following sections since a comprehensive survey is well beyond the scope of this chapter.

31.2 Chromatography (see Sect. 4.1)

A brief glimpse of advanced chromatographic separation techniques, which appeared in the literature during the late twentieth century, displays how the full potential of these techniques have been utilized through the exercise of minds and experiments. Many Nobel Laureates have used various chromatographic techniques in their work. The seed of the chromatographic separation (Sect. 4.1) was sown by Tswett (Appendix A, A-27) *while separating* ***plant pigments*** *using a column filled with chalk*.

31.3 Instrumental Analysis (see Sect. 4.2)

The instrumental analysis especially the NMR technique has been designed in such a sophisticated way that in most cases it has now been possible to get from its data the full information about the structure, stereochemistry, and conformation of the

molecules under investigation. The minor components isolated in mg quantities by advanced chromatographic techniques could be solved structurally from the NMR techniques. The improvement of the instrumental techniques especially *the NMR spectroscopy is triggered by the need of the structural complexity of natural products and sometimes by their isolation in very minute amounts*. In medical sciences, the sophisticated NMR and chromatographic methods are used with great success and benefits.

31.4 Synthesis. Asymmetric (Stereoselective and Stereospecific) Synthesis [5–14]

The occurrence of the natural products in one enantiomeric form (a few exceptions), the different biological properties of the enantiomeric pairs in living systems (Chap. 32) and their complex attractive and challenging structures inspired outstanding synthetic organic chemists to synthesize these molecules in the laboratory. In doing so new reagents, new methodologies, reactions, concepts, etc., were born and enriched the field of organic chemistry. *In most of the chapters at least one or more of the above findings will appear*. In this connection we quote E.J. Corey [5].

"Nature continues to be exceedingly generous to the synthetic chemist in providing ample opportunity for discovery and creative endeavor of highest magnitude and in surrounding him with an incredible variety of fascinating and complicated molecular structures.

...The creative possibilities of synthesis are manifold and embrace not only conceptual and strategic information but also methodological discovery in the form of new chemistry and new techniques for experimental execution." Interestingly, Nicolaou commented that the birth of organic synthesis is the product of structural misassignments. He mentioned the attempted synthesis by Wöhler of ammonium isocyanate. In a review article [6] he cited examples of a number of syntheses that corrected the wrongly assigned structures of natural products. Of course, the correct structures were also corroborated by their synthesis (for examples see Chaps. 6 to 14 and Chaps. 16 to 29).

The fundamental concepts of stereochemistry, conformation, etc., conceived during the work of natural products, especially the terpenoids, steroids, and sugars, have been outlined in Chap. 2.

31.4.1 Chemical Phenomena Like Some Reactions, Rules, Degradations, Rearrangements Methodologies, Etc., Emerged from Studies of Natural Products

In the chemical literature pertaining to natural products work there are innumerable examples of new chemistry, methodology, reagents, rules, etc., of which only a few

common examples emerging from studies on some natural products have been selected for brief discussion in the sequel. Hence, this treatment is far from a comprehensive one.

31.5 From Studies on Camphor or Related Compounds

31.5.1 Bredt's Rule (K. Julius Bredt, 1855–1937)

While studying the elimination reactions with molecules having camphane and pinane skeletons during 1920–1924, Bredt observed the non-formation of double bond at the bridgehead. He also observed that Nature does not elaborate compounds with double bond at the bridgehead of small bridgeheaded bicyclic molecules. He could not offer explanation to such observations, but formulated a rule known as Bredt's rule to bring this observation to the notice of chemists in general. Bredt's rule thus states that a double bond cannot be formed at the bridgehead of small bicycle compounds [15, 16][1]. A bridgehead double bond in the Bredt's rule implies that one of the rings containing the double bond must be an excessively strained *E*-cycloalkene. Later on, the non-formation of a double bond at the bridgehead has been attributed to the fact that at the bridgehead the p-orbitals are not pure, rather hybridized, and their proper overlap to form a π-bond *needing a flat geometry is not possible in a small ring*. It is difficult to construct a model of nonbornene (**A**) which will be spatially a highly strained one and does not exist (Fig. 31.2). However, such intermediate of transient existence may be formed in some chemical reactions. Bredt's rule is not absolute and may be violated when one of the rings containing the bridgehead *E*-double bond becomes longer, and its molecular scaffold moves from rigidity to flexibility. Several anti-Bredt compounds have been reported (Fig. 31.3). Bicyclo[3.3.1]-1-nonene (**B**) is a stable compound [17–19, 27, 28], as are other bridgehead alkenes (paradoxically called Bredt olefins containing E-cyclooctene rings (Fig. 31.3) [28]. 4-(1-Adamantyl)homoadamantyl-3-ene (**C**) is quite stable [23]. An *E*- double bond, if present as a part of a 5-, 6-, or 7-membered ring in a bridgeheaded bicyclic system, is severely strained and highly unstable.

On the basis of Bredt's rule, many structures have been modified and corrected in the literature.

[1] The reference numbers 17 to 28 are cited according to the sequence of the reference numbers in Figure 31.3.

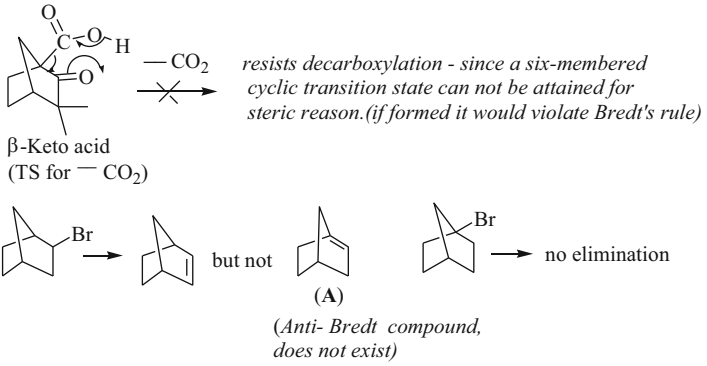


Fig. 31.2 Some consequences of Bredt's rule

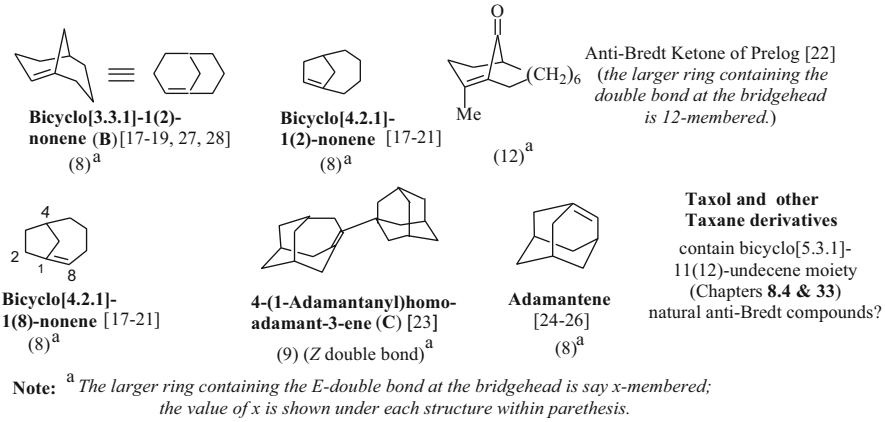


Fig. 31.3 Reported anti-Bredt compounds

31.5.2 Wagner (George Wagner, 1849–1903)—Meerwein (Hans Lebrecht Meerwein, 1879–1965) Rearrangement [29, 30]

During the conversion of α-pinene to camphor, Wagner first identified the formation of camphene from bornyl chloride as well as borneol [29]. Later, Meerwein realized [30] that a rearrangement was needed during this conversion. These concepts led to the genesis of Wagner–Meerwein (**W–M**) rearrangement of carbonium ion species (Sect. 6.3).

In most cases of W–M rearrangement a low energy tertiary carbocation [e.g., (**D**)] is formed from a high energy secondary [e.g., (**C**)] or primary carbocation through (1,2)-hydride shift (Fig. 31.4). Here 1,2-Me shift from (**C**) would produce the same cation. The overall reaction involves three stages: (i) generation of carbocation,

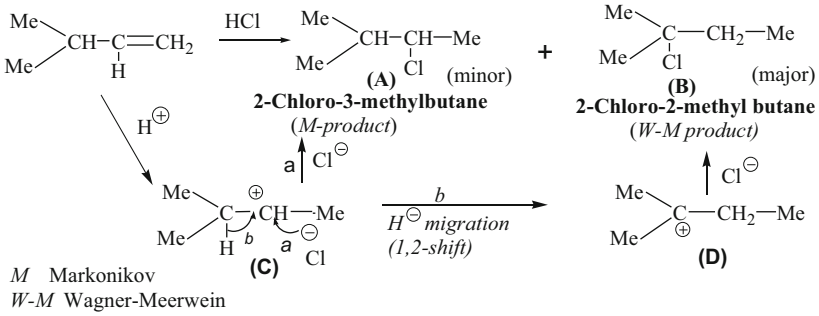


Fig. 31.4 Competitive products of low-energy carbocations (*M* versus *W–M* reactions)

(ii) 1,2 shift to generate low energy carbocation, and (iii) quenching of the carbocation to form a neutral molecule. The regiospecificity of the reaction depends on the stabilities of the intermediate carbocations based on resonance and inductive effect. The filled σ orbital (HOMO) of the alkyl group overlaps with empty 2p orbital (LUMO) of the carbocation. W–M rearrangement is a case of simplest and best known sigmatropic shift, and the allowed 1,2-shift occur suprafacially. Generally, the three stages of the W–M rearrangement are concerted to entail an inversion of configuration of both the migration origin and migration terminus, with retention of configuration of the migrating group in appropriate cases. In many cases multistep Wagner–Meerwein rearrangement takes place. Mechanistically many reactions (e. g., pinacol–pinacolone, Curtius–Wolf, Beckmann, Demyanov, Hofmann) may be considered as the special cases or variants of W–M rearrangement since they are routed through carbonium ions, although they may involve different leaving groups. During the biosynthesis of natural products especially terpenoids and steroids 1,2-migration occupies the central position (many examples are available in Chaps. 6–11 etc).

31.5.3 Nametkin Rearrangement

The rearrangement in which Me group migrates is sometimes called Nametkin rearrangement (1927). *W–M Rearrangements involving multi-migration provides immeasurable insight in the biosynthesis of natural products, mainly terpenoids and steroids, with their structural concepts*. Some examples of Nametkin rearrangement and some more examples of *W–M* rearrangements are shown in Fig. 31.5.

The W–M rearrangement and Nametkin reaction initiated by the carbocation and sterically suited bond migration (multi-W–M rearrangement) is almost a monopoly of terpenoid chemistry (Chaps. 6–11) especially of lower terpenoids.

In connection with the studies on the fate of steroids in the fossils, some W–M rearrangements of steroids have been studied with PTS in acetic acid. For example, when the steroid (**B**) is treated with PTS, a spiro compound is formed as a

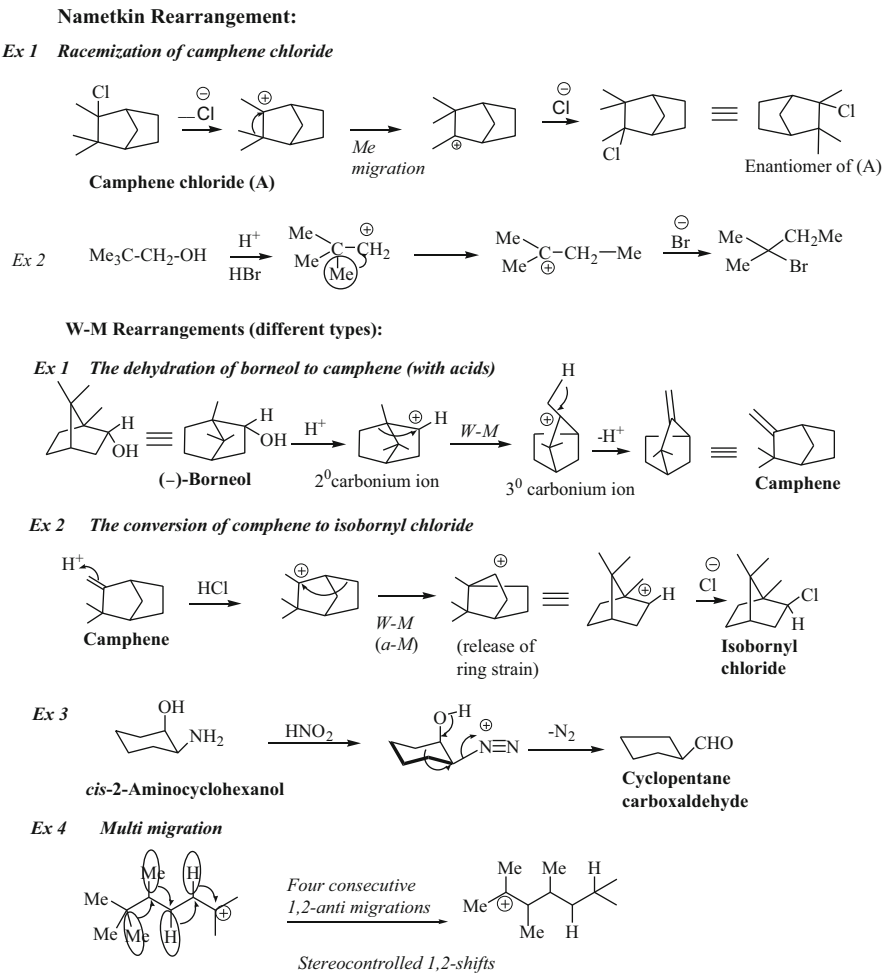


Fig. 31.5 Some examples of *W–M* and Nametkin rearrangements

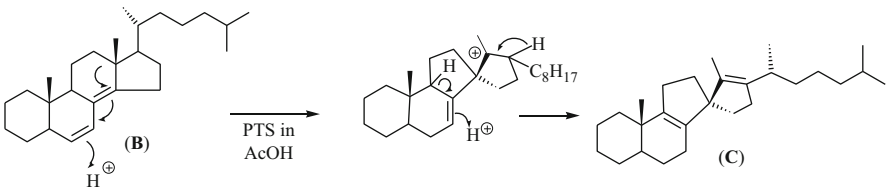


Fig. 31.6 A steroid (**B**) converted to spirosteroid (**C**) by W–M rearrangement

consequence of a W–M rearrangement initiated by protonation, followed by collapse of the generated carbonium ion by loss of an adjacent proton, and isomerization of the double bond formed to a more substituted one giving the final product (**C**) (Fig. 31.6) [31].

31.5.4 Chiral Auxiliary

Camphor and its derivatives have been extensively used as chiral auxiliaries (Chap. 32).

31.6 From Studies on Menthone

31.6.1 Baeyer–Villiger Oxidation

In 1899 Adolf von Baeyer (1835–1917, NL 1905) and Victor Villiger (1868–1934, a student of Baeyer) treated menthone with peroxymonosulfuric acid ($HOOSO_3H$, Caro's acid) and obtained a lactone [32, 33] (Fig. 31.7). The reaction was applied to camphor (Sect. 6.3, reaction **IX**) and other alicyclic ketones. Kurt Mislow (1923-) named the reaction as Baeyer–Villiger oxidation reaction. It has become one of the staple oxidation reactions [33] of aldehydes and cyclic, aromatic, as well as open-chain ketones. Carboxylic esters or lactones are formed due to *insertion of an oxygen atom* between the CO group and the C2 carbon (in case of aldehyde) or one of the carbons (in case of a ketone) attached to the carbonyl in the substrate molecule. Baeyer–Villiger oxidations of menthone, camphor and norcamphor are delineated in Fig. 31.7.

In absence of any complicating steric factor, for example, in case of norcamphor usually the more substituted and more electron-rich C1 migrates to O, forming the lactone (**C**) preferentially. But, in case of camphor the *exo* face being sterically hindered, initial attack by peracetic acid on the CO carbon preferentially takes place

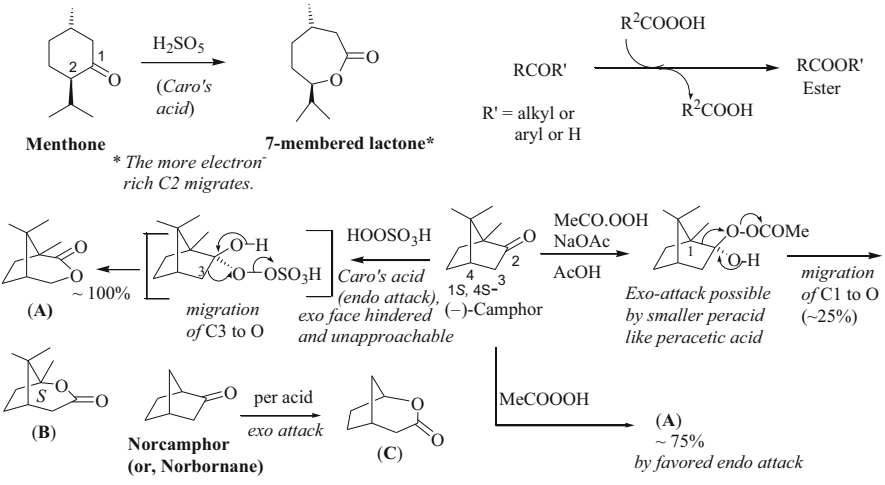


Fig. 31.7 Baeyer–Villiger (B–V) oxidations of menthone, camphor, and norcamphor

from the *endo* face resulting in the preferential migration of C1 to form the lactone (**A**) (75 %), but *endo* face attack occurs exclusively when the bulky Caro's acid is used; using peracetic acid 25 % of (**B**) is also formed. Steric effects in Baeyer–Villiger oxidation and dependence of the reaction course on the acidity of the medium have been studied [34].

Reagents Various reagents for Baeyer–Villiger oxidations, e.g., Caro's acid (H_2SO_5), a solution of peracetic acid ($MeCO_3H$) in acetic acid containing sulfuric acid or *p*-toluenesulphonic acid as a catalyst, *m*-chloroperbenzoic acid (MCPBA), peroxytrifluoroacetic acid, peroxymonophosphoric acid, or monopermaleic acid in methylenechloride solution, or 90 % H_2O_2 have been introduced and used for reaction with different ketones. The reaction with peroxytrifluoroacetic acid is rapid and clean, giving high yield of the product; it is thus usually the reagent of choice. However, to minimize any *transesterification* with the product ester a buffer such as disodium hydrogen phosphate, Na_2HPO_4 is added to the reaction mixture.

Migratory Aptitude From a study of various alkyl aryl ketones the relative ease of migration of various groups (anti to the leaving group) in the B–V reaction has been found to be of the following order:

$$3^{\circ}\text{-alkyl} > \text{cyclohexyl} \sim 2^{\circ}\text{-alkyl} \sim \text{benzyl} \sim \text{phenyl} > 1^{\circ}\text{-alkyl} > \text{cyclopropyl} > \text{methyl} > \text{H}$$

Among the aromatic groups, as expected, the more electron donating group preferentially migrates in absence of any steric factor:

$$p\text{-anisyl} > p\text{-tolyl} > \text{phenyl} > p\text{-chlorophenyl} > p\text{-nitrophenyl}$$

Much less regiospecific preferential migration takes place when 15 % H_2O_2/NaOH is used as the reagent.

Stereochemistry of Migration B–V rearrangement takes place with *retention of configuration of a chiral migrating group* and thus an optically active ketone leads to an optically active ester. Formation of (**B**) and (**C**) in Fig. 31.7 exemplifies this. The concerted O–O bond cleavage and migration is usually the rate determining step. The new σ-bond is formed on the same face of the migrating group leading to the retention of configuration. Some examples with simple acyclic and cyclic molecules are cited in Fig. 31.8.

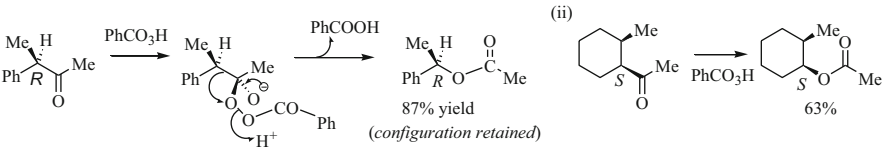


Fig. 31.8 Migration of a chiral group with retention of configuration in B–V reaction

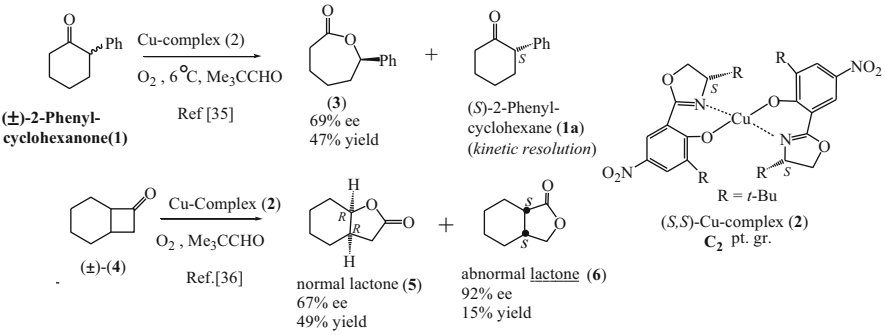


Fig. 31.9 Two examples of enantioselective metal-catalyzed B–V oxidation [35, 36]

31.6.2 Enantioselective Metal-Catalyzed Version of B–V Oxidation

B–V oxidation has been developed using molecular oxygen in presence of a metal complex catalyst and an oxygen acceptor, a bulky aldehyde such as isovaleraldehyde (Me_2CHCH_2CHO) or pivaldehyde (Me_3CCHO). Two examples are given here. Thus, racemic 2-phenylcyclohexanone when treated with molecular oxygen in presence of 1 mol% of the chiral copper-complex (**2**) and pivaldehyde gave the corresponding optically active lactone (**3**) with 69 % ee in 47 % yield at 6 ^{0}C along with the optically active starting ketone (1a), as a result of kinetic resolution (Fig. 31.9) [35].

The copper complex-catalyzed oxidation of the *rac*-cyclobutanone (**4**) with the active copper catalyst (**2**) transformed the two enantiomeric ketones into two regioisomeric lactones (**5**) and (**6**) (Fig. 31.9) [36]. The stereochemistry of such B–V reactions is reported to be controlled by two factors: (i) face selectivity during oxidant addition and (ii) enantiotopos selectivity during migration.

31.6.3 Microbial Baeyer–Villiger Oxidation [37–44]

Microbial Baeyer–Villiger (B–V) oxidation of cyclohexanone and substituted cyclohexanones using purified cyclohexanone oxygenase enzyme [37] has produced the corresponding lactones, analogous to the classical B–V rearrangement (Fig. 31.10). $NADP^+$ and H_2O are the bye-products in these reactions.

In recent years extensive studies have been reported on the substrate selectivities and enantioselectivities of many novel alkyl substituted *cyclohexanone monooxygenases* (CHMO) in microbial B–V oxidations [40–43], and evolution of enantioselective B–V monooxygenases for asymmetric catalysis [44].

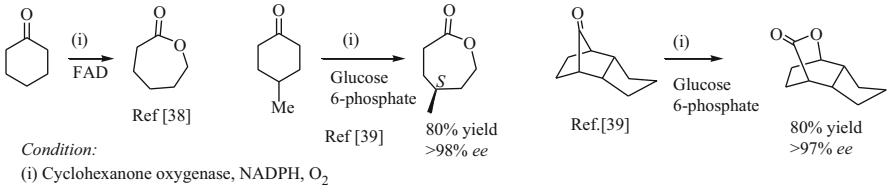


Fig. 31.10 Microbial B–V oxidation of cyclohexanone, methylcyclohexanone and a tricyclic ketone

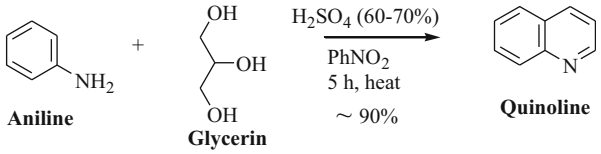


Fig. 31.11 Skraup reaction

31.7 From Studies on Quinine

Much chemistry and some important events evolved during the structural elucidation work on quinine and other related *Cinchona* alkaloids (Chap. 25).

31.7.1 Skraup Reaction

One such example is **Skraup reaction** [45–49] for quinoline synthesis. Czechoslovakian chemist Zdenko Hans Skraup (1850–1910) worked on quinoline alkaloids as an assistant of Rochleder and helped in the structural elucidation work. While working on quinoline alkaloids, he saw the work (1877) of Prudhomme on the dye alizarin blue, which was obtained by heating nitroalizarin with glycerin and sulfuric acid to form a pyridine ring. This prompted him to synthesize quinoline, using aniline, nitrobenzene, glycerin, and sulfuric acid, (Fig. 31.11) and he presented his work in 1880.

Mechanistically, acrolein (propenal) is generated in situ and a Michael-type addition takes place, followed by ring closure, dehydration and oxidative dehydrogenation (Fig. 31.12).

Various oxidizing agents have been used and the reaction has been carried out with modified substrates for better yield. This reaction has found wide application. The aniline as well as the Michael substrate glycerin may be substituted to yield differently substituted quinolines.

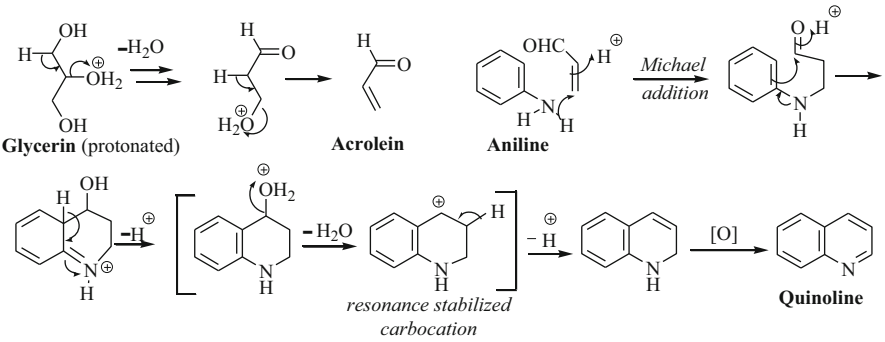


Fig. 31.12 Mechanistic rationale for Skraup's synthesis of quinoline

31.7.2 A Precursor of Meerwein–Pondorff–Verley (MPV) Reduction [50]

In search of Rabe's aluminium powder that yielded quinine from quinotoxine in 1907, it was found that that powder must have contained some Al^{III} impurities to give significant conversion of the ketone substrates to the secondary alcohols. A further inference from these studies is that Rabe–Kindler aluminium powder reduction may be viewed as an early "activated" progenitor of the Meerwein–Pondorff–Verley reduction (MPV) [50] which was published by these three investigators independently in 1925–1926.

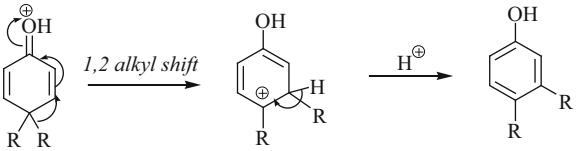
31.7.3 Some Other Outcomes

(i) *Discovery of the First Synthetic Dyestuff Mauve, a Serendipitous Product. Beginning of Chemical Industry* (Appendix B, 1856).
(ii) *The first drug of malaria. Beginning of Chemotherapy* (Chap. 33).
(iii) *Chemically manipulated quinine and its congeners serve as very important chiral auxiliaries* (Chap. 32), *the privileged catalysts and ligands for asymmetric catalysis.* The discovery of *Cinchona* alkaloids-derived chiral auxiliaries as excellent ligands (quinuclidine nucleus) for OsO_4 gave birth to the Sharpless *asymmetric dihydroxylation* (AD), one of the most reliable reactions in asymmetric synthetic strategies (Sect. 32.5.1).

31.8 From Studies on Santonin

31.8.1 Dienone–Phenol Rearrangement [51]

4,4-Disubstituted cyclohexadienones under the influence of an acid undergo 1,2-migration of a substituent to form 3,4-disubstituted phenols. The driving force of this rearrangement is the aromatization in the product as follows:



This reaction is an outcome of a reaction of santonin having a decalindienone moiety with an acid when a phenolic compound was obtained, identified, and named desmotoposantonin (cf. Figs. 7.42 and 7.44). This reaction received a generality for compounds having the structural feature of ring A of santonin or its equivalent and is known as dienone–phenol rearrangement (Fig. 31.13).

This type of rearrangement is also observed during the biosynthesis of aporphine alkaloids from the immediate precursors proaporphines, one example of which is given in Fig. 31.14. Usually aporphines are rationalized to be the enzyme catalyzed *ortho–ortho* or *ortho–para* oxidative coupling products of the suitably substituted benzyltetrahydroisoquinoline alkaloid precursors (Chap. 30). In case of the latter alkaloids possessing an OH only at the C3′ position of the benzyl part, the aporphine alkaloids can be biosynthesized through their immediate precursor, proaporphine by means of a dienone–phenol rearrangement (Fig. 31.14). Thus the oxidative coupling *ortho–para* to the phenol groups of (*S*)-*orientaline* forms the dienone (*S*)-*orientalinone* which upon dienone–phenol rearrangement would give an aporphine (**A**). (*S*)-Orientalinone upon reduction to the corresponding dienol, followed by dienol–benzene rearrangement produces a natural aporphine (*S*)-*isothebaine*, isolated from *Papaver orientale*. This rearrangement thus gives us an insight about the biosynthesis of aporphine alkaloids in plant cells.

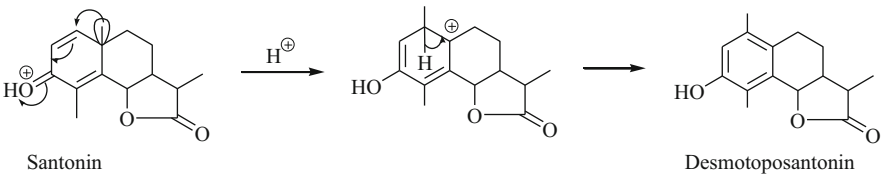


Fig. 31.13 Acid-catalyzed dienone–phenol rearrangement of santonin

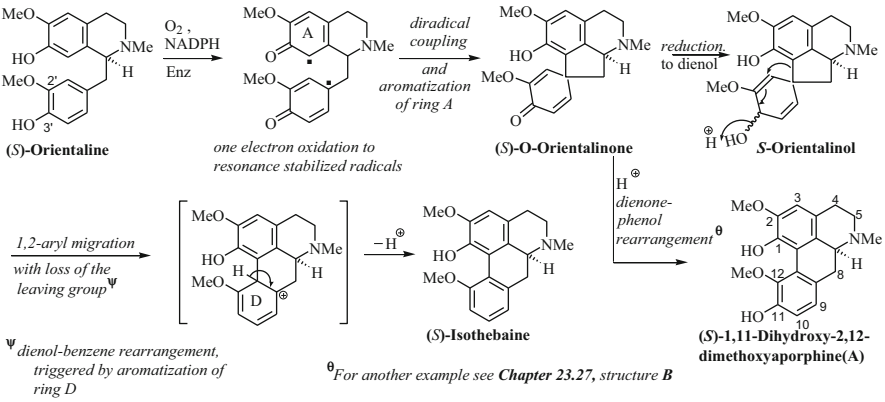


Fig. 31.14 Biosynthesis of aporphines involving dienone-phenol and dienol-benzene rearrangements

31.8.2 Intramolecular Michael Addition

During the structure elucidation of santonic acid Woodward discovered intramolecular Michael addition as a means of bridgehead formation via carbanion leading to a tricyclic skeleton. It turned out to be an important methodology. Corey utilized this methodology for the synthesis of the tricyclic framework of longifolene (Fig. 7.34).

31.9 From Studies on Coniine

31.9.1 Hofmann Degradation/Hofmann Elimination

While working with the structural elucidation of coniine (Chap. 17) A.W. Hofmann (1818–1892) observed that quaternized coniine methiodide (exhaustive methylation) when heated with Ag_2O and water forms a nitrogenous olefin. On repeating the process with this nitrogenous olefin a non-nitrogenous diene is formed and nitrogen is eliminated from the molecule (see Fig. 17.3). He further suggested that when nitrogen is present as a part of two rings, three times of such treatment will be necessary to eliminate nitrogen from the molecule to form a non-nitrogenous triene (Fig. 31.15). This degradative method is known as Hofmann degradation/elimination. It is a case of E2 elimination and a suitable β-H is needed. If more than one suitable β-Hs are present, a mixture of olefins is obtained. If a suitably disposed intramolecular nucleophile is present at a β-position to the quaternized nitrogen, an intramolecular S_N2 reaction takes place (e.g., see Fig. 17.5). Additionally, methanol is always co-formed.

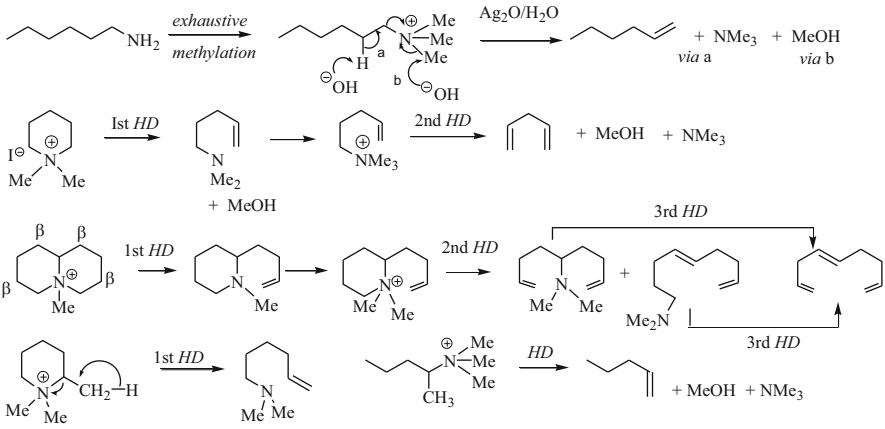


Fig. 31.15 Hofmann degradation of acyclic and cyclic amines and amines with ring juncture nitrogen

According to Hofmann rule the decreasing order of the ease of proton abstraction from the β-position is $–CH_3 > RCH_2– > R_2CH–$; the direction of olefin formation (E2 mechanism) is generally governed by steric factors in the transition state of the E2 elimination.

31.9.2 The Hofmann–Löffler–Freytag (H–L–F) Reaction (Reactions at Nonactivated Carbon Atom Involving Intramolecular H Abstraction by N Radical From δ- (or 4-) Position)

N-Bromoconiine upon heating with H_2SO_4 followed by treatment with a base formed δ-coneceine (Fig. 31.16). Hofmann made this observation [52] as early as 1883. This reaction was later shown to be a general process for the synthesis of pyrrolidines. (±)-Nicotine was synthesized by Löffler [53] in 1909 by this reaction. Formations of some other pyrrolidine and piperidine derivatives are shown in Fig. 31.16.

This is a general reaction of N-chloroamines or N-bromoamines possessing a δ-CH moiety. The mechanism of this reaction was initially suggested by Wawzonek in 1951. Later, it was studied in detail by Corey [54] and Neale [55, 56]. The formation of a trigonal radical is suggested for the optical inactivity of the products [54, 55] (Fig. 31.17).

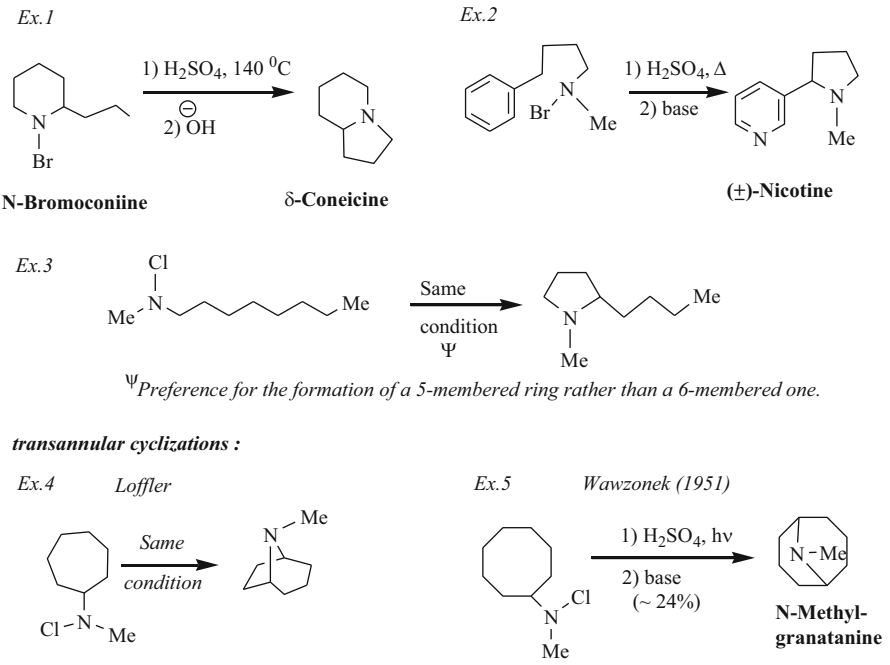


Fig. 31.16 Formations of pyrrolidine and piperidine derivatives with suitable *N*-haloamines

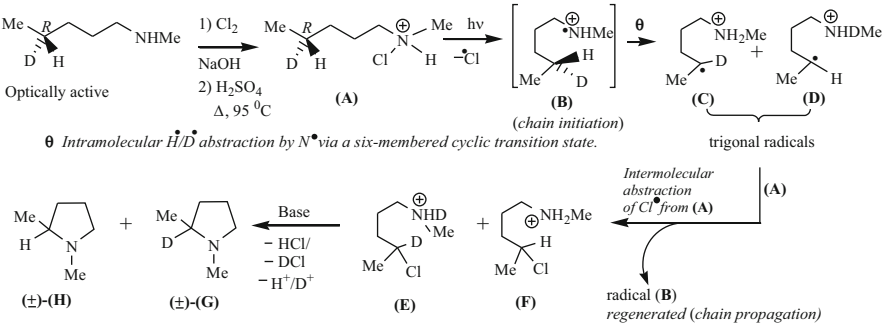


Fig. 31.17 Mechanism of photochemical H–L–F reaction. Conversion of (*R*)-(A) into (±)-G and (±)-H by chain reaction through trigonal radicals

Thus, protonated amines undergo photochemical or thermal decomposition to yield pyrrolidine derivatives from suitable substrates via radical intermediates.

Synthesis of Dihydroconessine [57–59]. The H–L–F reaction has been elegantly applied in the terminal stage of the synthesis of (±)-dihydroconessine (Fig. 31.18).

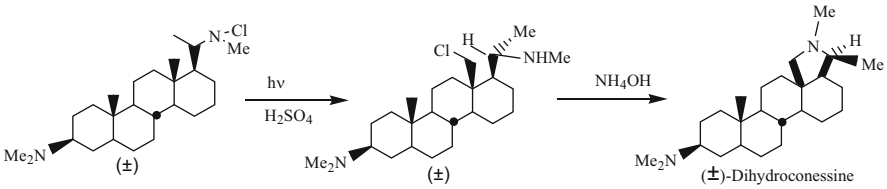


Fig. 31.18 Application of the H–L–F reaction in the terminal stage of dihydroconessine synthesis

31.10 From Studies on Morphine

31.10.1 Morphine, A Miraculous Pain Killer

Morphine finds wide application as a remarkable pain killer in cases of postsurgery patients (see Chaps. 23 and 33).

31.10.2 Phenanthrene Chemistry and Pschorr's Synthesis of Phenanthrene

During the structural elucidation of morphine a number of differently substituted phenanthrene derivatives were obtained (Chap. 23). They have direct bearing on the structure of morphine. Pschorr (Robert Pschorr, 1868–1930, German Chemist) who was interested in morphine chemistry, devised a method (1896) [60, 61] for the synthesis of these compounds. His method has become a general method of phenanthrene synthesis (Fig. 31.19) and is well known as Pschorr synthesis [62, 63]. Thus the chemistry of phenanthrenes started with the study of morphine molecule.

In this method an appropriate *o*-nitrobenzaldehyde is allowed to react with sodium phenyl acetate in presence of acetic anhydride when Perkin condensation takes place resulting into the corresponding *cis*-cinnamic acid. The nitro group is then reduced with ammoniacal ferrous sulfate solution to an amine. The latter is diazotized and subjected to Cu-catalyzed (Cu-powder) ring closure through the elimination of nitrogen to form phenanthrene carboxylic acid. The latter loses CO_2 on heating to give phenanthrene. Altogether four steps are involved (Fig. 31.19).

The reaction has shown a wide scope and has been used for the synthesis of variously substituted phenanthrenes. It has been extended to the synthesis of other aromatic systems [62, 63], *e.g.*, fluorenone, diphenyl derivatives, etc. (Fig. 31.20).

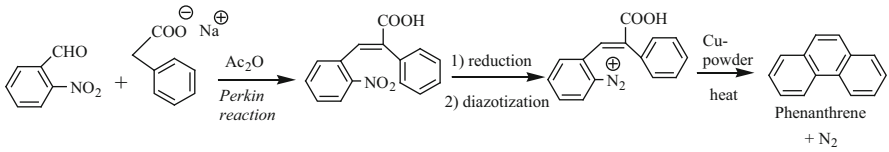
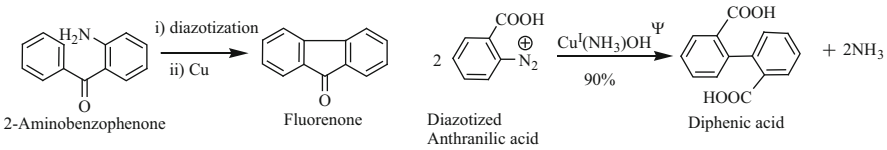


Fig. 31.19 Pschorr's synthesis of phenanthrene



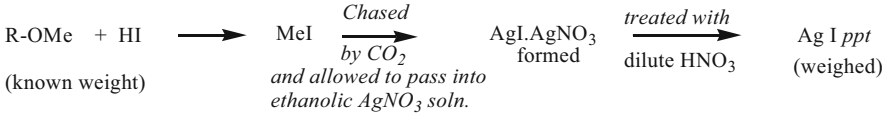
Ψ *It is prepared by treating $CuSO_4$ in NH_4OH with NH_2OH. The reaction probably occurs by dimerization of the aryl radicals generated by one-electron reduction of the diazonium ion.*

Fig. 31.20 Extension of the Pschorr reaction to the synthesis of fluorenone and diphenic acid

31.11 From Studies on Colchicine

31.11.1 Zeisel's Gravimetric Method for Methoxyl Group Estimation and Its Subsequent Modification to a Volumetric Method

During the structural work on Colchicine (Chap. 24), S. Zeisel [64–67] developed a method for the estimation of the methoxyl group present in an organic compound. The reactions are shown below:



However, the method was inapplicable in sulfur containing compounds. Later the method has been modified to a volumetric method by Vieböck.

Ψ *Generated MeI is chased by CO_2 into a solution of Br_2 made in AcOH/NaOAc*

1Br is oxidized to HIO_3 which liberates iodine from HI; the liberated iodine is estimated with standardized sodium thiosulfate ($Na_2S_2O_3$) solution

$$1Br + 2Br + 3H_2O \rightarrow HIO_3 + 5HBr$$
$$HIO_3 + 5HI(\text{from KI}) \rightarrow 3I_2 + 3H_2O$$
$$3I_2 + 6Na_2S_2O_3 \rightarrow 6NaI + 3Na_2S_4O_6 \text{ (Sodium tetrathionate)}$$
$$\text{Thus -OMe} = HIO_3 = 3I_2 = 6Na_2S_2O_3 \text{ (standardized)}$$

This method is applicable to sulfur containing compounds also. This method has further been modified. The modified Zeisel's method is sometimes known as *Zeisel–Pregl–Viebock and Brecher's* method although usually it is referred to as Zeisel method.

Prior to the NMR spectroscopic analysis the above method played an important role in the estimation of methoxyl group. Further, methoxyl group estimation of the chemically fragmented molecules sometimes gave an idea about the location of the methoxyl group/s in the parent molecule: Natural products hardly elaborate OEt group. However, if both methoxyl and ethoxyl groups are present they can be differentiated by Kuhn–Roth oxidation when ethoxyl group gets oxidized to acetic acid, while methoxyl group does not.

The presence of four methoxyl groups in papaverine (Chap. 22) was detected by Zeisel's method.

31.11.2 Tropolone Chemistry. Tropylium Ion

M.J.S. Dewar first suggested the presence of cycloheptatrienolone moiety in colchicine [68] on the basis of his work on *stipitatic acid* [69]. It should be mentioned that Tetsuo Nozoe assigned *hinoketiol,* isolated from the essential oil of *taiwanhinoki* in 1941, a seven-membered nonbenzoid aromatic structure based on Pauling's resonance concept. It was the first of the troponoids, but it went unnoticed till 1951 [70]. Dewar termed the cycloheptatrienone system as tropolone in 1945 and thus began tropolone chemistry [71]. Tropolone, also known as tropone, is planar, lacks ketonic properties, and in fact, has markedly different properties than aliphatic or alicyclic ketones. It is better represented by the dipolar structures which constitute the aromatic tropylium system stabilized by resonance, as shown in Fig. 31.21. Tropolone undergoes electrocyclic reactions. A colchicine photoproduct (Chap. 24) serves as the first example of an electrocyclic reaction. Again, cycloheptatrienyl bromide (tropylium bromide) is planar and is a salt, unlike aliphatic bromides, since cycloheptatrienyl (tropylium) cation is aromatic (6π electrons in cyclic conjugation) and hence strongly resonance stabilized (Fig. 31.21).

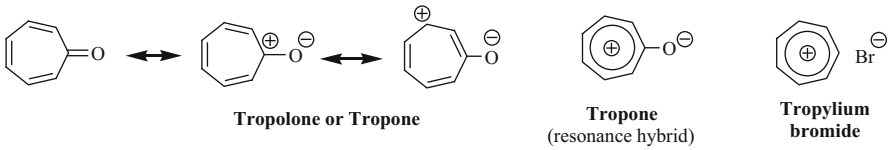


Fig. 31.21 Tropolone and tropylium ion

31.12 From Studies on Coumarins

31.12.1 Perkin Reaction [72–74]

Coumarin was isolated in 1820 from Tonka bean (*Coumarauma odorata*). Fittig gave its correct structure in 1868. The same year Perkin (William Henry Perkin, 1838–1907, British Chemist) synthesized coumarin in 34 % yield by heating salicyldehyde with acetic anhydride in presence of sodium acetate. The other product has been *o*-hydroxycinnamic acid (Fig. 31.22); *for mechanism, see* Sect. 13.2.3.1. Using a suitable aromatic aldehyde and heating the aldehyde with acetic anhydride and sodium acetate, variously substituted cinnamic acids could be synthesized (Fig. 31.22). This condensation of an aromatic aldehyde with an aliphatic acid anhydride in presence of sodium or potassium salt of the corresponding acid is known as *Perkin reaction*; the reaction serves as a general method for the synthesis of cinnamic acids. The cinnamic acids thus formed can be converted into other products—so Perkin reaction finds importance in getting a number of other types of compounds.

31.12.2 Pechmann Reaction [75–77]

Hans von Pechmann (1850–1902, German Chemist) synthesized *umbelliferone,* (7-hydroxycoumarin) (Sect. 13.2) in 1884 by heating malic acid with resorcinol in presence of conc. H_2SO_4 (Fig. 31.23).

The reaction has been extended to β-ketoesters (Fig. 31.23). Such acid-catalyzed condensation of phenols with β-ketoesters to produce coumarins is known as Pechmann reaction (Sect. 13.2.3). A variety of other condensing agents such as hydrogen fluoride, polyphosphoric acid, phosphorus pentoxide, aluminium chloride, and other Lewis acids have been used for the synthesis of coumarins. The Pechmann catalyst has also been largely modified (Sect. 13.2.3.4) and differently substituted coumarins have been synthesized.

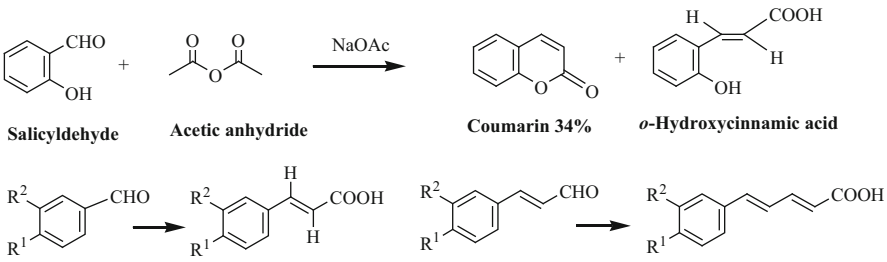


Fig. 31.22 Perkin reaction: some examples

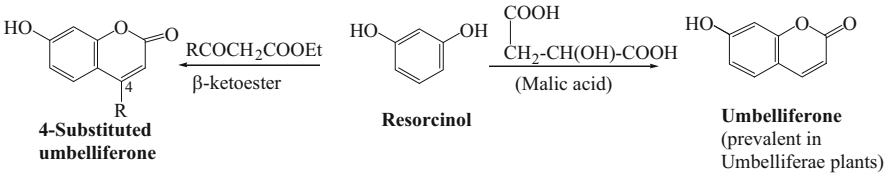
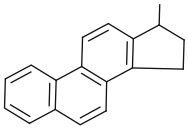


Fig. 31.23 Pechmann reaction

31.13 From Studies on Steroids

31.13.1 Diels Hydrocarbon. Se Dehydrogenation [78–80]

Otto Paul Hermann Diels [1876–1954, NL 1950—shared with his student Kurt Alder (1902–1958)] introduced the use of Se in the chemical dehydrogenation of polynuclear compounds. In 1925, he obtained a hydrocarbon, $C_{18}H_{16}$, m.p. 127 °C, by Se-dehydrogenation of cholesterol. Its structure was determined, and the compound is known as Diels hydrocarbon. This method was applied by Diels to other steroids; many of them produced the Diels hydrocarbon. Biogenetically important steroid groups were shown to have the same carbon scaffold since all of them on Se-dehydrogenation yielded the same Diels hydrocarbon.



Diels hydrocarbon

In 1934 Diels hydrocarbon was synthesized; its structure gave a vital clue to the structures of steroids; all of them contain four rings (methylcyclopentanoperhydrophenanthrene) and thus this skeletal pattern as derived by Diels has become the identifying feature of the steroids.

Later on, Se dehydrogenation has been applied to many triterpenes, diterpenes, and alkaloids when their skeletal orientation could be arrived at from the aromatic hydrocarbon produced (Sect. 4.2).

31.13.2 Barton Reaction (A New Photochemical Reaction for Remote Functionalization) [81–85]

While working with the synthesis of some steroids Barton invented a new photochemical reaction to functionalize a distal nonactivated methyl or methylene carbon at the δ-position of an alcohol. This reaction is known as Barton reaction. He photolyzed the *o*-nitroso (at C11) derivative of corticosterone acetate (**A**) in toluene solution to obtain the 11-hydroxy-18-aldoxime derivative. The latter upon hydrolysis produced aldosterone-21-acetate via the intermediate δ-hydroxyaldehyde. (Fig. 31.24). He very successfully employed this method of distal functionalization to convert 3β-acetoxy-5α-pregnan-20β-nitrite (**B**) to the oxime derivative (**C**) (Fig. 31.24) and in the case of β-amyrin synthesis (Chap. 10).

Thus in Barton reaction an OH is used to oxidize a saturated methyl or methylene four bonds away to an oxime through photolysis of its nitrite ester. The stepwise mechanistic rationale of this reaction is delineated in Fig. 31.25. *The*

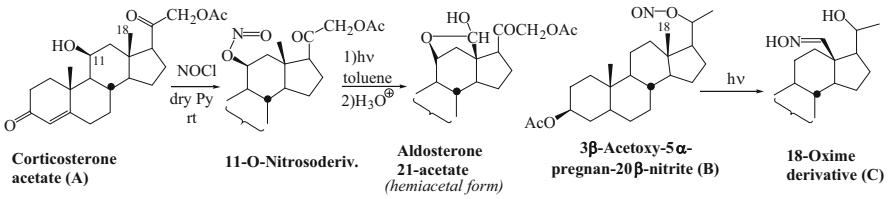
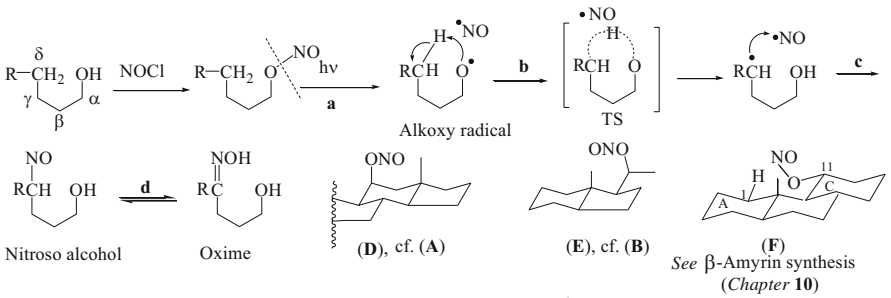


Fig. 31.24 Barton reaction in case of two steroids: functionalization of $18CH_3$



a homolytic cleavage of O-NO bond generates an alkoxy and an •NO radicals
b The alkoxy radical abstracts H from the carbon at δ–position through a 6-membered cyclic TS
c bonding between $\dot{N}O$ *and* $\delta\,\dot{C}HR$
d tautomerization

Fig. 31.25 Mechanistic rationale for Barton reaction (Fig. 31.24)

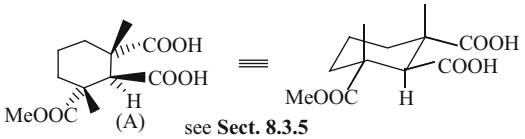
proximity of the reacting groups, as in 1,3-diaxial orientation in (**A**) or (**D**), or as in (**B**) or (**E**), or as in (**F**) [diequatorial in rings (**A**) and (**C**)] (Figs. 31.24 and 31.25), capable of forming a 6-membered cyclic transition state, *is a prerequisite*. A chain mechanism for this reaction could also be written, but a non-chain mechanism is appropriate since $^{\bullet}N{=}O$ is a stable free radical, which hangs nearby until it combines with the alkyl radical to form a strong C–N bond.

Photolysis is done by irradiation in a suitable nonhydroxylic solvent (like toluene) using a high pressure mercury arc lamp in N_2 atmosphere. A pyrex filter is used to get the radiation of wavelengths more than 300 nm.

31.14 From Studies on Abietic Acid [86–90]

31.14.1 Conformational Analysis [86–90]

Barton applied semiempirical and semiquantitative known methods of calculations of non-bonded interactions to ethane, to cyclohexane, and to the *trans*- and *cis*-decalins in 1948 [86, 87]. These methods were exceedingly arduous in absence of computers. To obviate this problem Barton constructed special models [88], which later proved to be very useful in working out the principles of conformational analysis. These models also led to the understanding, in conformational terms, the dissociation constant of the monomethyl ester (**A**), of the tricarboxylic acid obtained by the oxidative degradation of *abietic acid* [89]. In fact, this is an early example of conformational analysis [90] (for details, see Sect. 8.3.5, Sect. 2.12.1, and Appendix B, 1948–1950).



see **Sect. 8.3.5**

31.15 Biomimetic Synthesis

31.15.1 Biomimetic Synthesis of Tropolones

The concept of biomimetic synthesis was first elaborated by Robinson when he synthesized tropinone in a biomimetic fashion in a single pot [91] (see Sect. 19.3.2). This reaction also serves as the first example of a **multicomponent synthesis** [92] (*cf.* Appendix B, 1917). However, literature survey shows that **Strecker reaction**

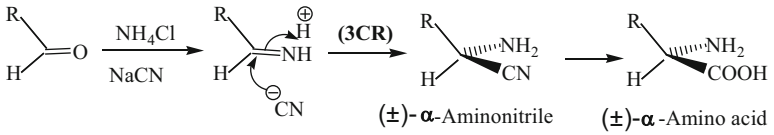


Fig. 31.26 Strecker Reaction (MCR)

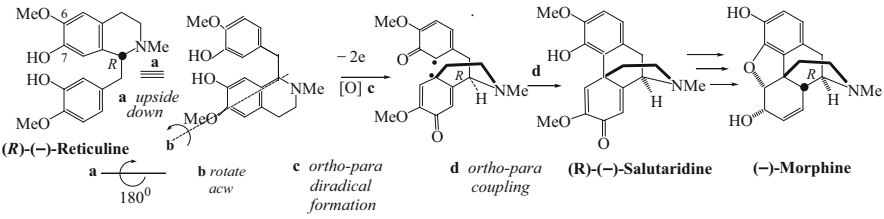


Fig. 31.27 Biomimetic conversion of (*R*)-(−)reticuline to (*R*)-(−)-salutaridine

(synthesis) in which an α-amino nitrile, an immediate synthon for the corresponding amino acid, has been prepared in one step in one pot using three reagents at a time. An aldehyde is treated with ammonium chloride, and sodium cyanide when ammonia is generated *in situ*; the latter reacts with the aldehyde to form an imine which reacts with the cyanide to form the corresponding α-aminonitrile (Fig. 31.26). The reaction was reported in 1850, and being a three-component reaction, may be looked upon as the first reported multicomponent reaction (MCR).

31.15.2 Biomimetic Oxidative Coupling of Phenols

The isolation and structural elucidation of a large number of biaryl compounds of great structural varieties isolated from plant materials have made the titled topic an exceptionally active area of research. Such oxidative coupling can be done in the laboratory. It is very close to the Nature's way to elaborate biaryls. Barton has contributed significantly in the field of biomimetic synthesis of this type. To cite an example, Barton has converted (*R*)-(−)-reticuline to salutaridine [93] where one electron transfer triggers the biaryl coupling, mimicking the biosynthesis of the latter (Fig. 31.27). This concept has been applied and extended to understand the biosynthesis of many natural products, e.g., to the biosynthesis of morphine alkaloids [94] (Fig. 31.27).

31.16 From Studies on β-Carotene

31.16.1 Kuhn-Roth Oxidation: Methyl Side-Chain Determination (1931)

During the structure elucidation of β-carotene it has been essential to know the exact number of vinyl methyls in the side chain. An oxidation method known as *Kuhn–Roth oxidation* has been devised using chromic acid–sulfuric acid to form acetic acid which could be determined quantitatively to give the number of vinyl methyls.

31.17 Woodward–Fieser–Scott Empirical Rules

31.17.1 Conjugated Polyene Absorptions

While studying the UV absorptions of a number of terpenoids and steroids (1941–1942) Woodward observed a consistent influence of substituents on the bathochromic shift of the UV absorption maxima of a number of terpenoids and steroids possessing conjugated polyene chromophores. He systematized these observations and found out the magnitudes of the shifts caused in the absorption maxima of such compounds by the substituents concerned (see Table 4.3). The calculated UV absorption maxima were found to be very close to those of the observed ones in cases of a large number of natural products having substituted polyene chromophores. Some such results are shown in the Table 31.1. The absorption maxima values for conjugated polyene systems were modified by Fieser and Scott and hence the name of the empirical rule.

31.17.2 Conjugated Ketone/Aldehyde Absorptions

Woodward made similar observations for conjugated ketones and aldehydes and framed the relevant rule, modified by Fieser and Scott (Table 4.4). However, the

Table 31.1 The UV absorption maxima of some compounds

Compound	Observed absorption maximum (nm)	Calculated absorption maximum (nm)[a]
Abietic acid	241	239
Cholesta-3,5-diene	235	234
Ergosta-4,6,8(14)-22-tetraene	283	284
Cholesta-2,4-diene	275	273

[a]For calculation see Sect. 4.2 (Table 4.4)

calculated values of absorption maxima of conjugated ketones or aldehydes were found to be less consistent with the observed absorptions than those for the conjugated polyenes. The comparison of the observed values of UV absorption maxima with the calculated ones for the proposed structures on many occasions helped to arrive at the correct structure. *This marks the beginning of the use of spectroscopy for the structural elucidation.*

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Chapter 32
Chiral Recognition in Biological Systems and Natural Chiral Auxiliaries

Imagination is more important than knowledge.
—Albert Einstein

32.1 Introduction

Chirality or asymmetry is the geometrical key to the fundamental *chiral recognition* of biochemical processes occurring in living systems. The biochemical processes involving anabolism, absorption, or catabolism take place almost always by the recognition of the stereospecific characters of the molecules. Fischer's attempts to characterize certain carbohydrate molecules resulted into the first enantioselective synthesis in 1894—a case of chiral recognition [1].

Enzymes which are formed from L-amino acids only (excepting achiral glycine, NH_2–CH_2COOH) are themselves single enantiomers, and hence *stereorecognition* is the hallmark of enzymes and stereospecificity is maintained to generate single enantiomers of natural chiral molecules.

Each enantiomer (natural or synthetic), being a different entity, exhibits different manifestation of chiral recognition. Drugs interact with four major types of protein targets: (1) enzymes, (2) membrane carriers, (3) ion channels, and (4) receptors [2]. All types of interactions involve, in most cases, chiral recognition of the active site of the protein by the drug molecule, often chiral. Thus, chiral recognition is the essence of pharmaceutical science and natural products chemistry.

Chiral recognition generally originates from noncovalent bonds such as hydrogen bond, π–π interactions, polar or electrostatic interactions, dispersion forces, etc. Through stereospecific interactions, a pharmaceutically active molecule may bind to the receptor site of a cell (*cf.* "*host-guest chemistry*") and may produce either agonistic (full, partial, inverse, or irreversible) or antagonistic (competitive, noncompetitive, uncompetitive, or reversible) effect leading to a particular pharmacological action.

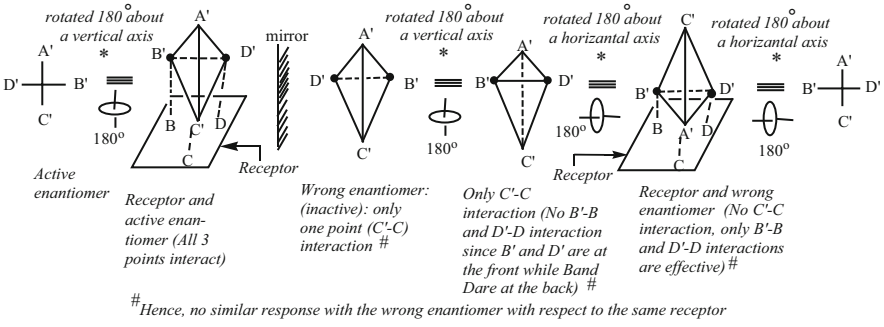
S.K. Talapatra and B. Talapatra, *Chemistry of Plant Natural Products*,
DOI 10.1007/978-3-642-45410-3_32,

A chiral auxiliary with its enantioselective catalytic property forms diastereomeric chiral complexes in unequal amounts with a substrate minimizing the free energy of the transition state leading to the desired isomer. This is followed by deblocking of the product(s) with introduced chirality.

This chapter elaborates enantiomeric stereospecificity with many examples and the role of chiral recognition using natural products or their derivatives as chiral auxiliaries in the formation of diastereoselective complexes.

32.2 Chiral Discrimination. Enantiomeric Stereospecificity

The biological properties of enantiomers play an important role in cases of the application of chiral drugs, pharmaceuticals, insecticides, other agrochemicals, etc. Since chirality is correlated directly to the biological activity, and the desired action of bioactive chiral molecules takes place in only one enantiomeric form, the delivery of the right active enantiomer is absolutely necessary for the desired activity [3–7]. When the racemate [(±)-variety] of a chiral drug is used, the right enantiomer goes to the right receptor site (the site of drug action responsible for the particular pharmaceutical effect) for the desired action, while the other enantiomer may act differently (Figs. 32.1 and 32.2). If the latter is inactive, it may be removed from the system, or it may reach at some other receptor site and may even act in an unwanted way and becomes toxic. Sometimes the pair of enantiomers show the same type of activity, but their magnitude remains different [e.g., warfarin, (Sect. 13.2.9.5), dexchlorpheniramine] (Fig. 32.2). This may be rationalized by the formation of diastereomers (binding complex) by the two enantiomers with the same receptor. Since the relationship of the binding complexes is diastereomeric they will have different energy, chemical properties, and dissociation constants



*Note: *For the vicissitude of a tetrahedral representation and its relation with a Fischer projection formula see* **Figure 2.14**

Fig. 32.1 A simple model displaying the complimentary recognition mechanism between the receptor and the potential substrate

causing different rates of their action. With respect to a particular receptor site the active form of a chiral drug showing the desired activity is called *eutomer*, its inactive or less active enantiomer is called *distomer*, and their ratio (eutomer: distomer) in terms of activities is known as *eudesmic ratio*, which gives a measure of the stereoselectivity of the enantiomer in the biological system. The increase in the eudesmic ratio signifies the increase in the potency of the eutomer. Sometimes a distomer for one receptor may act as eutomer for another receptor. The distomer/eutomer designation regarding a specified bioactivity may be reversed with reference to the receptor site. The difference in the potency of enantiomeric drugs may also involve different binding sites and the receptors (Chap. 33). When a eutomer contains a stereogenic center in its pharmacophore, then its complementarity with the receptor is high, while for a distomer it is low and hence the eudismic ratio of the drug is high.

A notorious and most perplexing example of the drug is thalidomide [3, 8, 9] (Fig. 32.2). Racemic thalidomide (synthetic) was released in West Germany on October1, 1957, and by 1961 it became the best selling sleeping pill in West Germany and the UK. It was declared completely safe without any data [8]. It was being used as a sedative and antinausea agent especially during early pregnancy (first trimester, 3 months). Unfortunately, it was soon found that the children born to the mothers taking thalidomide had a high incidence of deformities in their

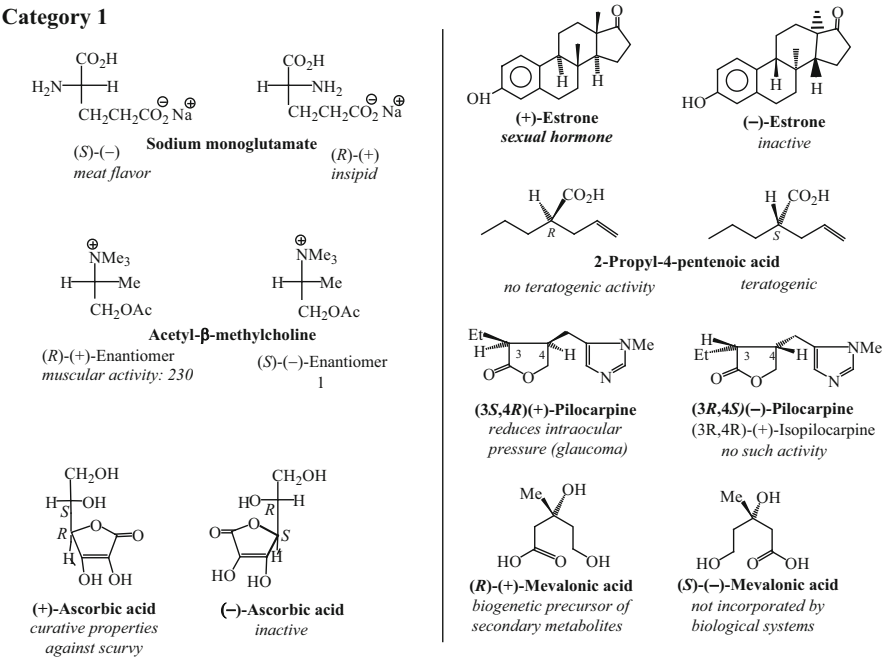


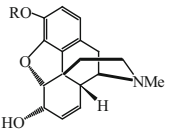
Fig. 32.2 (continued)

Category 1 (contd.)

α-Phenoxypropionic acid

(R)-D-(+)-Enantiomer
herbicidal activity

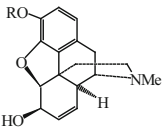
(S)-**L**-(−)-Enantiomer
no herbicidal activity;
has undesirable ecological impact



R = H, (−)-Morphine
R = Me, (−)-Codeine

(−)-Morphine is a powerful analgesic and narcotic, hence its major use is only for terminal pain.

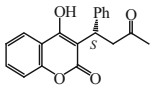
(−)-Codeine is also analgesic (potency 1/10 of (−)-morphine), but non-addictive; also used for preventing coughing



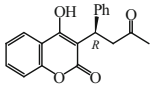
(+)-Morphine
(+)-Codeine

(+)-Morphine *has no such activity*

(+)-Codeine *has no such activity*

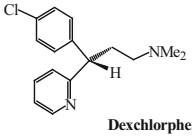


(*S*)-(−)-Warfarin
effective anticoagulant



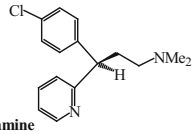
(*R*)-(+)-Warfarin
less effective anticoagulant

S-Isomer is 4 times more potent than R-isomer



Dexchlorpheniramine

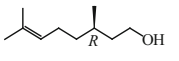
(*S*)-(+)-Enantiomer



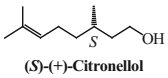
(*R*)-(−)Enantiomer

antihistamine activity

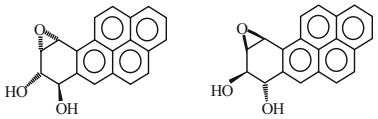
Potency: 200 1



(*R*)-(−)-Citronellol
light rosy petal-like odor



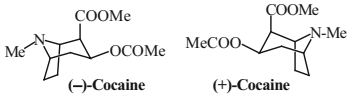
(*S*)-(+)-Citronellol
very fresh, light and clean rosy-leafy petal-like odor (closer to the odor of natural rose oil)



Metabolites of benzo[A]pyrene

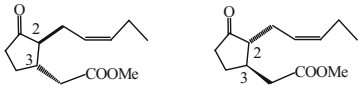
(+)-Isomer
carcinogen

(−)-Isomer
innocuous



(−)-Cocaine
psychoactive

(+)-Cocaine
inactive



(2*S*,3*S*)-Jasmonate
strong jasmine like odor

2*R*,3*R*-jasmonate
weak odor

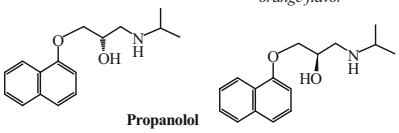
Category 2

(*R*)-(−)-Carvone
spearmint flavor

(*S*)-(+)-Carvone
caraway flavor

(*S*)-(−)-Limonene
lemon flavor

(*R*)-(+)-Limonene
orange flavor



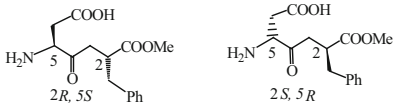
Propanolol

(*S*)-(−)-Enantiomer

*β-*blocker used in heart disease*

(*R*)-(+)-Enantiomer
contraceptive

Potency 40 : 1 (may be included in Category **1**)



Asparmate

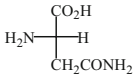
sweetener

slightly bitter

** Sir James W. Black shared Nobel prize in Medicine for this discovery in 1988.*

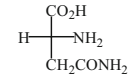
Fig. 32.2 (continued)

Category 2 (contd.)

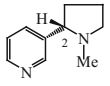


Asparagine

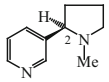
(S)-**L**-*Enantiomer (bitter)*



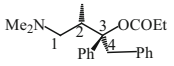
(R)-**D**-*Enantiomer (sweet)*



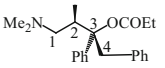
***(−)*-Nicotine (2*S*)**
(Natural)
insecticidal property
highly toxic to human



(+)-Nicotine (2*R*)
different activity
(not properly assessed)

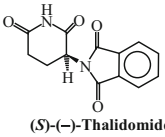


Novrad
2*S*,3*R*-(+)-Propoxyphene
anticough

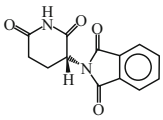


Darvon
2*R*,3*S*-(+)-Propoxyphene
analgesic

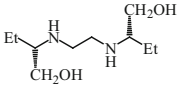
Note that the trade names are also of enantiomeric order!



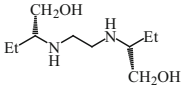
(*S*)-(−)-Thalidomide
teratogen (causes fetal abnormalities) in limb bones, phocomelia



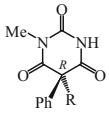
(*R*)-(+)-Thalidomide
sedative, antinausea and anti-inflammatory agent

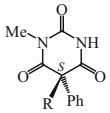


(*S*),(*S*)-(+)-Ethambutol
tuberculostatic



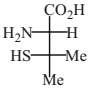
(*R*),(*R*)-(−)-Ethambutol
causes blindness





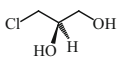
Antipodal barbituric acid derivatives

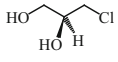
R =*n*-Bu: *narcotic* *anticonvulsant*
R =*n*-Pr: *anesthetic* *convulsant*



L-Penicillamine
mutagen

D-Penicillamine
antiarthritic

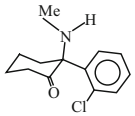




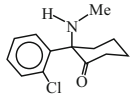
1-Chloro-2,3,-propanediol

(*R*)-*Enantiomer*
poison

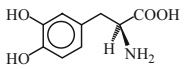
(*S*)-Enantiomer
medicinal drug



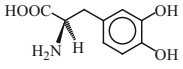
(*S*)-(+)-Ketamine
hypnotic and analgesic



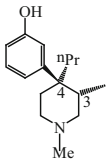
(*R*)-(−)-Ketamine
hallucinogen



(*S*)-L-(−)-Dopa
anti-parkinson

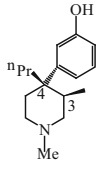


(*R*)-D-(+)-Dopa
serious side effects



(3*S*,4*R*)-(+) *opiate agonist*
(*narcotic analgesic*)

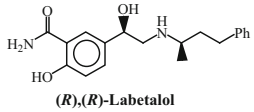
Picenadol



(3*R*,4*S*)-(−)
narcotic antagonist

[*The racemate is a partial agonist* **(Category.3)**]

Category 3



(*R*),(*R*)-Labetalol
β-blocker

The (R),(R)-enantiomer is more hepatotoxic than the racemate. The (S),(R)-diastereomer is an α_1-blocker.

Category 4

(i) *For some local anesthetic and antiarrhythmic activities the enantiomers are essentially equipotent*

(ii) *For 2-propyl-4-pentenoic acid no stereoselectivity of anticonvulsant activity was observed.*

Fig. 32.2 Contrasting biological behavior of enantiomeric substrates (exhibiting stereospecificity)

limb bones (phocomelia). The drug must be a very potent teratogen causing fetal abnormalities. Eventually, the teratogenicity (birth defect) was traced to the (S)-(−)-thalidomide enantiomer, whereas the (*R*)-(+)-enantiomer is a powerful tranquilizer, antinausea, and anti-inflammatory agent (Fig. 32.2). It is claimed not to cause deformities in animals even in high doses. The tragedy could have been avoided if the physiological properties of the individual enantiomer and of the racemate had been tested before marketing the drug. *Thus resolution of synthetic racemate drugs became indispensible*.

The racemic thalidomide was soon withdrawn from the market. In spite of such horrible effects the racemic drug has been reintroduced in the market for the treatment of an advanced inflammatory complication of leprosy known as ENL (Erythema Nodosum Laprosum). It alleviates the severe pain caused by such leprosy and is a wonder drug of choice for ENL. It has also been found to inhibit some cancers [8, 9]. *However, even the (R)-enantiomer cannot be prescribed to the pregnant mothers since liver contains an enzyme that converts the (R)- to the (S)-enantiomer* [8, 9].

A simplified representation of chiral discrimination of enantiomers in biological systems (enantiomeric stereospecificity) [3] in an extended form is shown in Fig. 32.1. A number of enantiomeric pairs exhibiting different biological properties classified under four heads are listed in Fig. 32.2 [3, 4]. In identifying fragrance [5, 7], odor [6], taste, etc., we unknowingly exhibit chiral recognition in our system.

Thus, the enantiomeric stereospecificity of natural as well as synthetic drugs and also the stereospecific biogenesis of bioactive natural chiral molecules can be visualized by a simple model for the *complementary recognition mechanism between the receptor and the potential substrate* as represented in Fig. 32.1 in a simple way. The actual pathway is, however, more complex.

Enantiomers may differ both qualitatively and quantitatively in their biological activities. The stereoselective differences may arise not only from the diastereomeric relation of the drug at pharmacological receptors but also from pharmacokinetic events, including protein binding, of drug metabolism and transport. Because the undesired enantiomer may be a medicinal pollutant or an unscreened substance, endeavors have been going on all over the world since 1980 to achieve chiral synthesis of the desired enantiomer (or stereoisomer) in cases of all known or new chiral drugs starting from appropriate chiral synthons from natural sources or from a prochiral synthon through asymmetric synthesis.

The biological activities of the enantiomeric pairs resulting from pharmacodynamic and pharmacokinetic considerations (Chap. 33) *may be broadly categorized as follows* (Fig. 33.2) [3, 4].

Category 1. The enantiomers differ quantitatively in their biological activities; in the extreme case one isomer is totally devoid of the measured bioactivity.

Category 2. The enantiomers differ qualitatively in their activities and exhibit different bioactivities at the same or different receptors.

Category 3. The racemate exhibits different behavior compared to that of either enantiomer alone.

Category 4. Both (all) isomers are equally active and no stereoselectivity of interaction is observed.

The situation of category 4 is rarely observed: even general anesthetics show stereoselectivity, although of moderate magnitude.

Enantiomeric stereospecificity is illustrated with a number of bioactive molecules, both synthetic and natural (Fig. 32.2). The majority of the natural drugs are available in a single stereoisomeric form, while the majority of the semisynthetic or totally synthetic drugs used to be synthesized in racemic forms. However, the extent of availability of synthetic single enantiomer chiral drugs is increasing. In 1982 nearly 15 % of synthetic racemic drugs were available in single enantiomers; by 1991 this has increased to ~40 %. At present, it is likely to have increased significantly because of the decisions (1) to pursue single enantiomers rather than racemates and (2) to switch the existing racemic drugs to single enantiomers.

32.3 Asymmetric Synthesis In Vivo and In Vitro

Because of the thalidomide tragedy, the importance of enantiomeric purity of chiral drugs, long ignored, was realized. A great need is thus felt to synthesize enantiomerically pure compounds and the enantiomeric pristine purity of chiral natural products allured synthetic organic chemists to direct their efforts toward asymmetric synthesis [10]—a methodology which starts with a prochiral substrate (possessing a plane of symmetry); the prochiral functional group becomes diastereotopic on attachment to a chiral auxiliary. Under a chiral influence causing controlled face discrimination, the reaction ends up into the formation of two enantiomers (plane of symmetry destroyed) in unequal amounts and the efficiency of the methodology depends on the percentage of the desired enantiomer over the other, as well as on the chemical yield of the process.

Nature elaborates chiral organic molecules in one of the enantiomeric forms with a very few exceptions as we do not know yet about the existence of enantiomeric pairs of a particular enzyme. This is not possible because of the homochirality of the building blocks (L-amino acids for proteins, D-sugars of DNA, RNA, ATP, etc.). D-amino acids are not known in higher organisms. Thus two parallel mirror image enzymatic reactions could not be conceived for the biosynthesis of two natural enantiomers of a chiral compound. However, literature suggests that in vivo there might be some enzymes which could convert one enantiomer to the other one. It has been shown that the (*R*)-thalidomide (synthetic) is converted into (*S*)-thalidomide by a liver enzyme [8, 9]. It is a case of substrate specificity.

Likewise, (*S*)-reticuline is reported to be epimerized to (*R*)-reticuline by an enzyme (Fig. 23.25) during the biosyntheses of morphine. A less defined physical picture of enzymatic asymmetric reactions in vivo can be visualized as follows: The enzyme participates either alone or in combination with a coenzyme in holding the substrate as well as sometimes the cosubstrate in a sterically rigid conformation and configuration so as to protect one face of the reaction site of the substrate from the approach of the attacking reagent, and only the other face remains available for the attack, thus a situation for 100 % enantiofacial selectivity is developed, the characteristic of enzyme catalyzed reactions in vivo.

It was realized that the molecular dissymmetry and enantiomeric purity of some natural products and their derivatives might confer on them this type of enzymatic properties affecting the enantioface selectivity of the substrate. Advantageously, unlike enzymes which are mostly substrate specific, the nonenzymatic species would be expected to be more substrate tolerant and are thus capable of accepting substrates of different structures; hence the generality and versatility are more in this respect compared to enzymatic reactions. Incidentally, it may be mentioned that the enantioselectivity is so high in many asymmetric reactions, (e.g., Sharpless epoxidation) that they appear to be comparable to but more general than enzymatic reaction. *The enzymatic reaction of course has high turn over in situ and also can achieve selective functionalization at apparently non-activated positions of organic molecules which are not ordinarily possible by this type of asymmetric synthesis. Further, enzymatic reactions do not need blocking and deblocking of some functional group/s prone to be involved during 100 % stereoselective synthesis in the biological systems.*

32.4 Resolution by Chiral Ligands. Chiral Recognition [11]

Chiral "solvents" have been used for the resolution of suitable racemic substrates *via* extraction with high selectivity. Many ion selective ligands called *ionophores* have been prepared and used with success. This is also a specific case of chiral recognition. The racemic solute and the "solvent" (a solution of an optically active compound diluted by, *e.g.*, chloroform) are often called *guest* and *host*, respectively, and the specific and tight interaction may even allow the isolation of the diastereomeric complexes thus formed. The inclusion of solute molecules in the cavity of the "solvent" gives rise to cavitates [11]. For common chiral reagents used for resolution see Sect. 4.1.

Thus, the key step of resolution of many racemic substrates [12] is chiral recognition. In addition to suitable chiral solvents the following two methods have also been used.

(a) Kinetic resolution by lipases and amidases of racemic alcohols, esters, amides, etc.

(b) Through co-crystal formation where the co-crystal former recognizes only one enantiomer over the other and the co-crystals are physically separated.

32.5 Natural Products and Natural Products-Derived Chiral Auxiliaries

Though the number of natural products selected for the purpose of chiral auxiliaries is limited to a few skeletal patterns till date, some synthetic manipulations by way of derivatizing or putting some chemical handles on their chiral backbone increase their numbers in the list of chiral reagents. In fact, these auxiliaries are important contributors in the field of nonmetallic organic catalysis—an importantly urgent area of asymmetric synthesis. (+)-Camphor, (−)-camphor, (−)-α-pinene, (+)-a-pinene, (−)-carvone, etc., have been synthetically manipulated extensively to develop a large number of better equipped chiral auxiliaries. *Cinchona* alkaloids with an advantageous presence of diastereomeric chirality in their natural occurrence have been exploited with great success, and they have been widely used in asymmetric synthesis [13, 14]. The literature is deluged with their reports and the exponential growth in this area began in 2000. Thus organic chiral auxiliary controlled face discriminating reactions appear as a powerful strategy in asymmetric synthesis.

A schematic diagram of the process of asymmetric synthesis using natural products-derived chiral auxiliaries is shown in Fig. 32.3.

The term asymmetric synthesis was first used by Emil Fischer in 1894. Morrison and Mosher [15] and Brown [16] defined asymmetric synthesis more explicitly (*cf.* Sect. 2.11). During the formation of unequal amounts of enantiomeric end products (Fig. 32.3), the enantiomeric excess (cf. Chap. 2) will be dictated by the favorable diastereomeric relationship with the chiral center/s of the chiral auxiliary and the newly formed chiral center in the product. The product could then be easily detached from the auxiliary without losing its/their stereochemical integrity. Prior to the detachment of the products from diastereomeric intermediates, quite often diastereomers are chromatographically purified to ensure better purity of the product.

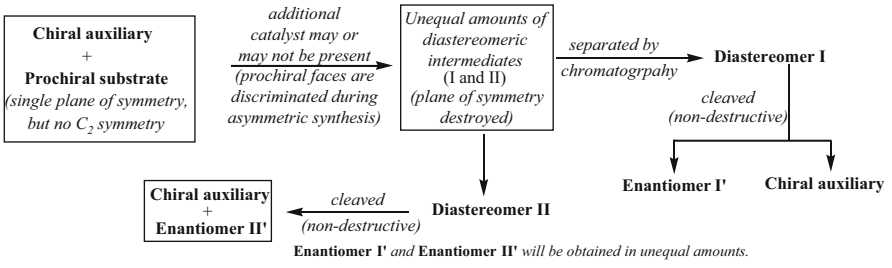


Fig. 32.3 Asymmetric synthesis using chiral auxiliary

32.5.1 Cinchona *Alkaloids as Chiral Auxiliaries. Phase Transfer Catalysis*

Appropriate chiral inducers/controllers have been developed for C–C bond formation, functional group transformation, cycloaddition, Michael addition, alkylation, condensation, etc. [17]. Chiral auxiliaries to be discussed here briefly involve their applications only. *Mechanism of their action, transition states, and their preparations will not be discussed.* Reactions are provided with pertinent references for details. *Cinchona* alkaloids have been conveniently used as resolving agents since the early nineteenth century. However, the first report of asymmetric organocatalysis was made by Bredig and Fiske [18, 19] in 1912 when they studied *Cinchona* alkaloid-catalyzed HCN addition to benzaldehyde, an asymmetric cyanohydration reaction extended to other carbonyls with low enantiomeric excess. An organocatalytic transformation (methanolysis) of phenyl methyl ketene with remarkably improved enantiomeric excess (76 %) has been reported by Pracejus [20, 21] in 1960 using O-benzoylquinine (Fig. 32.4).

The versatile natural scaffolds of *Cinchona* alkaloids holding enantiomeric as well as diastereomeric relationship among the chiral centers were chemically manipulated so much that they came into wide use in asymmetric synthesis. Wynberg [13, 14] and others have chemically manipulated these alkaloids for better and desired chiral induction and control.

Cinchona alkaloids catalyze stereoselective addition of diethylzinc to aldehydes. The configuration of the catalysts determines the configurations of the products (Fig. 32.5) [22].

Quininium benzyl chloride (Quibec) catalyzes different substrates to epoxides. Reactions are carried out in phase transfer catalytic conditions [23] (Figs. 32.6).

Tertiary amines like quinuclidine form considerably stronger complexes with OsO_4. Sharpless' group discovered that derivatives of natural *Cinchona* alkaloids are substantially better ligands for osmium, and hence significantly higher enantioselectivity (~90 %) was obtained in the dihydroxylation reaction. Thus *Sharpless Asymmetric Dihydroxylation* (AD) was born. This is one of the most reliable methods available in asymmetric synthesis [24]. Sharpless designed the $(DHQ)_2$-PHAL and $(DHQD)_2$-PHAL-ligands for osmium-catalyzed asymmetric dihydroxylation (AD) of various alkenes (32.7) [25, 26].

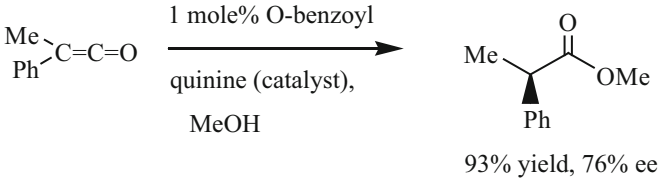
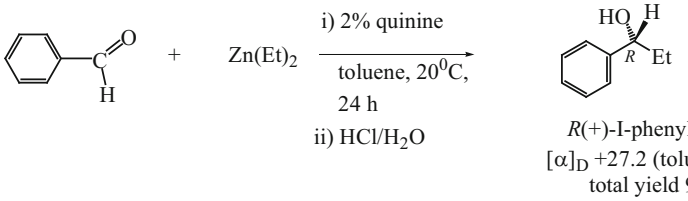
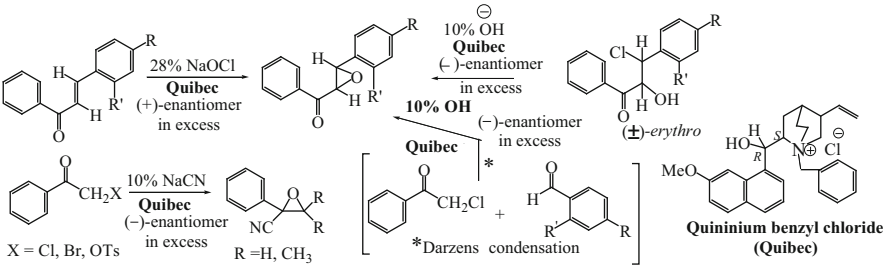


Fig. 32.4 Organocatalytic methanolysis of phenyl methyl ketene



Catalyst	Yield %	Configuration	ee %
Quinine	92	*R*	68
Quinidine	90	*S*	48
Cinchonidine	89	*R*	58
Cinchonine	90	*S*	46

Fig. 32.5 *Cinchona* alkaloids catalyzed asymmetric aldol condensation



Note: *Optical purity of the epoxide in each case was low. Absolute configuration was not known in any case.*

Fig. 32.6 Quibec-catalyzed epoxidation [23]

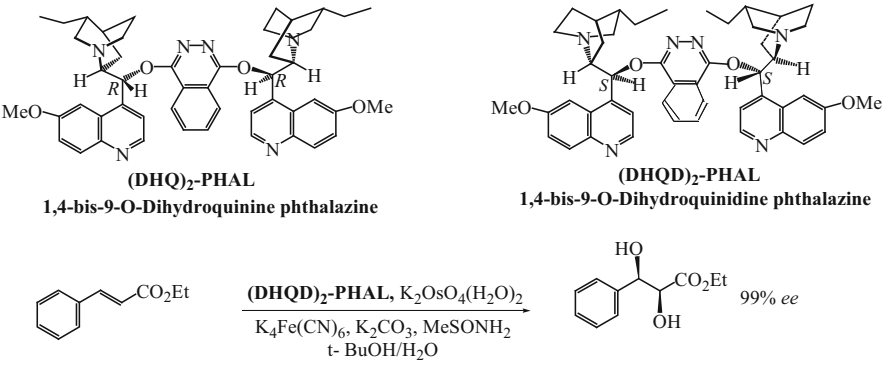


Fig. 32.7 Osmium-catalyzed AD of some alkenes

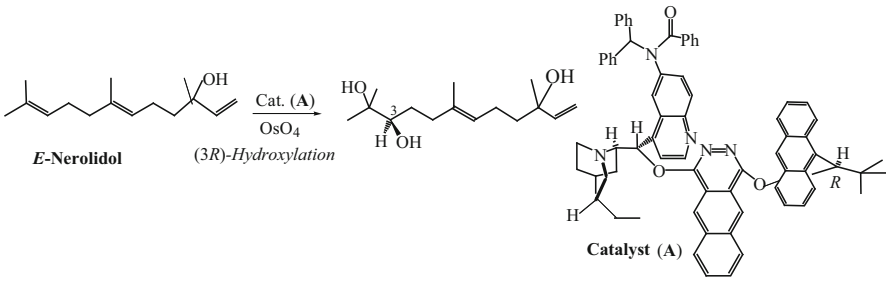


Fig. 32.8 (3*R*)-Hydroxylation product of *E*-nerolidol

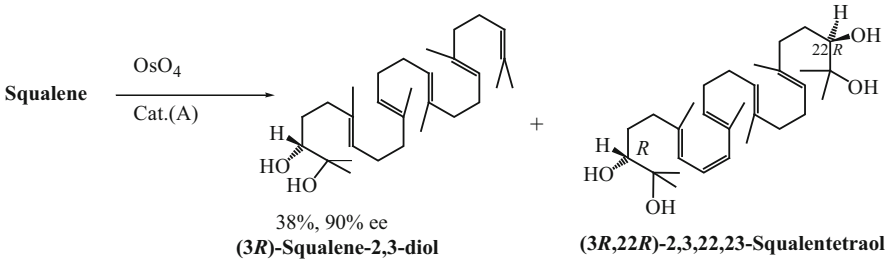
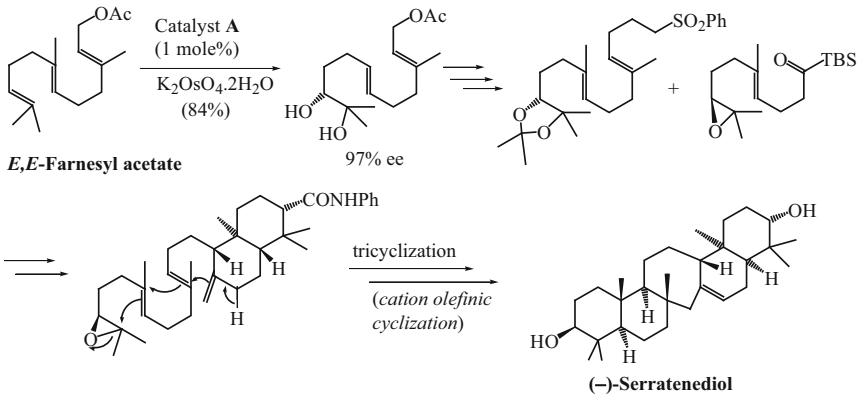


Fig. 32.9 Regio- and enantioselective dihydroxylation and tetrahydroxylation of squalene

Catalyst structure (**A**) a monocinchona derivative (Fig. 32.8) designed by Corey [27] has been found to be of great use in combination with OsO_4 for the enantio- and position-selective dihydroxylation of the terminal isopropylidene group of polyisopropenoids. *E*-Nerolidol is thus oxidized by OsO_4 in the presence of the catalyst (**A**) to give dihydroxylation product of the terminal isopropylidene group (Fig. 32.8).

(2,3S)-Squalene epoxide, an important biosynthetic precursor of triterpenoids and steroids, has been conveniently prepared from squalene via its 2,3-diol using the catalyst (**A**) [27]. The other product was the corresponding tetraol (Fig. 32.9). Squalene being sparingly soluble in aqueous *t*-BuOH, the hydroxylation is slow at 0 °C. Corey discovered the acceleration of the reaction by the use of 0.5 equiv. of n-Bu4N$^+$OH as surfactant (relative to squalene). The yield was 38 %. 90 % ee of 3*R*-diol thus obtained.

The first enantioselective synthesis of (−)-*serratenediol* (Fig. 32.10), a pentacyclic triterpene containing a unique seven-membered central ring has been achieved by a short biomimetic pathway involving a tricyclization of an N-phenylcarbamate derivative [28] (Fig. 32.10). The latter has been prepared through a number of steps starting from *E,E*-farnesyl acetate using the catalyst (A) in the first step. (For details of all the steps and conditions of each step, see [28].)



Note : For reaction conditions of an N-phenyl carbamate derivative see ref. [28].

Fig. 32.10 Synthesis of serratenediol using the monocinchona catalyst (**A**)

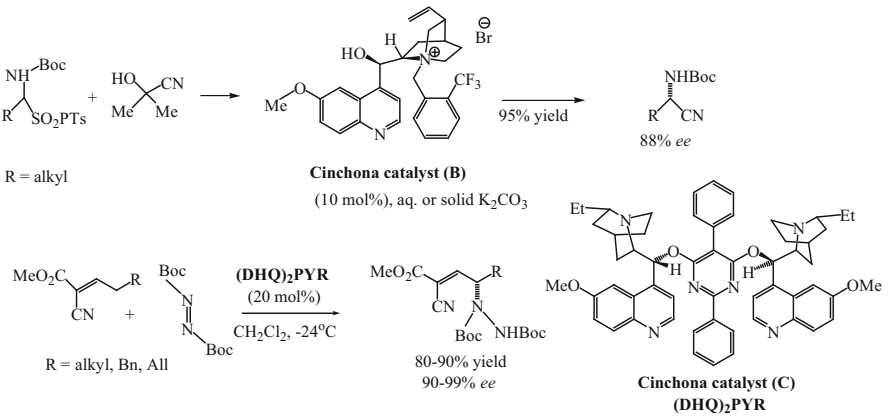


Fig. 32.11 Structure of the phase transfer catalyst (**B**) and catalyst (**C**)

Asymmetric phase transfer catalysts (PTC) serve as the powerful organocatalysts for substrate activation [29].

The phase transfer *Cinchona* catalyst (B) (Fig. 32.11) has been used in enantioselective Strecker reactions of α-aminosulfones with cyanohydrins, acting as a source of CN group [29].

Highly enantioselective allylic amination has been achieved for the first time using a dimeric Cinchona alkaloid $(DHQ)_2PYR$[Cinchona catalyst (**C**)] (Fig. 32.11) in high yield (80–90 %) and excellent enantiomeric excess (90–99 %) [30].

Asymmetric [3 + 2] formal cycloaddition between α-substituted isocyanoesters and nitroolefins is catalyzed by *cinchona* alkaloid derivatives to yield 2,3-dihydropyrroles with high diastereo- and enantioselectivities [31] (Fig. 32.12).

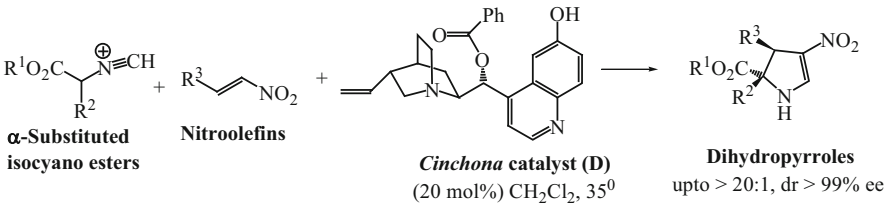


Fig. 32.12 Asymmetric synthesis of dihydropyrrole derivative with the help of *cinchona* catalyst (**D**)

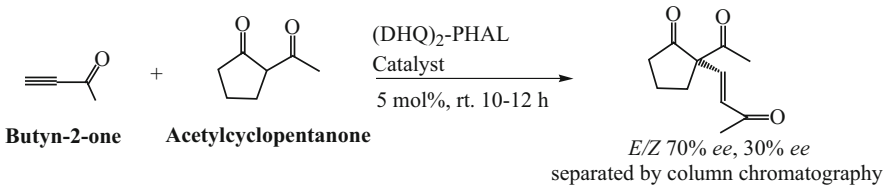


Fig. 32.13 Use of $(DHQ)_2$-PHAL ligand for enantioselective addition of a β-dicarbonyl to an alkynone

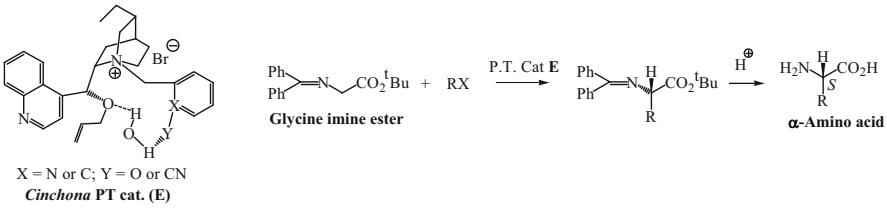


Fig. 32.14 Asymmetric phase transfer catalytic synthesis of α-amino acids [33]

Enantioselective conjugate addition of a β-dicarbonyl to alkynones (Fig. 32.13) catalyzed by specially designed *cinchona*-derived catalyst [$(DHQ)_2$-PHAL] (See Fig. 32.7) has been reported [32].

The electronically modified quaternary ammonium salts derived from *Cinchona* alkaloids containing 2′-*N*-oxypyridine and 2′-cyanobenzene moieties act as the asymmetric phase transfer (PT) catalyst in the enantioselective alkylation of glycine imine ester (Fig. 32.14). The 2′-*N*-oxypyridine and 2′-cyanobenzene might form a rigid conformation with H_2O via hydrogen bonding effecting the high enantioselectivity (97–99 % ee) and providing evidence for electronic factors for such high enantioselectivity.

For more examples of *Cinchona* alkaloid-derived asymmetric PTCs, see [34] and also synthesis of R-(+)-hygrine (Chap. 16, Ref. [5]).

32.6 Chiral Organoboranes

Organoboranes prepared from simple commercially available natural products like (+)-α-pinene and (−)-α-pinene are so versatile that they occupy a significant place in the literature of chiral inducers. Brown has been the pioneer in using them as organic boranes [35]. Hydroboration is a [2 + 2] cycloaddition between a boron hydrogen bond acting as an electron acceptor and a π C=C double bond acting as an electron donor. Boron is attached to the sterically more accessible least substituted carbon atom, while hydrogen goes to the most substituted carbon atom (*anti*- Markonikov addition). The reactions are simple and chiral boranes could be prepared from commercially available natural products like (+)-α-pinene. Di-3-pinanylborane prepared from (1R,5R)-(+)-2-pinene is the most readily available as well as versatile chiral reagent. The other reagent isopinocompheylborane (IPC_2BH), exhibiting a remarkable ability to achieve asymmetric synthesis, could be obtained in (−) as well as (+) forms when prepared form (+)-α-pinene and (−)-α-pinene, respectively [35] (Fig. 32.15).

Report on the asymmetric reduction of prochiral ketones with the chiral trialkylborane (B-(3-pinanyl)-9-borabicyclo[3.3.1]nonane) to chiral α-hydroxy esters in optical purities approaching 100 % has been made [36]. The rate of reduction has been increased by substituting electron withdrawing group on the carbonyl compound (Fig. 32.16).

A wide range of olefins could be converted to chiral secondary alcohols by treatment with (−)-$IPCBH_2$ (monoisopinocampheylborane) (prepared from (+)-α-pinene) [37] (Fig. 32.17), followed by oxidation.

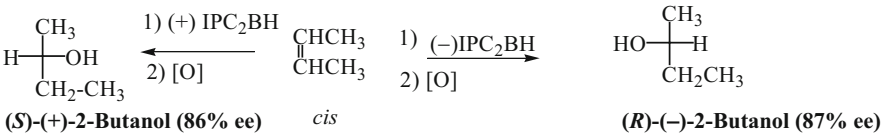
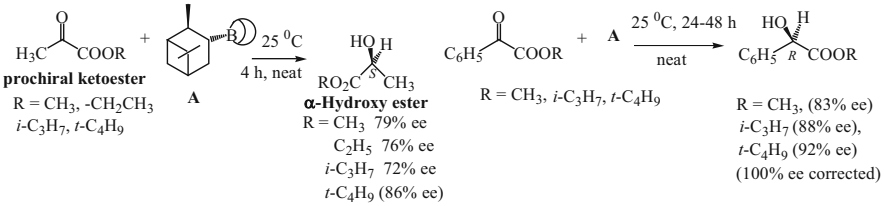


Fig. 32.15 Asymmetric synthesis using isopinocampheylborane (IPC_2BH)



The reversal of the quantitative optical induction realized with different esters in aliphatic and aromatic systems is a mystery [36]

Fig. 32.16 Asymmetric reduction of prochiral ketones by chiral organoborane

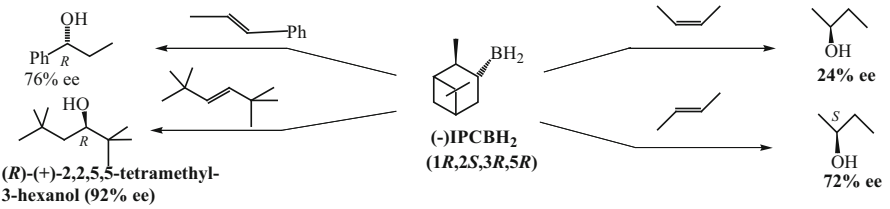


Fig. 32.17 Conversion of olefins to chiral secondary alcohols

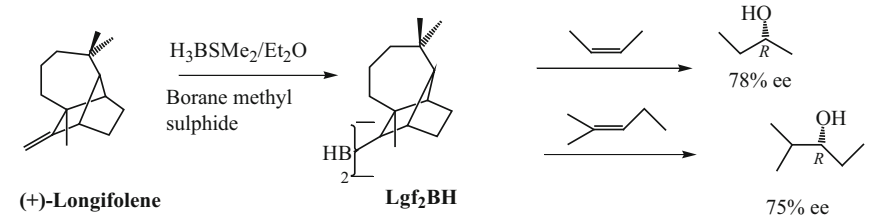


Fig. 32.18 Use of organoborane from longifolene for enantioselective conversion of olefins to secondary alcohols

Lgf_2BH obtained from natural (+)-longifolene has been used to form secondary alcohols from di/tri-substituted olefins via hydroboration in fairly good enantiomeric excess [38]. However, (−)-longifolene rarely occurs and is not readily available. Therefore, it is not possible to prepare chiral products of opposite configuration [16] (Fig. 32.18).

32.6.1 Another Useful Application of (+)-di-3-Pinanylborane

The iridoid glucoside Loganin, (a monoterpene glucoside) (Sect. 6.1.6) occurring in *Strychnos nux vomica* and other *Strychnos* species (Fam. Loganiaceae) *Menyanthes trifoliata* (Gentianaceae), *Vinca rosea* (Apocynaceae) and various species of *Gentiana* (Gentianaceae), *Hydrangea* (Saxifragaceae), *Lonicera* (Caprifoliaceae), *Mytangyna* (Rubiaceae), and *Swertia* (Gentianaceae) occupies a central position in the biosynthesis of indole alkaloids and secoiridoids.

Asymmetric synthesis of this very important biogenetic precursor has been achieved by the useful application of di-3-pinanylborane, IPC_2BH [39]. In this synthesis, the key intermediate (1*S*,2*R*)-2-methyl-3-cyclopenten-1 (**4**) is obtained from 5-methylcyclopentadiene (**1**) by its hydroboration with (+)-di-3-pinanylborane to (1*R*,2*R*)-2-methyl-3-cyclopenten-1-ol (**2**) followed by its inversion at C1 through its methane sulfonate derivative (**3**) (Fig. 32.19).

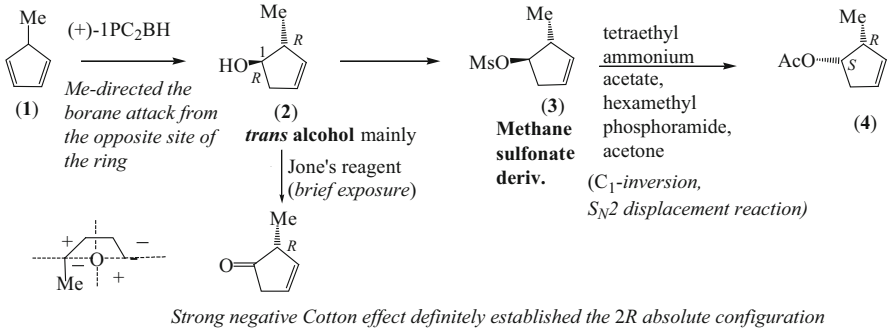


Fig. 32.19 Synthesis of (**4**) the key intermediate for loganin synthesis by use of (+)-di-3-pinanylborane

In this connection it may be mentioned that the paper on another total synthesis of loganine by G. Büchi group appeared [40] as the next paper of [39].

32.7 Camphor-Derived Chiral Auxiliaries

Camphor (Sect. 6.3) is a versatile as well as inexpensive readily available material. It has been employed to prepare a large number of chiral auxiliaries. A few examples are given in the sequel.

Ex.1 ***Highly enantioselective Darzens*** (Georges Darzens, 1867–1954) ***Reactions***. Asymmetric synthesis of (2R,3S)-2,3-epoxyamides (**B**) has been achieved via camphor-derived sulphonium salt (**A**) (Fig. 32.20) [41]

Ex.2 ***Highly enantioselective synthesis of chiral α-amino acids***. A tricyclic iminolactone (C) has been prepared from (1*R*)-(+)-camphor which could be alkylated in good yield (~74–96 %) and with excellent diastereoselectivities (>98 %). On hydrolysis of the *endo* iminolactone, *R*-α-amino acid could be obtained in good yields and enantioselectivities with nearly quantitative recovery of the chiral auxiliary (Fig. 32.21) [42]. Likewise, *S-a*mino acid will result from the chiral auxiliary derived from (1*S*)-(−)-camphor, following the same two reactions.

Ex.3 ***Highly enantioselective synthesis of secondary alcohols by nucleophilic addition of dialkylzinc to carbonyls catalyzed by camphor-derived chiral auxiliary***

Dimethylaminoisoborneol (DAIB) (1*R*,2*S*,3*R*,4*S*)-3-Dimethylamino-1,7,7-trimethylbicyclo [2.2.1]heptan-2-ol) is a chiral catalyst prepared from (1*R*,4*R*)-(+)-camphor. It has been used as a catalyst for the enantioselective nucleophilic addition of dialkyl zinc to carbonyls. Dialkyl zinc acts as the alkyl donor. Zn is

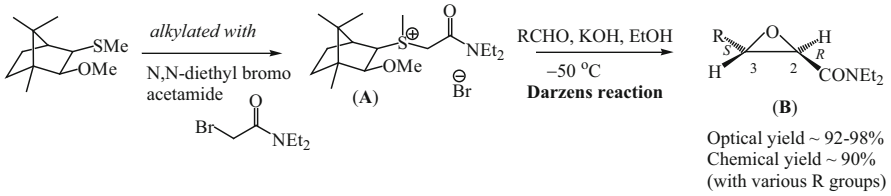


Fig. 32.20 Highly enantioselective Darzens reaction using (+)-camphor-derived chiral auxiliary (**A**)

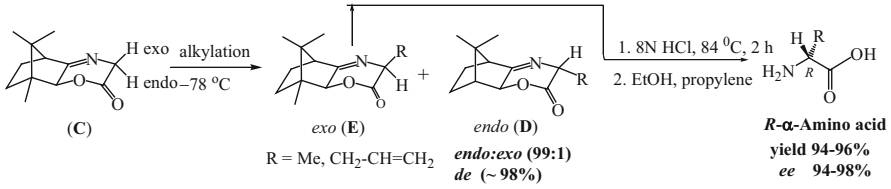


Fig. 32.21 Highly enantioselective synthesis of α-amino acids using a camphor derived auxiliary

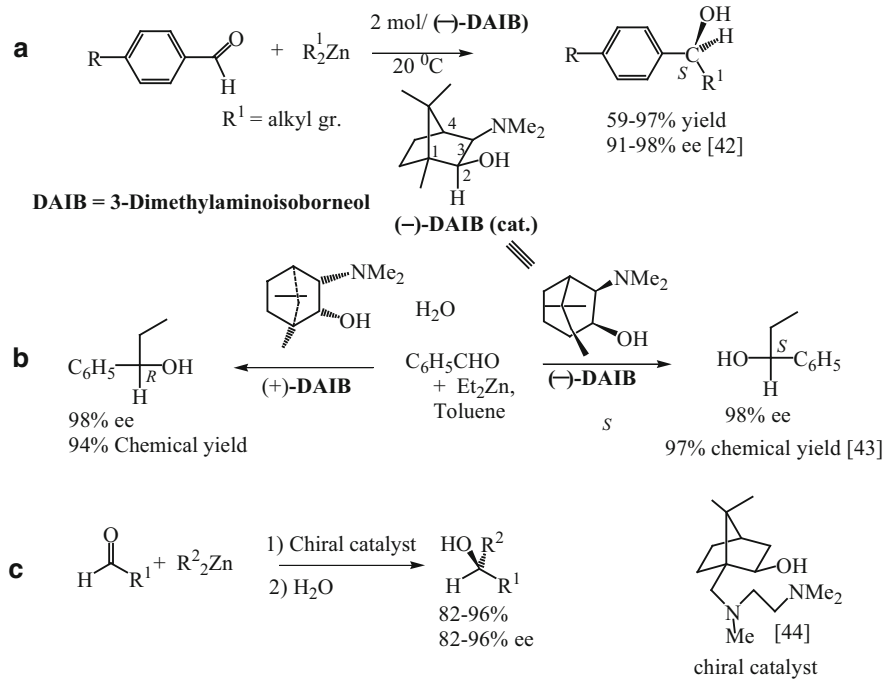


Fig. 32.22 Highly enantioselective synthesis of secondary alcohols by use of camphor-derived chiral catalyst

sp hybridized and hence linear and inert to carbonyl. The catalyst helps in enhancing the bond polarity and p-character of Zn. This makes the dialkylzinc active as a nucleophile toward carbonyl (Fig. 32.22) [43–45].

Ex.4 ***Highly enantioselective synthesis of vinylcyclopropanes*** [46] (Fig. 32.23).
Several other applications of chiral auxiliaries are included in Fig. 32.24.

Ex.5 ***Enantiomerically pure aldolization*** [47]
N-Propionylsultam ***(1)*** *(when SO_2NH-/SO_2N is present as a part of a ring, it is called sultam) on O-silylation yields Z-O-silyl-N, O-ketene acetal* ***(2).*** *The latter undergoes Lewis acid promoted addition of aromatic and aliphatic aldeydes to give diastereomerically pure, crystalline "anti" aldols* ***(3)*** *or their silylethers* ***(4)*** (Fig. 32.24).

Ex.6 ***Enantiofacial Michael addition*** (Fig. 32.25)

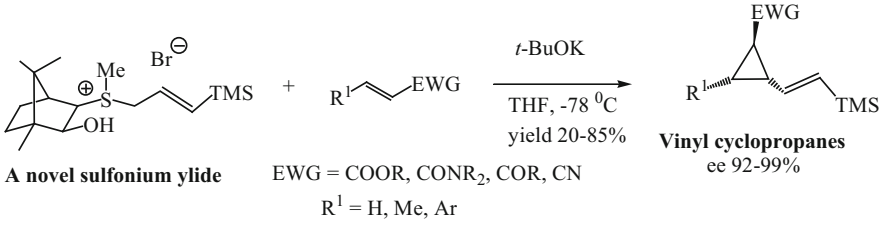


Fig. 32.23 Highly enantioselective synthesis of vinylcyclopropanes

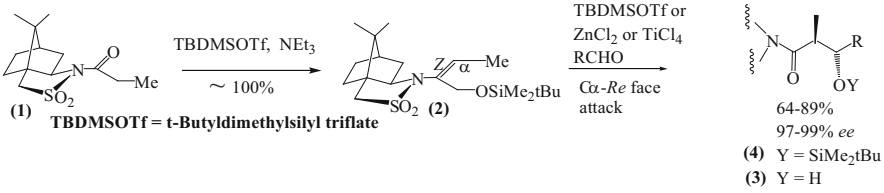


Fig. 32.24 Formation of enantiomerically pure anti aldols and their silyl ethers

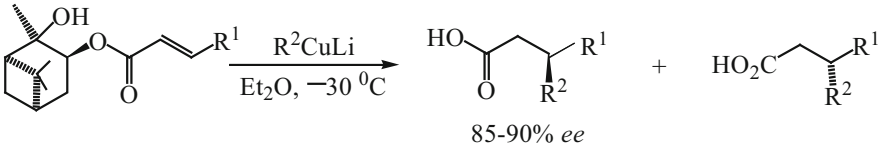


Fig. 32.25 More applications of camphor-derived chiral auxiliaries

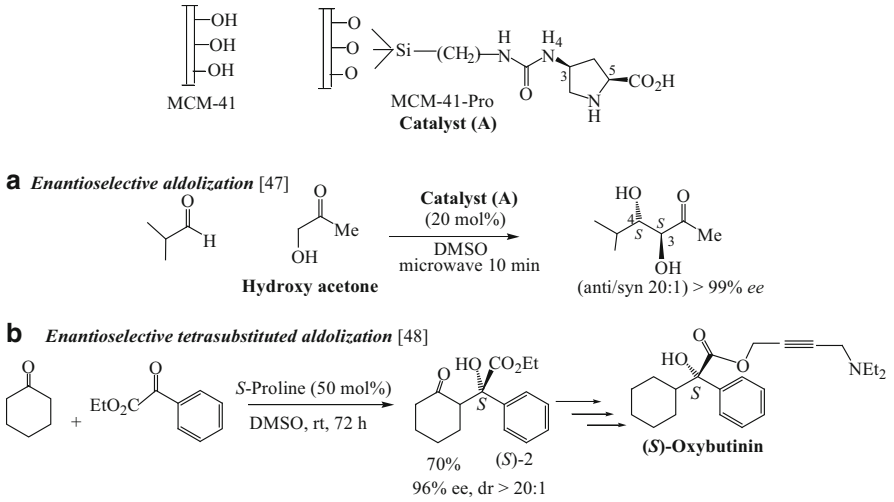


Fig. 32.26 Enantioselective aldolizations using a mesoporous silica-supported proline catalyst

32.8 A Few Proline-Derived Chiral Auxilliaries

(a) Mesoporous silica-supported reusable proline catalyst (**A**) has been developed for direct aldol reaction between aldehyde and hydroxyacetone when the product (3*S*,4*S*) is obtained in 60 % yield and >99 % ee (Fig. 32.26) [48].
(b) An asymmetric tetrasubstituted carbon center was constructed with excellent stereoselectivity (96 % *ee*) through the proline-catalyzed direct asymmetric aldol reaction between cyclohexanone and ethyl phenyl glyoxalate under mild condition to give (S)-2-cyclohexyl-2-phenylglycolic acid [49] (Fig. 32.26). This served as the precursor for the synthesis in several stages of an important drug (*S*)-oxybutinin (Fig. 32.26) used for the treatment of urinary frequency.

In a review on proline-catalyzed asymmetric reactions [50] the widely and successfully used proline and its derivatives as chiral auxiliaries in synthetically powerful asymmetric reactions such as aldol reactions, Mannich reactions, Michael additions, direct electrophilic aminations, Diels-Alder type reactions, Robinson annulations, Baylis–Hillman reactions, oxidation and reduction reactions, etc., have been illustrated with examples.

32.9 Concluding Remarks

The involvement of metal catalysts in asymmetric synthesis has been well established. The work carried out by eminent scientists like Sharpless, Noyori, Knowles, Jacobsen, Watanabe, and others has taken this area of chemistry to a highly sophisticated level.

Most of the stereospecific catalysts are organometallic in nature, thereby creating problems in purification of health-related products like pharmaceuticals. Hence endeavors are being made for employing organic molecules as asymmetric catalysts. Thus, use of natural chiral auxiliaries such as proline, *Cinchona* alkaloids, and their derivatives (not containing any metal) form an important part of *green chemistry*. The products derived from the use of such auxiliaries generally do not face much problem with purification, especially in cases of health-related products like pharmaceuticals requiring much care for purification.

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Chapter 33
Natural Products in the Parlor of Pharmaceuticals

"I do not want to die in this beautiful world,
I want to live amongst the human beings."

Rabindranath Tagore (1861–1941, NL 1913)
(translated from Bengali by the authors)

"No one wants to die. Even people who want to go to heaven don't want to die to get there. And yet death is the destination we all share."

Steve Jobs (1955–2011)
(Convocation Speech at Stanford University on 12 June, 2005)

33.1 Introduction. Historical Background

Whenever there is life there are diseases, decay, and death. Death is the most inevitable biological event of a living system. Since life is the most precious gift of Nature, people want to preserve it as along as possible. In an endeavor to arrest the approach of death, our ancestors had not only to withstand the fury of Nature but also had to fight against diseases and decay. People were thus in search of remedial measures since the dawn of human intellect. The relationship of man and Nature was symbiotic and the immediate source of such remedial measures had been the forests, the home of our ancestors. They used to roam in and around the forests, and through thousands of years of interactions with Nature by trial and error methods, and under the pressure of experience and need they could discover a large number of plants with varying healing properties. These plants are referred to as the medicinal plants, and the people having the knowledge of their curative applications were known as medicine men. Thus a wealth of information on the curative properties of plants resulted.

Even today the medicine men exist in far and remote places where the privileges of the modern medical treatments are yet to reach. The local people solely rely on them and their prescribed herbal medicines for the cure. Our ancestors sometimes used to chew different parts of the plants, drink decoctions, smear the paste prepared from plants on the wounds as poultice, and even inhale the smoke of

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burnt plants, in an effort to accelerate the healing. Sometimes they thought that the healing power of plants would be augmented if taken during some religious rituals blended with witchcraft, mysticism, astrology, and along with some animal products like animal fats and oils. Their knowledge on medicinal plants remained confined within their own clans, and they handed down the information from one generation to the next of their clan verbally like cooking recipe evolving some rudimentary formulation. We came to know about their knowledge on medicinal plants to some extent from the relics they left at the ancient sites of their dwellings. In absence of proper recording and communication, much genuine knowledge of many medicinal plants was lost during knowledge transfer and also with some vanished civilizations.

However, with the advent of civilization, and the development of the art of writing, people started recording various types of information on medicinal plants. This information thus percolated down into the population at large. The records revealed man's attempt to free himself of diseases and physical distresses for several thousands of years and their deep beliefs about the significance and importance of plants in their life. The evidence is abundant in the history and culture of South Asia especially in China, India, Tibet, Arabian countries, African country like Egypt, and European countries like Greece and Italy. Babylonian clay tablets as old as 3000 BC contained the records of the various uses of plants in diseases. The earliest uses of medicinal plants are found in "Rig Veda"—perhaps the oldest repository of human knowledge having being written around 3000–2500 BC.

In the work that followed particularly in *Ayurveda* (*Ayus—life*, *veda—knowledge*), which is stated to have been written around 2000 BC. The properties of various crude drugs have been described and compiled which laid the foundation stone of Indian medicine. *Charaka Samhita* [1] and *Susruta Samhita* [2] were written around 1000–900 BC and ~600 BC, respectively. These are the two hallmarks of Ayurvedic concepts and practice. The latter gave more emphasis on surgery [3] though it contains 395 medicinal plants as therapeutic agents. Different civilizations that came to India either as invaders or friends and many monks, preachers, and travelers who came to India brought with them various herbal drugs of their countries and contributed to the development of vegetable drugs of India. During the Buddhist period a large number of herbal drugs were introduced. China is a very important contributor to the herbal medicine with a long honorable historical background (Sect. 7.9) [4, 5]. The catalog of Chinese herbs by Shen Nung Pen Ts'ao Ching is regarded as the oldest record which has been subsequently revised and enlarged during different dynasties [4].

A recent review [6] gives an account of some of the traditional Chinese medicines, their chemical constituents and biological properties along with pertinent references. Ginseng (*Panax ginseng*) is one of the most important herbs in Chinese medicine for thousands of years. It also spread throughout Korea and has moved into the main stream of drugs. It has been widely used to normalize abnormalities in body function, against blood and heart ailments, in nerve disease, gynecological problems, and respiratory disorder. The first record of ginseng's prescription as Chinese medicine appeared in Zhang Zhong Jing's book "*Shanghan Lun*"

[7]. A brief but excellent account of herbal medicines of different cultures has been given by Nakanishi [4] and also by Cragg and Newman [8]. Herbal drugs prefer the composite prescription (combination of herbs and hence mixture of chemical constituents) rather than one or two active ingredients. Herbal drugs (including drugs from shrubs) thus have synergistic therapeutic effect. However, randomized and controlled examination of different batches of a particular herbal drug in several cases show inconsistency in the presence/absence and extent of the same chemical components and hence show difference in compatibility in efficacy. Ecological factors like place and climate of growth, maturity of plants, season of collection, etc., play important role in the chemical compositions of the secondary metabolites occurring in plants (Sect. 4.1). The above factors pose a great problem in standardization. The standardization basically needs to include [9] (i) the proper identification of plants; (ii) their method of cultivation, collection, and storage; (iii) analytical profile of the plant extracts (the marker and some other major components should be present); (iv) toxicological tests and formulations for their use; (v) shelf-life (generally expiry date is not written on the label probably because herbal drugs contain mixture of different chemical components); and finally (vi) quality control. Thus drug standardization, manufacture, and control are essential for rationalization.

It is particularly appropriate at the present moment when pharmaceutical concerns all over the globe are emitting ceaseless flow of synthetic drugs, many of which possess considerable side effects; attention should be turned to the indigenous herbs. (Herbs may be defined as flowering plants whose stems are never woody, rather succulent, die after flowering, and inhibit propagative vegetation.)

Higher plants are also chemically searched for the possible remedies of the sufferings of human being. Now there is a growing realization to this need. Standardization procedure should be followed for the clinical efficacy and reproducible results. This will create greater confidence amongst the users and will cause greater acceptance for herbal drugs.

33.2 Modern Drugs. Ethnotherapeutics. Bioactivity

Medication by drug molecules is essentially based on a single molecular entity with specificity for target disorder. Plants are selected from Ayurveda and various folk and traditional medicinal sources of different countries and cultures and chemically investigated for the active principles many of which have entered our modern medicine in the form of a single entity. A bird's eye view of some extremely useful bioactive molecules, their plant sources, and bioactivities will be provided by Tables 33.1 and 33.2. *The structures of the compounds not appearing elsewhere in the present book are shown in Fig.* 33.1. It is evident from these Tables that plants represent a promising and expanding platform for biologically active natural products with therapeutic implications and are superb sources of molecular diversity [6, 10–12]. *Combinatorial chemistry* (*vide* Appendix B) consists of appending/

Table 33.1 Ethnotherapeutics and some traditional modern drugs

Drug	Basis of investigation
Codeine, morphine (effective pain killers)	Opium, the latex of *Papaver somniferum* used by ancient Egyptians, Greeks, and Sumerians for the treatment of headaches, arthritis, and for inducing sleep
Aspirin derived from salicylic acid (used as pain killer)	Extracts of the leaves of wintergreen plant used as pain reliever. Herbal tea made from willow bark (*Salix alba*) was used as an antipyretic
(±) Atropine[a] (−) Hyoscyamine (dilatation of pupil)	*Atropa belladonna* and *Hyoscyamus niger* were important drugs in Babylonian folklore. (Ladies of Venice used it for converting them into lustrous wide-eyed beauties)
Ephedrine (antiallergic, used for respiratory trouble)	Crude drug, Ma-huang (astringent yellow) derived from *Ephedra sinica* had been used by the Chinese for respiratory ailments since 2700 BC
Quinine (antimalarial)	*Cinchona* species were used by Peruvian Indians for the treatment of fever
Artemisinin (used for malaria)	*Artemisia annua* (herb) used traditionally in China for the treatment of shivering fever
Colchicine (used for gout)	Use of *Colchicum* in the treatment of gout has been known in Europe since 78 AD
Emetine (used for amoebic dysentery)	Roots and rhizomes of *Ipecacuanha* were used by the Brazilian Indians and several other S. American tribes to induce vomiting and cure dysentery
Digoxin (used for heart ailments)	*Digitalis* leaves were being used in Europe in heart therapy during the 18th century
Reserpine (excellent transquilizer)	*Rauwolfia serpentine* ("Sarpagandha") roots were widely used in India for the treatment of hypertension, insomnia and mental disorder

[a]In modern medicine atropine is classified as an anticholinergic drug, *i.e*, an agent which blocks the neurotransmitter acetylcholine in the central and peripheral nervous system

arranging the various structural features of the pharmocophore part (*vide infra*) of the natural drug or its equivalent in a biologically optimal way in order to have the improved biological properties of the targeted drug. Thus, combinatorial chemistry may help in designing the desired drugs with rapid speed.

A few of the drugs from the list (Tables 33.1 and 33.2) and some other drugs will be discussed in some detail.

Modern drug has been defined by Sukh Dev as "*a chemically efficacious* (having undergone pharmacological, toxicological and clinical screening as per accepted parameters of modern medical sciences) c*hemical entity or mixture, of synthetic or natural origin, administered as such or admixed with other entities or vehicles, and being produced in a reproducible form under good analytical control*" [10, 11].

Table 33.2 The sources and bioactivities[a] of some useful natural molecules

Name	Source (plant)	Effective for the treatment of activity	Leading References/ Chapter
Reserpine (CIBA, USA, 1953) (Indole alkaloid)	*Rauwolfia serpentina* (Sans.: *Sarpagandha*)	Hypertension, mental disorder; used as tranquilizer	Chap. 27
Vinblastine (Eli Lilly, 1961) Vincristine (Eli Lilly, 1963) (Dimeric indole alkaloids)	*Catharanthus roseus* (Syn.: *Vinca roseus*) (Beng.: *Nayantara*) (few hundred mg from few tons of dried plant materials)	Hodgkin's disease, lymphosarcoma, leukemia in children	Chap. 28
Podophyllotoxin	*Podophyllum hexandrum; P. peltatum*	Antimitotic, anticancer but toxic	[13–15]
Teniposide Etoposide (Lignans)	Developed from podophyllotoxin	Testicular cancer, small cell lung cancer, and lymphomas	
Taxol (Paclitaxol)	*Taxus brevifolia,* Yew tree (bark)	Metastatic ovarian cancer, lung cancer, breast cancer, malignant melanoma	Sect. 8.4
Taxotere (Diterpenoids)	Semisynthetic analogue of taxol	As active as taxol	
Betulinic acid	*Vauquelinia corymbosa*; common in higher plants	Anticancer cytotoxic effect on human melanoma cell line	[16]
Camptothecin/e (Quinoline alkaloid)	*Camptotheca acuminata* (Chinese tree) (bark) *Nothapodytes nimmoniana* (Syn.: *Mappia foetida*) (Indian tree)	Antileucoplastic activity with serious side effects, such as bleeding in bladder	Chap. 29
Irenotecan Topotecan 9-Aminocamptothecin	Semisynthetic analogues of camptothecin	Lung, ovarian, colon, cervical cancer Similar effects	Chap. 29

(continued)

Table 33.2 (continued)

Name	Source (plant)	Effective for the treatment of activity	Leading References/ Chapter
Artemisinin (Sesquiterpene peroxide lactone, introduced world-wide in 1987)	*Artemisia annua* (herb) (used traditionally in China against fever)	Effective against cerebral malaria; active against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* and *P. vivax*	Sect. 7.9
Artemether Arteether	Simple derivatives of artemisinin	Have better clinical profile and now are being actively developed	Sect. 33.4 (*vide infra*)
Yingzhaosu A Yingzhaosu C (Sesquiterpene peroxides)	*Artabotrys uncinatus* (roots) (used in China for malaria)	Like artimisinin	
Flinderole A	*Flindersia acuminata*	Selective antimalerial activity against *P. falciparum*	[17]
Flinderoles B and C	*Flindersia corymbosa*		[17, 18]
Gomisin A (Polyoxygenated biphenyl derivative)	*Schizandra chinensis* (fruits) (used in China for stomach trouble)	Hepatoprotective	[10, 11]
Sophoradin (Chalcone)	*Sophora substrata*	Significant antigastric ulcer activity	[10, 11]
Plaunotol (Acyclic diterpene)	*Croton sulyrantus* Thai medicinal plant "*plau-noi*"	Cytoprotective antiulcer agent	[10, 11]
Plaunol	*C. sublyratus, C. columnaris* (Thai species)	Antipeptic ulcer activity	[19]
Huperzine A	*Huperzia serrata* (used in China to alleviate memory disorders of the old)	Alzheimer disease; powerful acetylcholinesterase (AchE) inhibitor	[20–23]
Huperzine B	*H. serrata*	Marked anticholinesterase activity	[24]
Curcumin	*Curcuma longa*	Alzheimer disease anti-oxidant, anti-inflammatory	[20] Chap. 34
Capsaicin	*Capsicum annum*	Analgesic	Chap. 34
Pilocarpine	*Pilocarpus jaborandi*	Chlorinergic agent used for glaucoma; stimulates salivary and lacrimene secretion; used during radiotherapy	Chap. 21

Resveratrol	Grape skin; blue berry	Antioxidant in AD as it promotes the decomposition and clearance of intracellular AB aggregates	Chap. 34
Steviol glycosides (Stevioside)	*Stevia rebaudiana*	Sweet in taste; sweeteners to diabetics and others on carbohydrate-controlled diets	[25]
From some Ayurvedic plants receiving clinical support for their therapeutic claims:			
Z-Guggulsterone	Gum-resin called "*gugglu*," (renowned in Ayurveda) from *Commiphora wightii* (Syn.: *C. mukul*)	Inhibits cholesterol biosynthesis	[26]
E-Guggulsterone	*C. wightte*	Similar activity	[26]
Andrographolide (a diterpene)	*Andrographis paniculata* (*Bhuinimka*)	Chronic hepatitis; liver cirrhosis	[27, 28]
Picroside-I Picroside-II (Monoterpene glucosides)	*Picrorrhiza kurroa*	Hepatoprotector liver ailments	[29, 30]
Psoralen (a furocoumarin)	*Psoralea corylifolia*	Antileucoderma, antibacterial (stimulates formation of melanin)	[31, 32]
Bakuchiol (C_{12}-side-chain carrying *p*-hydroxybenzene)	*Psoralea corylifolia*	Antibacterial, effective against psoriasis	[33–35]
Palasonin	*Butea frondosa* (San.: *Palasha*)	Anthelmintic activity	[10, 11, 36]
Gedunin (a tetranortriterpenoid)	*Azadirachta indica* (Sans.: *nimb* Beng.: *neem*)	Antimalarial agents; good *in vitro* activity on certain clones of *Plasmodium falciparum*	[37, 38]

Also references [1, 3]

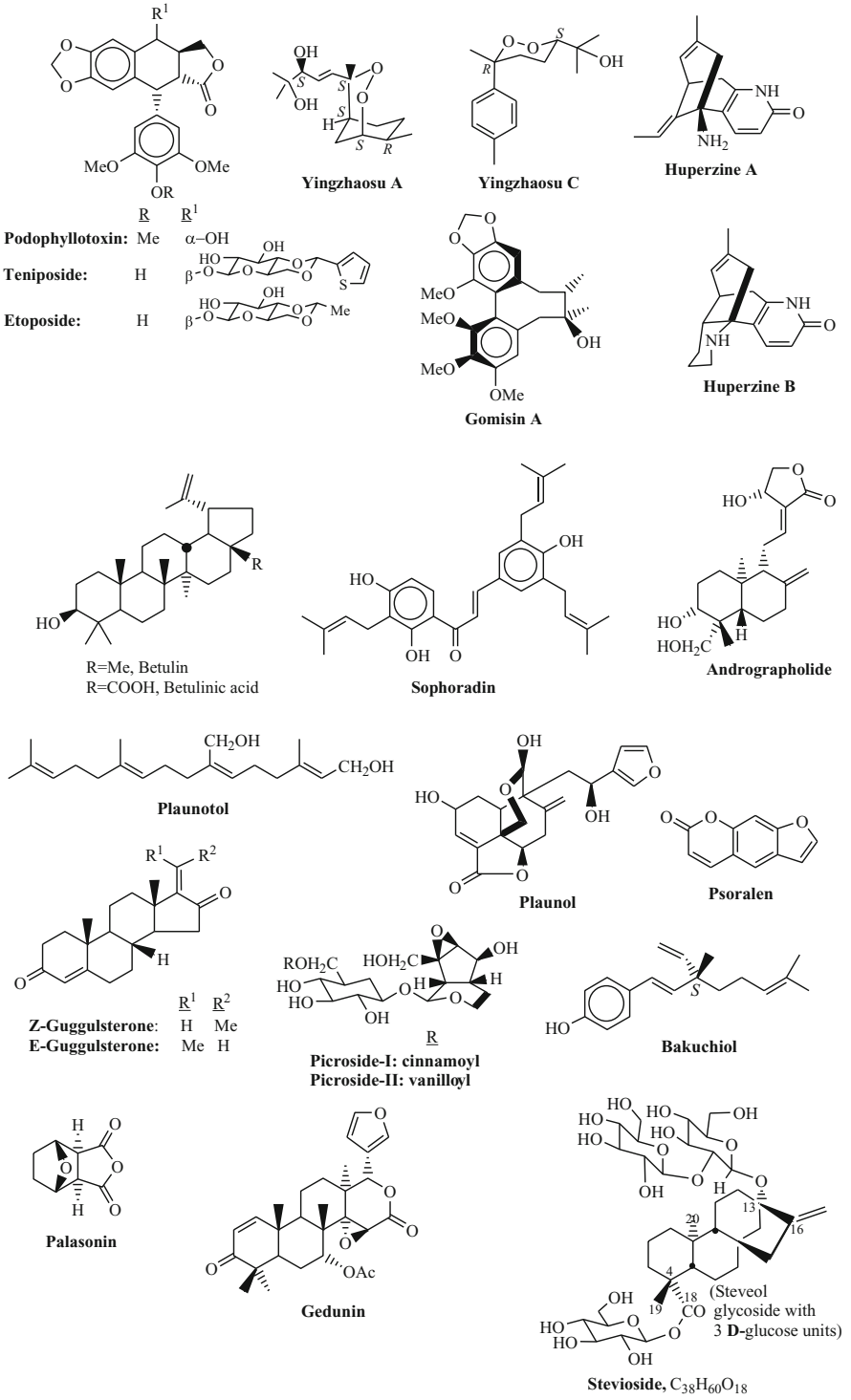


Fig. 33.1 The stereostructures of some useful bioactive molecules

33.2.1 Drug Discovery Process

Drug discovery process involves basically three stages—(i) preclinical studies when an Investigational New Drug Application (**INDA**) is submitted to Regulatory authorities for obtaining approval for carrying out clinical studies, (ii) clinical development of the drug candidate through **Phase I to Phase III** studies, on successful completion of which (iii) a New Drug Application (**NDA**) is submitted for manufacture/marketing of the drug substance in the jurisdiction of Regulatory Authority (Fig. 33.2)

Discovery and early development or preclinical study generally starts with *target identification* which needs thorough knowledge of pathophysiology of a disease. Once the target is decided the next step is synthesis of compounds and selection of the best interacting compound. Likewise, natural products and their derivatives, or their simplified chemical versions are selected. Compound interaction with the target/receptor may confer positive or negative effect on the physiological function of the receptor, which would in turn lead to relief from the disease. Initial lead identification and optimization are generally done using animals.

Clinical development of the drug candidate obtained using preclinical screening is done in the three phase studies. **Phase I study** involves safety tests, pharmacokinetic parameters, and pharmacodynamics of the drug candidate in healthy human

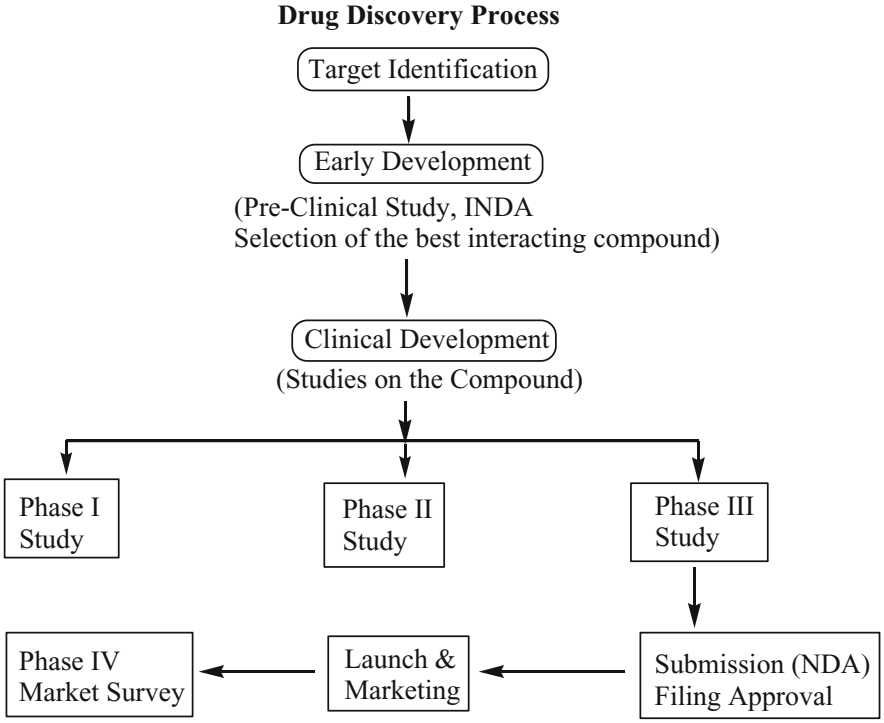


Fig. 33.2 Stages in the drug discovery process

volunteers. This study also provides information about optimum dosage during **Phase II study,** which is a proof of concept study and is aimed to obtain information related to efficacy of the compound in patients. In **Phase III Study** of drug development extensive studies against placebo/standard therapy is carried out. This study provides confirmation of efficacy and safety of the drug candidate.

Once the drug candidate sails through clinical studies one can apply for a new drug application (**NDA**). The regulatory body of the state then thoroughly reviews the studies related to safety and efficacy of the drug candidate and then *approves the application if the data produced are sufficient and unambiguous*. The applicant can then launch the product in the state and also starts the postmarketing surveillance (**Phase IV**) once the product is marketed.

33.2.2 Drug Administration and Its Journey to Receptor

Drugs are generally administered orally (although there are various other ways of drug administration) in the form of tablets/pills/capsules, etc. Oral drugs contain additional additives like binder and filler to press into tablets and to make the drug large enough for handling. Coloring and flavoring components are added to make them acceptable to the patients.

When the drug enters the system it passes through many phases: faces many enzymes and physiological conditions including different pHs and passes through many membranes. In course of its journey the drug molecule bursts open from the additives and would face many interactions, which might affect its structural integrity. Because of its long journey, the drug may be distributed to the different tissues of the body and thus only a small part of it will reach at and interact with the targeted receptor. However, prior to the absorption the drug is topologically considered to be outside the system.

The phases the drug molecule enters have been classified in terms of the interactions of the drug molecule with the body constituents, involvement of the latter, and the fate of the drug. In most cases the mode of action of the drug for its pharmaceutical properties is not complete; rather fragmented knowledge is available. However, the mode of action available for some important plant-derived drugs will be discussed briefly and prior to that a brief account of some of terminology will be given to familiarize them to the students.

For further details a few excellent books [39–45] may be consulted.

The current state of affairs in the drug discovery and development process has been briefly discussed by K. C. Nicolaou in an illuminating article (see reference [13] in p. 1055].

33.2.3 Prodrugs

The designed chemically masked drugs possessing, compared to the original drug, increased solubility, bioavailability, absorption and distribution capability, less toxicity, stability in the phases they pass through, etc., are known as *prodrugs*. The masking depends on the knowledge of body's own metabolism since from the prodrug the true drug should be metabolically unveiled prior to its interaction with the receptor. Further, the drug part of the prodrug should not have any built-in steric shield to offer blocking during demasking (*e.g.*, by hydrolysis). Therefore masking should be done very carefully. There are various types of prodrugs and their ways of releasing the drug molecules. Prodrugs are not active themselves in vitro, but are active in vivo. Some common examples*:* Heroin is a prodrug for morphine (increases the solubility). Aspirin and its ester, which lower toxicity and gastric irritation of the drug, are the prodrugs for deacetylaspirin (salicylic acid). A prodrug of taxol has been included under taxol (Fig. 33.10).

For details, the reference books [39, 40] may be consulted.

33.3 Important Terminologies in the Study of Drugs [44, 45]

33.3.1 Pharmacophore. Pharmacophoric Pattern, Auxophore, Receptor Map

Drugs are pharmacologically active molecules. During drug action, the drug interacts with the macromolecular receptor. The latter recognizes only a part of the drug molecule, which is stereochemically (three-dimensional contours and electron density) compatible with it. This part consisting of interactive (mostly nonbonded) functional group/s which sit/s on the receptor, is called **pharmacophore** (the active or binding site of the drug molecule) and the three dimensional orientation of the groups of the pharmacophore is called the **pharmacophoric pattern**. The IUPAC definition of pharmacophore is " an ensemble of steric and electronic features that are necessary to ensure the optimal supramolecular interactions with specific biological target and to trigger (or block) its biological response." Some atoms are essential to maintain the structural integrity of the pharmacophore and are called **auxophores**. Of the several possible conformers of the pharmacophore, the one which binds the receptor best is known as **bioactive conformation**. The endoperoxy containing ring of artemisinin group of drugs is the pharmacophore of the drugs. The complementary structural part of the receptor is called **receptor map**. When the pharmacophore contains one or more chiral center/s or dissymmetry the optically active drug involves in the stereospecific interaction (enantiomeric

preference) with the drug receptor. The more effective is the interaction, i.e., compatibility, the less is the toxicity.

Auxophores are the atoms which help in keeping the pharmacophore in the right disposition, while some auxophores are non-involving and may be pruned while designing a drug with the natural drug model.

Cis–trans isomerism also plays an important role in the biological system as we find in human eye (Chap. 34).

33.3.2 Pharmacokinetics

Pharmacokinetics deals with the rate of delivery, distribution, absorption, and disappearance of the drug from the site of action, i.e., how the concentration of the drug in the body fluid and tissues varies with time. When the drug is released from its additives, i.e., from its formulation it enters into the pharmacokinetic phase. The journey of the drug molecule up to the target receptor falls under the purview of pharmacokinetic phase which thus covers the duration of its absorption in the body to the receptor. The efficiency of the drug depends much on this phase, since as a consequence of various interactions, the chemical integrity of the drug may be to some extent affected and the concentration of the drug that reaches the receptor is much reduced.

33.3.2.1 Therapeutic Window

A well-defined range of a drug's serum concentration at which a desired effect occurs, below which there is little effect, and above which toxicity occurs, is known as the *therapeutic window* (Fig. 33.3). C_{MAX} is the maximum serum concentration achieved by the drug, T_{MAX} is the time required to achieve maximum concentration in serum, and T_{MTC} is the time required by the drug to achieve minimum therapeutic concentration.

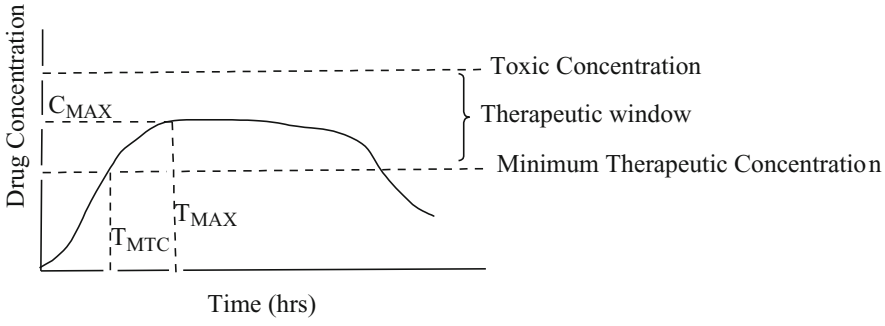


Fig. 33.3 Range of drug's serum concentration

33.3.3 Pharmacodynamic Phase

The pharmacodynamic phase involves the interactions of the drug with the receptor, i.e., the bindings of the pharmacophore at the receptor site when interactions take place both at physiological and molecular levels exerting its biological effect. The drug molecule binds the receptor site based on geometrical complimentarity and interactions (ionic interactions: dipole-dipole interaction, hydrogen bonding, π-stacking, etc.).

33.3.4 Toxicology. LD_{50}. IC_{50}. ID_{50}. ED_{50}

Toxicology refers to the study of poison. This is a very important aspect of a drug. A compound may serve as a therapeutic agent when used in small doses but may be toxic when used in a large dose. A compound responding therapeutically may exhibit side effects like bleeding, e.g., camptothecin/e and podophyllotoxin. The following terms are usually used to denote the toxicity: LD_{50} (a dose which is lethal to 50 % of the experimental animals); IC_{50} (50 % inhibitory concentration, *i.e.*, the concentration of the drug which causes 50 % inhibition of an enzyme activity of a receptor); ID_{50} (50 % inhibitory dose). ED_{50} represents the dose of a drug which exhibits the desired effect on 50 % of the experimental animals. The ratio of LD_{5o} to ED_{50} is known as the *therapeutic ratio*/*index* of the drug.

33.3.5 Pharmacogenetics

The study of the unusual or unanticipated response of the system upon application of a drug is called pharmacogenetics. This effect differs from side effect or toxicological effect but may have a hereditary cause inherited by some in-born erroneous metabolism.

33.3.6 Bioavailability and Bioequivalence

Fraction of the total drug administered, available in the blood supply and for the interaction with the receptor, is known as bioavailability.

The term bioequivalence is used for comparing the bioavailability for a listed drug, *i.e.*, the drug invented by the inventor with that for the same drug formulation made by a generic supplier, and the ratio between these two should be one, *i.e.*, both must have the same pharmacokinetic pattern.

A few important examples of plant natural products and their derivatives and their uses as drugs/prodrugs are given in Sections 33.4–33.15 that follow.

33.4 Anti-inflammatory, Antipyretic, and Pain Reliever. Aspirin, the Miracle Drug (Baeyer, 1897)

One of the earliest and most important success stories in developing a drug from a natural product is that of aspirin [46, 47]. The extract of the leaves of wintergreen plants {*Gaultheria procumbens* (indigenous to USA) and *G. fragrantissima* (Indian winter green) (Ericaceae) [48]} had long been used as a pain reliever. In fact, Hippocrates prescribed willow bark extract for pain and fever more than 1,000 years ago. Even around 3,500 years ago Egyptian doctors first made a report on such an extract as pain reliever [47]. Tea made from willow bark (*Salix alba*, Salicaceae) was used as antipyretic. In an attempt to isolate the active principles responsible for the above properties salicin (willow bark) and methyl salicylate (wintergreen leaves) have been isolated. Salicin (Fig. 33.4) is responsible for analgesic and antipyretic properties of willow bark extract. Methyl salicylate is the major component of various balms used as pain reliever.

Salicin is converted to salicylic acid and was used as a pain reliever. An inexpensive synthesis of salicylic acid from sodium phenoxide and CO_2 under pressure at 180–200°C was achieved by Adolph Wilhelm Herman Kolbe (1818–1884) at Leipzig at one tenth of the price of natural extract. However, salicylic acid attacks the mucus membrane lining of mouth, gullet, and stomach and causes severe irritation. To make it less acidic it is converted to sodium salicylate. The substance was less irritative but its objectionable sweet taste made it unacceptable to the patients. However, a breakthrough came at the turn of 1897 when Felix Hoffmann a synthetic organic chemist at Baeyer, synthesized acetyl salicylic acid (ASA) known as *aspirin* which has the same medicinal properties but is free from side effects (irritation and objectionable taste) and till date maintains its fame as a miracle drug. The name aspirin is derived from *a* for acetyl and the root "spir" from the Latin name of mellow sweet plant "*spiraca*." Aspirin became the first major medicine to be sold as tablets and till 2006 one trillion (10^{12}) aspirin tablets have been

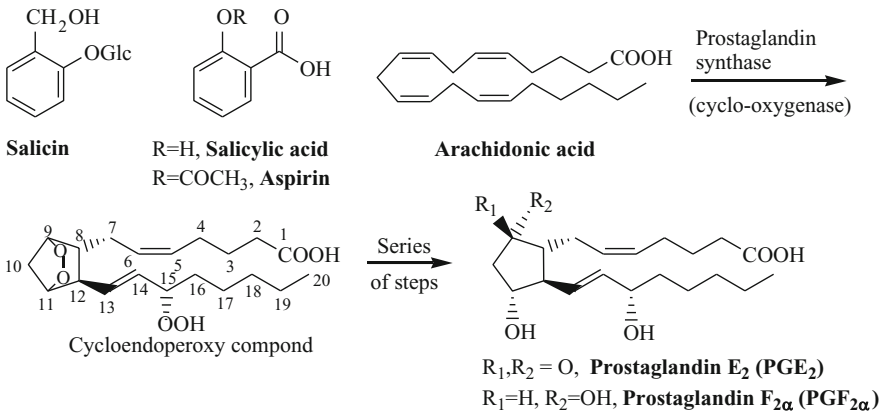


Fig. 33.4 Biosynthesis of prostaglandins

consumed [46–48]. Aspirin tablets are usually compounded of about 0.32 g of acetylsalicylic acid pressed together with a small amount of starch for binding. Kolbe's synthesis of salicylic acid and its conversion to aspirin led to its industrial production by Baeyer Company in 1897 which still remains one of the popular drugs. *For the last several decades it is being widely used as the major constituent of some drugs acting as a blood thinner, since aspirin prevents the initial activation of the blood clotting mechanism, enhancing blood flow through narrowed arteries and thus reducing the probability of the heart attack.*

The remarkable mechanism of the anti-inflammatory effect of aspirin was discovered by John R. Vane (1927-) in 1974. He showed that aspirin inhibits the biosynthesis of prostaglandins (Fig. 33.4) by acetylating a serine residue (active site) in prostaglandin synthase [49–51]. Vane shared the 1982 Nobel Prize in medicine with Sune K. Bergström and Bengt I. Samuelsson for this discovery.

The initial conversion of arachidonic acid to a cycloendoperoxy compound (Fig. 33.4) is catalyzed by microsomal enzyme/s present in numerous tissues and human platelets. The microsomal enzyme that metabolizes arachidonic acid has been termed prostaglandin synthase or cyclooxygenase (Fig. 33.4). Roth [52] showed that sheep vascular gland prostaglandin synthase (cyclooxygenase) is irreversibly inactivated by aspirin. Prostaglandins are thought to be responsible for evoking pain, fever, and local inflammation (body's immune responses). Aspirin thus prevents the bodily synthesis of prostaglandins and consequently alleviates the symptoms (fever, pain, inflammation, menstrual cramps) of the body's immune responses. The chronic use of aspirin leads to ulceration of stomach lining [49, 53]. The pregnant ladies should not be prescribed aspirin.

In 1994, sodium salicylate and its semisynthetic derivative were reported to be the first plant-derived compound to show ($NF\text{-}_K\beta$) activity.

33.5 Antimalarials

33.5.1 Introduction

Malaria is one of the most important tropical diseases and one of the most terrible scourges of tropical and subtropical region that claimed millions of lives. During the Second World War, a huge number of British and Indian soldiers died of malaria. In fact, malaria casualty was more than the people died in the battle. European countries were affected during seventeenth to eighteenth centuries as well. There were more than 200 million cases of malaria and at least 655,000 deaths reports WHO in 2010. The disease is caused by the protozoa of the genus *Plasmodium* which remains as spores in the gut of *Anopheles* mosquito. The female mosquito (*Anopheles maculipunnis*), acting as the carrier, injects the protozoal parasites through her bite to human when sucking the blood meal. The life cycle of the parasite is complex and is not completely understood. During the course of its life cycle the parasite resides in both human host and the mosquito vector at various

developmental stages. One of the most extensively studied stages is that of the red blood cell (RBC). The spores spread in human. They break the hemoglobin and use its amino acids and iron as food. The parasite *Plasmodium* enters the human blood and hence in the red blood corpuscles. Human blood is affected mainly by four different *Plasmodium* species (*P. vivax*, *P. falciparum*, *P. ovale*, and *P. malariae*) and occasionally by P. knowlesi. Of these *P. falciparum* is the most dangerous one. It infects the blood cells, makes them sticky and clumpy in the capillaries of deep organs, and arrests the microcirculation. When it occurs in the brain it causes sweating, dizziness, diarrhea, delirium, coma, convulsion, and finally death. Of all types of malaria, cerebral malaria is the worst [54]. Malaria management requires the right drug in one hand and secondly the mosquito breeding locations—the stagnant water in all its forms. With long use of the marketed drugs, the parasite becomes drug resistant. Chloroquine-resistant *P. falciparum* is a matter of great concern and new improved drugs are badly needed.

33.5.2 Quinine

All the major drugs of malaria are plant based. The first natural drug introduced for malaria treatment has been quinine, the most used one; it has become a household name. Malaria is the first disease treated with a pure compound quinine and this event heralded the chemotherapy. Prior to the isolation of quinine in the pure form by Pelletier and Caventau in 1820, the bark and the extracts were used to be exported to European countries. Quinine served as the lead molecule for several synthetic antimalarial drugs (Fig. 33.5). A new molecule ELQ-300 [55] has been developed by Michael Riscoe's group at Oregon Health and Science University that

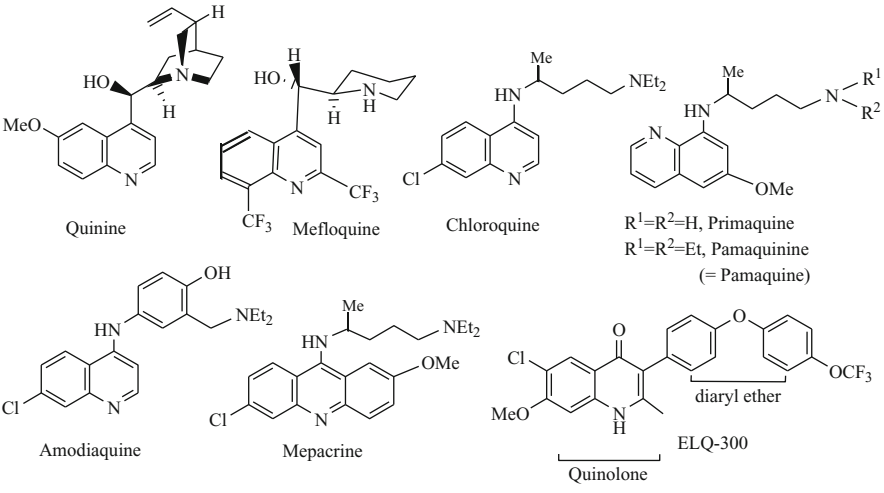


Fig. 33.5 Quinine and some synthetic antimalarials

can kill malaria parasite at several different stages of its life cycle by preventing the synthesis of its DNA bases thymine and cytosine. Thus prevents its reproduction and so it dies. Early studies show that the parasites might need longer time to develop resistance against this drug.

Paul Ehrlich (NL 1908) cured two malaria patients with a dye (methylene blue). This is the first time a synthetic drug was ever used in human. By modifying methylene blue pamaquine was synthesized in 1925 [56].

Quinine is still effective today. However, it has some adverse side effects namely ringing in the ears—sometimes leading to partial deafness. Its use in malaria has currently been limited. It is rather used as a *prophylactic* (a prophylactic is a protective agent, which when administered can prevent a disease from its occurring). Chloroquine largely displaced quinine, because of its excellent efficacy, low toxicity, and low manufacturing cost. It was introduced in 1950. However, possibly for its widespread use, the parasite became resistant to chloroquine and the drug accumulation in the food vacuole is reduced. Resistance appears to be the result of the parasite having a cell membrane protein that can pump the drug out of the cell.

The malaria management thus badly needs new drug/s.

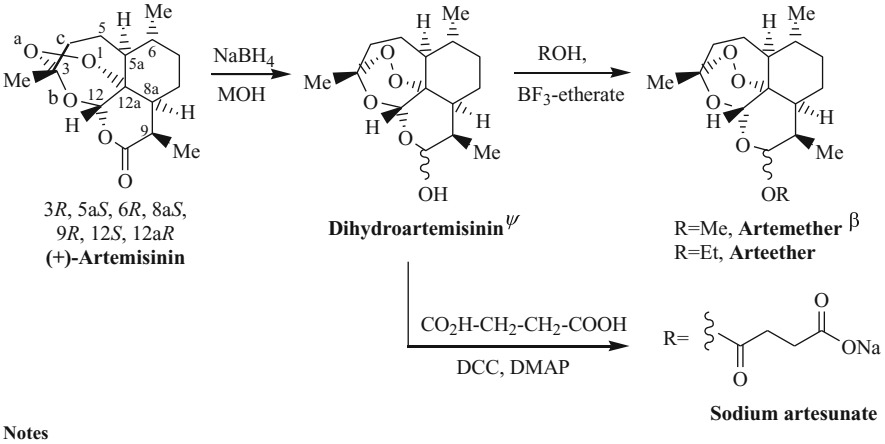
33.5.3 Artemisinin

The timely discovery of an antimalarial artemisinin from *Artemisia annua* came as a great boon. *Historically it is not a new drug. The plant had been in use as a herbal medicine in China for thousands of years and some data were documented* (Sect. 7.9). It is the first non-nitrogenous antimalarial with a unique structural scaffold endowed with an endoperoxy linkage. The natural (+)-artemisinin possesses 7 chiral centers (3*R*, 5a*S*, 6*R*, 8a*S*, 9R, 12S, 12aR) and profound antimalarial activity. It is interesting to note that since ancient days a formulation containing powdered *Artemisia* leaves, tobacco leaves, and arsenic were used to be prescribed as a mosquito repellant in China.

The therapeutic value of artemisinin is limited because of its poor solubility in water and oil and of its short half-life in plasma. High dose is needed because of its poor oral activity. To circumvent these shortcomings of artemisinin, its structural analogues have been semisynthesized. The lactone group of artemisinin is reduced and derivatized to produce dihydroartemisinin, artemether, arteether, and sodium artesunate (Fig. 33.6), which are found to be more active than artemisinin itself. Lactone group is thus not necessary for the activity. The endoperoxy group has been found to be essential for its activity and represents the pharmacophore of the drug.

The structures of three modified artemisinin derivatives and two other compounds having peroxy and oxy linkages like artemisinin which exhibit profound even better antimalarial activity are shown in Fig. 33.7.

In view of the occurrence of more than 200 million cases of malaria and at least 655,000 deaths as reported in 2010 by WHO, the organization has recommended



Notes

ψβ-epimer, which is more active, can be separated by column chromatography or fractional crystallization

βIn case of severe malaria, initially 200 mg artemether is given, followed by 100 mg for every 6 h for 1 day and then 100 mg after 4 days; thus total dose 700 mg is administered to clear the parasite rapidly with no side effects. However, high rate of recrudescence was reported.

Fig. 33.6 Artemisinin and its more active derivatives

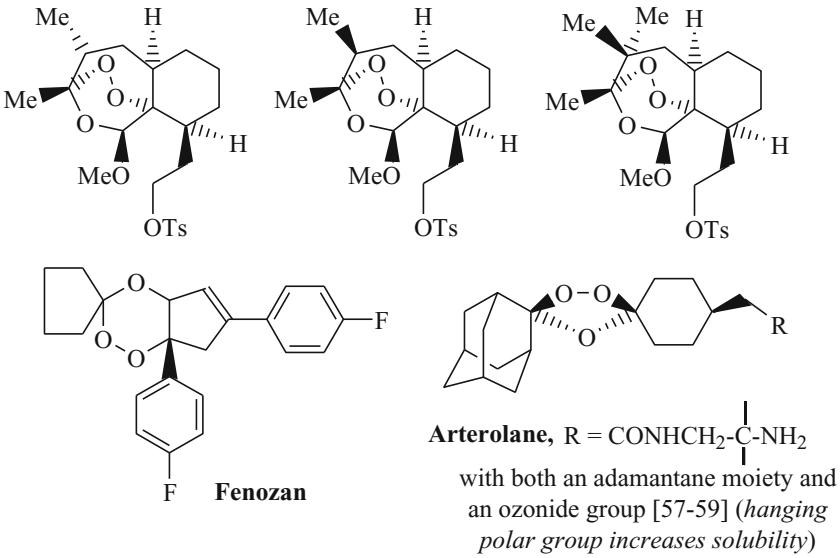


Fig. 33.7 Modified artemisinin and other peroxide compounds [57–59]

artemisinin based combination therapy (ACT) (ref. [92] of Chap. 7). Large-scale preparation of artemisinin by employing biological and chemical synthesis has been reported (Sect. 7.9). This method serves as a stable and affordable source of artemisinin.

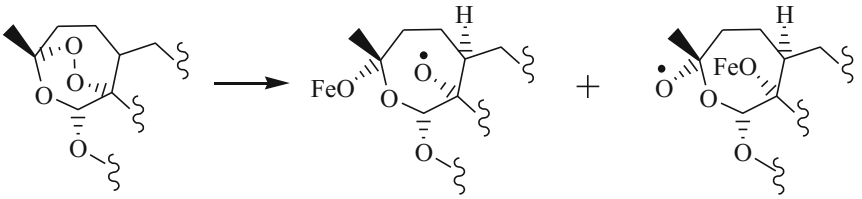


Fig. 33.8 Radicals generated from peroxide linkage

33.5.4 Probable Modes of Action of Antimalarials

The parasites, while breaking hemoglobin and using amino acids and the released heme cells, provide ferrous ion, which reduce the peroxide linkage of artemisinin to yield two types of radicals (Fig. 33.8). These radical attack the vital biomolecule within the parasite and destroy them. These radicals do not affect the cellular polymer of human. Artemisinin is thus used in the treatment of drug-resistant malaria.

The globin is enzymatically cleaved into small peptides, producing free heme as a byproduct toxic to the parasite. The heme undergoes a one electron oxidation to produce *ferriprotoporphyrin* that precipitates in the digestive food vacuole of the parasite as submicrometer to micrometer sized crystals, known as *hemozoin*, which is harmless to the parasite. Hemozoin [60], the malaria pigment, is a marker of malaria infection in RBC assays.

The antimalarials are known to act during the degradation of hemoglobin and subsequent production of hemozoin crystals.

Quinine derivatives and other antimalarials play the role of inhibiting the crystal nucleation and growth of hemozoin. The quinine and quinoline drugs stereospecifically bind the specific hemozoin crystal faces acting as the capping agent and subsequently inhibit the growth of the crystals. On the other hand artemisinin group of drugs form adduct to all the principal faces of hemozoin and causes the inhibition of the growth effect.

33.5.5 Flinderoles A–C

Since plants gave two major antimalarials and lead molecules, a screening program was undertaken under malarial natural products screening program with compounds isolated from Australian and Papua New Guinean plants. Bisindole alkaloids isolated in 2009 from *Flindersia* species and designated Flinderoles A–C [17, 18] (Fig. 33.9) showed impressive selective antimalarial activity against *P. falciparum*. They showed inhibition of parasite growth with IC_{50} values between 0.08 and 1.42 μm against a chloroquine resistant *P. falciparum* strain. Further work

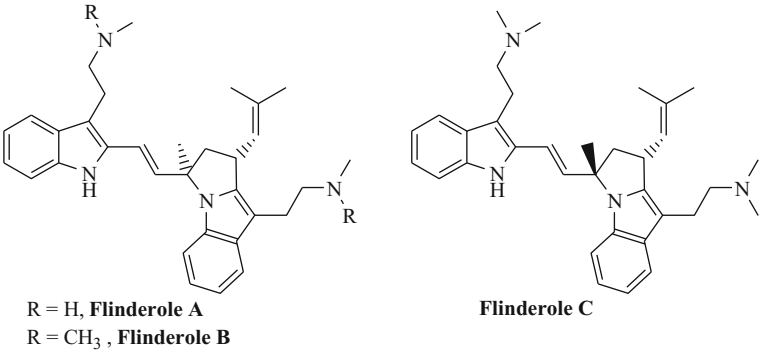


Fig. 33.9 Stereostructures of flinderoles **A-C**

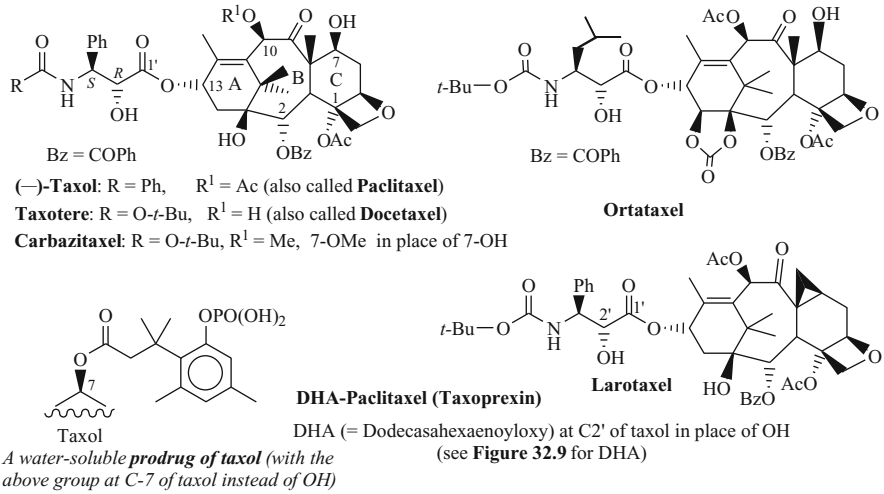


Fig. 33.10 Some active analogues of taxol and a prodrug

on the antimalarial activity of this novel class of antimalarials is in progress. The compounds have recently been synthesized [18].

33.6 Anticancer Drugs

Taxol (Paclitaxel) (Fig. 33.10) (*Taxus brevifolia*, Taxaceae) (Sect. 8.4) vinblastine, vincristine (*Madagaskar periwinkle* plant, *Vinca rosea* syn. *Catharanthus roseus*, Apocynaceae (Chap. 28), podophyllotoxin (a lignan from *Podophyllum peltatum* and Himalayan *Podophyllum emodi*, Podophyllaceae), camptothecin/e (Chinese

plant *Camptotheca acuminata*, Nyssaceae) (Chap. 29), betulic acid, and betulin (*Anemone raddeana, Lycopodium cernuum,* Lycopodiaceae, *Betula alba*, Betulaceae, and various other higher plants) exhibit profound anticancer activity. The last two are still in the testing phase [61]. The uncontrolled growth of highly heterogeneous malignant cell population is the characteristics of most of the cancer diseases. The biobehavior of the natural anticancer agents has been discovered during screening programs focused on the search for plant based anticancer agents.

33.6.1 Vinblastine, Vincristine, and Semisynthetic Analogues

The discovery of *Vinca* anticancer agents especially vinblastine is a case of serendipity. Extracts of *Vinca roseus* (Madagascar periwinkle) leaves and flower are used to treat diabetic patients. When these extracts were fed to the experimental animals (mice induced with diabetics) all of them died because of losing immunity caused by the dramatic downfall of white blood cell count. This observation was made by Dr. Robert Noble, University of Western Ontario, on the extracts of *Catharanthus roseus* leaves (*periwinkle* plant, Jamaica) sent by his elder brother Dr. Clark Noble from Jamaica. This struck him as a potential drug source for leukemia which is caused by abnormal rise in WBC count. By judicial dose, the abnormally high WCB count could be brought to normal count. Thus vinblastine, one of the main active principles of *C. roseus*, was born as an effective anticancer drug and almost immediately marketed. Even today after more than 50 years of its discovery it is one of the most important anticancer drugs [62].

The compounds with anticancer properties entered into the realm of therapeutic agents as such and also served as the lead molecules for more powerful analogs, with the benefit of the parent compounds without their disadvantage. They are semisynthesized from parent compounds or their appropriate chemical fragments. Synthetic simple molecules carrying the pharmacophore or its chemical and conformational equivalents have also been developed. Attention has always been focused to their low-cost production to make them available to the users. They are expected to have much reduced toxicity and pharmacokinetically and pharmacodynamically more effective in the system.

33.6.2 Mechanism of Action of Anticancer Drugs [63]

The mechanism of the action has been studied for many anticancer drugs; Tubulin, a structural protein is present in the nucleus of all plant and animal cells (eukaryotic cells). Tubulin plays an important role in cell division. It polymerizes to small tubes called microtubules in cell cytoplasm, and microtubules release neurotransmitters

involved in the mobility of cells. When the cell division is about to take place microtubules depolymerize back to tubulin and repolymerize to form spindles of cell division. The spindles push apart the newly formed cells and help in distributing the chromosomes from the original cells to the newly formed daughter cells, *i.e.*, *mitosis* takes place. Compared to the normal cells which infrequently divide, the division of cancer cells is rapid producing layers of cells forming lumps and tumors. The process of cell division could be arrested by interfering with either of the processes of polymerization of tubulin to microtubules or depolymerization of microtubules to tubulin.

Anticancer drugs target either of the steps. The drugs stop polymerization by binding tubulin in the metaphase, an event that induces *apoptosis* (suicide) by halting spindle formation during mitosis. Depolymerization of microtubules to tubulin stops when the drug helps in stabilizing microtubules. Drugs which prevent polymerization do not prevent depolymerization, and drugs which stop depolymerization cannot prevent polymerization [63].

Vincristine, vinblastin, vinorelbine, and vindesine (Fig. 28.1) (the latter two are semisynthetic derivatives of vinblastine) inhibit cell division by binding the tubulin protein and prevent the polymerization.

Vinblastine is used in the treatment of Hodgkin's disease, lymphocytic lymphoma, histiocytic lymphoma, testicular cancer, and breast cancer at an advanced stage.

Vincristine is used in leukemia, Hodgkin's disease, and other lymphomas. Pregnant women should not be prescribed vincristine and vinblastine as they cause defects.

Vindesine is used in melanoma, lung cancer, and uterine cancer. **Vinorelbine** has been tested successfully for ovarian cancer. **Podophyllotoxin,** also a tubulin interactive agent prevents polymerization of tubulin, but it has undesirable side effects. However, two of its derivatives *teniposide* and *etoposide* and *etophos* (a prodrug of etoposide) are effectively used in combination with other drugs in lung cancer, testicular cancer, and lymphoma. 4-Demethylepipodophyllotoxin drugs do not bind tubulin but inhibit the enzyme topoisomerase II needed in DNA synthesis and replication.

33.6.3 Taxol and Other Taxoids. Their Bioactivity

Taxol (Paclitaxel) (Sect. 8.4) [64–67] and other taxoid drugs show high activity in leukemia assay. Subsequent observations exhibited its superior spectrum of activities. Taxol is an outstanding therapeutic agent against solid tumor (breast and ovarian cancer, non small-/small-cell lung cancer, and cancers of head and neck) and also approved for Kaposi sarcoma (malignant cells appear as red or purple patches under the skin or mucous membrane (Dr. Moritz Kaposi, a Hungarian dermatologist first described it in 1872 as a rare disease). The unique mechanism of its action has been discovered by Dr. Susan Horwitz [63] and coworkers who demonstrated that taxol and other taxoid drugs bind β-subunit of tubulin protein and

accelerate the polymerization of tubulin to microtubules, stabilize the microtubules, and thus prevent their depolymerization. This phenomenon stops cell division. Taxol is the only plant product known to promote the assembly of microtubule and inhibit the tubulin disassembly process and, thus, appears to be the prototype of a new class of cancer chemotherapeutic agents.

Since the taxol-microtubule complex is noncrystalline, its X-ray crystallographic study is not available. T-Taxol has been proposed as the bioactive conformation on β-tubulin supported by modeling studies of the taxol–β-tubulin interactions based on electron crystallographic density [68] within the tubulin-binding site. The discovery of T-taxol conformation opened a new avenue for the synthesis of taxol analogues that fit this conformation and hence binds with tubulin.

The *structure activity relationship* (SAR) for taxol has been studied [69, 70]. Extensive SAR studies showed that the core containing the ester groups (C2-benzoyl, C4-acetyl, C13-side chain) and oxetane ring are responsible for biological activity (lower part of the molecule as shown in the normal diagram). The lower part of taxol plays an important role for taxol at its microtubule target through binding interactions. Hence substantial variation at the lower part is restricted, while wide variations in the nature and stereochemistry of the C13-side chain and C7 can be tolerated and could be done in the upper half of the molecule for improving solubility and potency (pharmacokinetics) of the drug [71, 72].

Some active analogues of taxol changed in the upper half of the molecule and a **prodrug** of taxol are shown in Fig. 33.10. **Docetaxel** (taxotere), a side chain analogue of taxol, is one of the intermediates produced during a semisynthesis of taxol from 10-deacetylbaccatin III. It is found to be more active than taxol and is more water soluble. Several taxol analogues have been subjected to clinical trials. Some of the compounds which have attained advanced clinical trials are **docetaxel, ortataxel, larotaxel,** and **carbazitaxel** (Fig. 33.10). The NHCOPh group of 10-deacetyltaxol when replaced by NHCOO*t*Bu gives docetaxel, also known as taxotere. Carbazitaxel is the 7,10-dimethyl ether of docetaxel, and ortataxel is an orally active cyclic carbonate analogue. An ester of taxol at the C13 side-chain hydroxyl with the polyunsaturated fatty acid DHA (see Fig. 34.10) known as **DHA-paclitaxel (taxoprexin)** is also in advanced clinical trials. A water-soluble prodrug of taxol (7-OH of taxol is esterified) and a peptide-bound **prodrug** of taxol have been reported.

The function of the oxetane ring (at C4 and C5) is (i) to rigidify the tetracyclic taxol core to provide an appropriate framework for presenting C2, C4, and C13 side chain to the microtubule protein and (ii) to serve as a H-bond acceptor. The oxetane ring is regarded to be essential for biological activity [69].

Global survey on *Taxus* species shows that the Asian species *Taxus yunnanensis* and *T. wallichianna* have high concentration of taxol in their bark and can be used for commercial production of taxol [73].

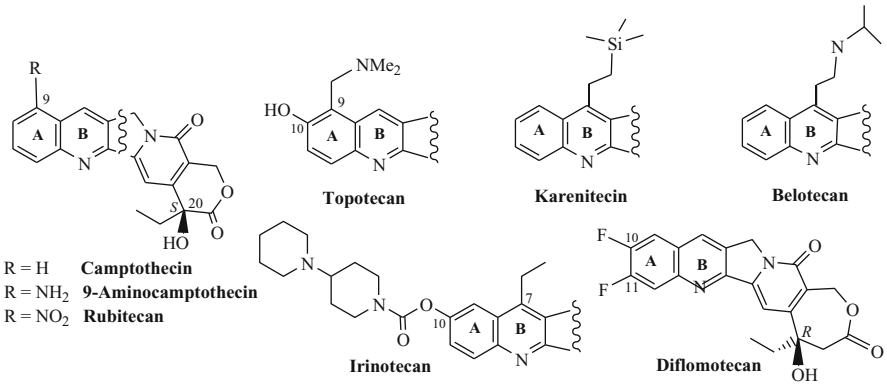


Fig. 33.11 Camptothecin and its some important derivatives (synthetic)

33.6.4 Camptothecins [64, 67]

Camptothecin possesses a broad spectrum of anticancer activity (breast malignant melanoma), but it showed some side effects. However its one derivative **9-amino camptothecin** showed good responses in a number of cancers. The water-soluble derivatives **topotecan** and **irinotecan** (Fig. 33.11) show effective anticancer activity against ovarian cancer and colorectal cancer, respectively. **Irinotecan** is a carbamate *prodrug* of 10-hydroxy-7-ethylcamptothecin, which is converted into the active drug by liver enzymes. It is recognized that camptothecin derived drugs inhibit topoisomerase I enzyme (involved in cleaving and resealing one strand of DNA during the replication process). They cause substantial DNA damage in tumor cells and prevent their growth. The *S-configuration at C20 appears to be essential for activity,* but the α-hydroxy-δ-lactone in these drugs is rapidly hydrolyzed under physiological condition to an almost inactive form. Structures of some recently introduced camptothecin-derived drugs are shown in Fig. 33.11. **Diflomotecan** having a 7-membered β-hydroxy-ε-lactone has enhanced stability toward hydrolysis and hence is a promising drug.

33.6.5 Colchicine, A Tubulin-Interacting Potential Anticancer Drug

Colchicine (Chap. 24) has been used in the treatment of gout since ancient time. The pharmacophore of colchicine has been identified to be the non-coplanar two aromatic rings. The tropolone system of colchicine tightly binds tubulin and prevents its polymerization to microtubules and hence the mitotic spindle formation necessary for cell division [74].

During the process, colchicine forms a complex with soluble tubulin [75]. Colchicine reduces the mobility of neutrophils into the joints. Unfortunately, colchicine has side effects which restrict its liberal use. Only in cases of acute pain it is prescribed. Colchicine serves as a lead molecule for compounds which inhibit tubulin polymerization and act as mitotic spindle inhibitor [76]. The behavior of colchicine and its derivatives is similar to those of vinblastine, vincristine, and podophyllotoxin; hence they may be *potential anticancer agents.*

In plants generally the eukaryotic cells are diplod, *i.e.*, they contain two sets of chromosomes (one set from mother and one set from father). During anaphase the spindle distributes the chromosomes of the mother cell to the two newly formed daughter cells. However, the inhibition of spindle formation by colchicine ends up into the supply of all the chromosomes into one of the daughter cells when the latter possesses four sets of chromosomes making it tetraplod. Thus colchicine finds wide-spread use in the tetraplod varieties of flowering plants [77].

33.7 Reserpine (Chap. 26)

Rauwolfia serpentia (Apocynaceae) extract (roots) has been prescribed from ancient times in India to patients suffering from hypertension, insomnia, and insanity [78]. It is one of the few examples of enthopharmacologic agents successfully introduced in Western therapeutics [79]. Later, reserpine (Chap. 26), an indole alkaloid has been found to be the active principle of *Rauwolfia serpentina* and is responsible for its biological activity. It interferes with the membrane of synaptic vesicles and deplete nerve endings, dopamine, serotonin—thus causes the depletion of neurotransmitter resulting into hypotension and sedation. Prolonged use of reserpine is restricted because of its side effects and is hardly prescribed now. According to some, the decoction of roots is better than the pure drug. Perhaps the enzymes and other components present in the decoction offer synergestic effect. *Rauwolfia* genus is a storehouse of indole alkaloids.

33.8 Opioid Analgesic Drugs. Morphine, the Active Principle of Opium, and its Analogues

A brief history about the use of opium and morphine has been given in Chap. 23. Morphine is an excellent pain killer but it has several undesired side effects. To overcome its disadvantages, various derivatives of morphine were prepared by adding to or deleting some chemical entities from its skeleton with the hope of getting higher potency analgesics. Even some very simple piperidine derivatives with morphine as the model have been synthesized and used. Unlike morphine these semisynthetic and synthetic drugs are to be non-addictive as far as possible.

Introduction of a hydroxyl group at C14 increases its activity. Perhaps the hydroxyl group provides an extra source for hydrogen bonding with the binding site. The variation of the side chain at nitrogen leads to important results. N-phenylethylmorphine is 14 times potent than morphine. Some N-substituted alkyl derivatives with 14-hydroxyl group also act as antagonists [80], *i.e.*, they block the receptor and morphine fails to bind and could no more exhibit its analgesic properties. In the event of overdose of morphine to patients, the antagonists play a positive role. They block the receptor and push away excess morphine from the binding site and save the patients from the risk of death by suffocation. Respiratory trouble is a side effect of morphine. Phenolic OH at C3 is also found to be an important functional group. Perhaps it helps in binding at the receptor site by Van der Waals forces. Some semisynthetic derivatives of morphine and simple piperidine derivatives are shown in Fig. 33.12. British chemist K.W. Bentley has prepared a number of morphine derivatives having much more potency than morphine. Some possess potency as high as 1,000 times more than morphine and have been used to subdue elephants in African Game Reserve with only 1 mg of the

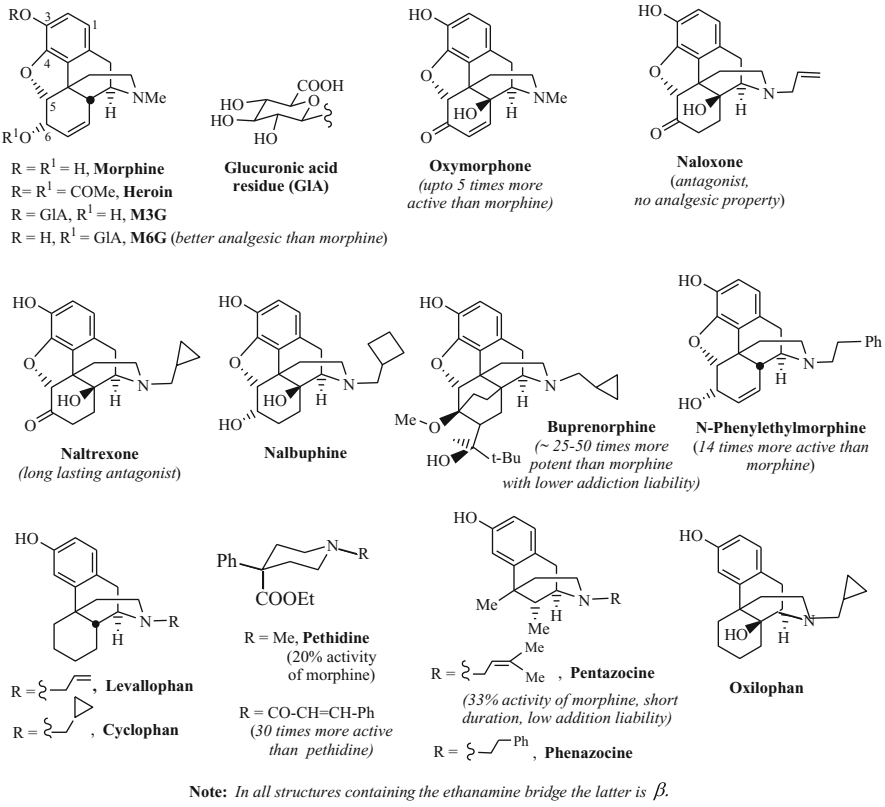


Fig. 33.12 Morphine and some of its semisynthetic derivatives and a few piperidine derivatives

material per elephant. However, their dangerous addictivity precludes their use on human [81].

Heroin, a 3,6-diacetyl derivative of morphine is highly addictive. It is more soluble, lipophilic in character, and gets absorbed and transported in a better way than morphine. It is two times more active than the latter. It is hydrolyzed to morphine, hence is considered to be a *prodrug*. It is used in the terminal diseases. Morphine is metabolized into *morphine 3-glucuronide* (M3G) and *morphine 6-glucuronide* (M6G). M6G shows analgesic effect, while M3G shows very low affinity for opioid receptor and analgesic property. Phase I, phase II, and phase III trials prove M6G to be a better drug than morphine. It has reduced side effects (vomiting tendency and nausea) in the patients [82].

Codeine, (Chap. 23), a methyl ether of morphine, much less analgesic than morphine, is used in cough syrups.

33.9 Huperzine A (Table 33.2) [12, 83, 84]

Huperzine **A** is a selagine [21] type alkaloid. It shows powerful and reversible anticholinesterase activity. It is used in the treatment of *Myasthenia gravis* (a rare neuromuscular disease which increases the weakness of the voluntary muscles specially of eye lids and part of mouth and throat) and for the improvement of the memories, senile dementia, and Alzheimer disease (Dr. Alois Alzheimer, a German neuropathologist first described the presenile dementia on the basis of amyloid as early as 1906). It showed good pharmacokinetics and good absorptions within the body. It is hoped to be better tolerated by the patients compared to the drugs in the market.

33.10 Curcumin (Table 33.2) (Chap. 34) [84]

Curcumin has been used for various health benefits and also in AD. It inhibits Aβ-deposition, Aβ-oligomerization, and tau phosphorylation in the brain of AD-induced animals. Curmumin shows great promise as a medicine in the treatment of AD.

33.11 Natural Products Affecting the Production of Nitric Oxide

In 1990 nitric oxide (NO) has been identified as a neurotransmitter within central nervous system and its involvement in amazingly wide range of physiological processes made NO, the molecule of the year 1992, as decided by the editors of Science. In 1998 Furchgott, Ignarro, and Murad received Nobel Prize in Physiology and Medicine in recognition of their pioneering work on NO. Being a very small simple molecule its analogues could not be conceived. Attempts thus have been made to generate and to inhibit the production of NO within the system using compounds under physiological conditions. Both inadequate formation as well uncontrolled production of NO is responsible for various vital disorders [85].

The potential of a number of natural products as inhibitors of mammalian NOs (nitric oxide synthase) and also for the decrease of the transcription level of NOs gene has been explored. These natural products include coumarins [86], terpenoids, flavonoids and compounds like curcuminoids (diarylheptanoids) [87, 88], and various stilbene derivatives.

33.12 Natural Products as NF-kB Inhibitors

Sodium salicylate and its semisynthetic derivative aspirin were the first plant-derived compounds reported in 1994 to modulate NF-kB-activity [89]. A large number of natural products isolated from plants, used in traditional medicine, are shown to interfere with cascade leading to NF-kB activity. In this physiological process the effect of diterpenoids (kaurane diterpenes) and sesquiterpene lactones have been studied [89].

33.13 *Aloe vera* (Liliaceae)

Aloe is a genus of 160 species indigenous to East and South Africa. Several species, *viz*, *Aloe vera, A. vulgaris, A. barbadensis*, and *A. officinalis* have been introduced in India. *A. vera is* a native of North Africa and spreaded to East and West Indies, India, China, and other countries [90]. The major constituents of *A. vera* are the hydroxyanthrone glycosides, *e.g.,* aloin A, xanthones, and chromones [3, 12]. It also contains glycosides, vitamins, amino acids, anthraquinones, saponins, and minerals [91]. It is a powerful laxative. It increases menstrual flow in nongravid uterus and is used for abortion. It is anthelmintic. The juice is used in minor burns and is now extensively used in various cosmetic creams and lotions [3, 12].

33.14 Flavanoids as Antioxidants

See Chap. 34

33.15 Pharmaceutical Applications of Some Other Drugs

The biological activities and pharmaceutical applications of **camphor** (Sect. 6.3.1), **menthol** (Sect. 6.4), **dicoumarol** and **warfarin** (Sect. 13.2.9.5), **atropine** (Sect. 19.7), **cocaine** (Sect. 19.9.4), **ephedrine** (Sect. 20.10), **pilocarpine** (Sect. 21.6), and **papaverine** (Sect. 22.7) have been discussed in the respective chapter and section given in the parenthesis.

Psoralen and **bakuchiol** (Fig. 33.1) in various formulations as well as the oil from the seeds of *Pongamia glabra* (Leguminosae) which contains flavones, furanoflavones, and other phenolic compounds are extensively used (Ayurvedic medicine) in the treatment of various skin diseases including psoriasis. From the flowers of *Pongamia glabra* several new hydroxyfuranoflavones have been reported [92, 93]. **Dodecahydrosqualene** obtained by catalytic hydrogenation of squalene on platinum or nickel catalyst is widely used in cosmetics.

33.16 Concluding Remarks

The biodiversity of natural products and the long traditional medicinal uses of their sources call for attention to a systematic venture for drug discovery with the help of combinatorial chemistry and pharmacophores from natural products. All pharmaceutical companies are involved in such researches, and the results may offer great hopes to the sufferers. For prime Ayurvedic plant drugs and their modern scientific appraisal reference [3] may be consulted [94].

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Chapter 34
Organic Phytonutrients, Vitamins, and Antioxidants

34.1 Introduction

All living beings need food to grow and survive. Through ages animals have collected food from the surroundings matching the need of the body. Man being supreme of all animals selected his food on the basis of taste, flavor, appearance and aroma, compatibility with the body system, and health benefit. Thus tongue, nose, and eyes are the primary bodily organs needed to select a food.

Every time we consume food, it contributes toward the renewal of many aspects of our body. Food provides us with both energy and materials needed to build and maintain body cells. The basic purpose of food is thus to provide nourishment.

The primary source of food of man is of plant origin that may be obtained directly or sometimes indirectly. We will discuss briefly only the types of phytochemicals that get incorporated into the body through food and beverages and provide benefits or otherwise toward human health. The fibers (mostly cellulose, hemicellulose, etc., which the body cannot digest) and some biomacromolecules like proteins, complex carbohydrates, and metal ions present in the food will not be discussed here, though each of them plays a vital role in human system.

The biochemicals like cholesterol, sugars, fats, vitamins, etc., are needed by the body system, but they may cause damage when present in excess; their optimal content is necessary for good health. Deficiency of some specific hormones, vitamins, and similar chemicals may be replenished by food or medicine or by both. Sometimes diseases developed due to deficiency of some biochemical/s could be treated with foods rich in those chemicals or their precursors. For example, **vitamin C** in the form of lime juice can be utilized to treat scurvy; carrot juice containing **β-carotene** (a precursor of **vitamin A**) will be helpful in cases of diseases caused by vitamin A deficiency: night blindness, macular degeneration, and other diseases of eye vision. In their initial stages these diseases may be treated as nutritional diseases. Raw food like fruits and some green vegetables are preferred for being fresh, thus preserving the food values.

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Our current knowledge of the metabolic system and the chemical constituents of food that we take, dictates us what to take and what to avoid. Though our nutritional life is expected to be designed by our current knowledge, it is difficult to follow meticulously the strict daily diet regime. Liking and disliking are the most important personal factors that dictate food habits. We live in an age of globalization. Almost everything including food is globalized. Now-a-days preparation of food is no more fully confined to the domestic kitchens, but it also finds its way into the processed food industry. Large companies have become key players in global food market. It is interesting to note that humans are capable of adapting their eating habits according to the availabilities and immediate surroundings. For example, there are large differences in food habits between Eskimos, rain forest inhabitants, and village and city dwellers; human beings are thus dietary versatile compared to other animals.

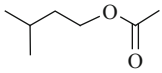
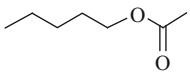
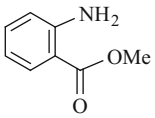
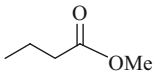
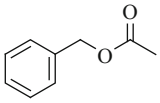
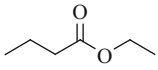
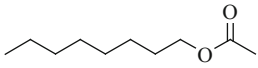
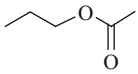
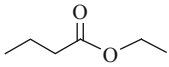
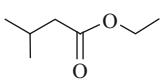
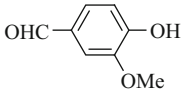
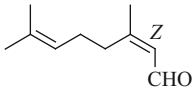
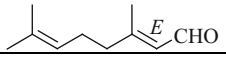
34.2 Flavors of Some Fruits, Nuts, Beans, and Vegetables (Table 34.1)

Fruits are included in the universal diet. The production of different types of fruits requires different types of climatic conditions. Fruits are consumed both in the raw form and as processed products such as juice, squash, jam, jelly, dried fruits, etc. The major attraction of fruits lies in their pleasing flavor, taste, and the universal appeal of sweetness. Table 34.1 shows the major chemical compounds identifying the flavor of some common fruits, nuts, and beans. Incidentally, it should be pointed out that *flavor is caused by a combination of many chemicals, of which a single or multiple ones may be the major contributor/s*. The identifying flavoring compounds are synthetically prepared and are used as artificial flavors commercially. However, the fullness of natural flavor can never be exactly duplicated because of the synergistic effect of many components that co-occur with the natural major flavoring agent/s. Professional flavor experts can identify the differences—whatever may be the closeness of the synthetic flavor to the corresponding natural one.

Mango (*Mangifera indica,* Fam. Anacardiaceae), the most tasty and likeable tropical fruit, is widely grown in India and other tropical countries. It contains monoterpene hydrocarbons. Some are major contributors to its flavor. They are *α*- and *β-pinene*, *myrcene,* and *limonene*. In Indian variety mango *cis-ocimene and myrcene* are responsible for aroma. It has also been reported that the ratio of *palmitic acid* ($Me(CH_2)_{14}COOH$) to *palmitoleic acid* ($Me(CH_2)_5CH{=}CH(cis)\text{-}(CH_2)_7COOH$) is responsible for the quality of flavor—specially when the ratio is less than one. In India many varieties of mangoes grow in different regions with varied types of flavor.

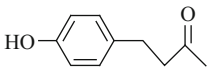
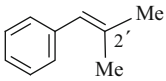
Venezuela mango contains *α-pinene*, *car-3-ene*, *limonene*, *β-selinene, acetophenone, benzaldehyde*, and *2′,2′-dimethylstyrene*, of which car-3-ene and 2′,2′-dimethylstyrene are the major contributors to its flavor.

Table 34.1 Major chemical compounds identifying the flavor of some common fruits, nuts, and beans

Banana (*Musa paradisiaca* and other species, Fam. Musaceae/Araceae)	Isoamyl acetate	
	Amyl acetate	
Grape (*Vitis venifera*, Fam. Vitaceae)	Methyl anthranilate and volatile isoprenoids	
Apple (*Malus sylvestris*, Fam. Rosaceae)	Methyl butyrate	
Peach (*Prunus armeniaca, P. percica*, Fam. Rosaceae)	Benzyl acetate	
Pineapple (*Ananas comosus*, *A. sativus*, Fam. Bromeliaceae)	Ethyl butyrate	
Orange (*Citrus reticulata*, Fam. Rutaceae)	Octyl acetate	
Pear (*Pyrys communis*, Fam. Rosaceae)	*n*-Propyl acetate	
Strawberry (*Sragaria vesca*, Fam. Rosaceae)	Ethyl butyrate	
	Ethyl isovalerate	
Almond (*Prunus amygdalus*, Fam. Rosaceae)	Benzaldehyde	C_6H_5CHO
Vanilla (bean) (*Vanilla planiflora,* Fam. Orchidaceae)	Vanillin	
Lemon (*Citrus limon*, Fam. Rutaceae)	*Z*-Citral (Neral)	
	E-Citral (Geranial)	

(continued)

Table 34.1 (continued)

Raspberry (*Rubus peasis*, Fam. Rosaceae)	4-*p*-Hydroxyphenylbutan-2-one (Raspberry ketone)	
Mango (Indian)	α- and β-Pinene, *cis*-Ocimene, Myrcene	For structures see Sect. 6.1
(*Mangifera indica*, Fam. Anacardiaceae)	Car-3-ene (Sect. 6.1) and 2,2′-Dimethylstyrene	
Mango (Venezuela)		

A very common flavoring agent **vanillin** is extracted from vanilla (pods of the orchid *Vanilla planiflora*, Fam. Orchidaceae). The crude extract contains many components, of which vanillin is the major one. It has a very agreeable pleasant smell and is extensively used in ice-cream and as condiment in various preparations. Vanillin is manufactured at a very low cost and the synthetic vanillin is extensively used. Sometimes *coumarin* is added to it to improve the flavor. Natural vanillin is costly. **Monosodium glutamate (MSG)** with umami taste, a flavor intensifier, occurs in fermented *soya bean, soya curd*, and *soya sauce,* which are extensively used in Japan and China and now globally to intensify the flavor of meat and some vegetables. Tomatoes and cheese also contain MSG.

Coriander and *broccoli* green leaves have some volatile components (volatile terpenoid mixture) and their composite flavors make the food attractive and agreeable and are used worldwide. Raw flavor and sometimes blending of flavors play a great role in our enjoyment of food. *Onion* flavor is enjoyed in stew, meat, and in some preparations of vegetables. *Dihydroxycinnamic acids, gallic* acid, *and quinic acid* in esterified forms are present in various vegetables. The green leaves of *Murraya koenigi* (Fam. Rutaceae), commonly known as *curry leaves* possess some attractive flavor, for which they are widely used in many food preparations all over India and now worldwide, mostly by people of Indian origin. Recently it has been reported that curry leaves possess antidiabetic properties. The taste buds and olfactory organs of *tobacco* smokers become less sensitive, and they lose appetite. However, the quitters pick up the appetite. To them food tastes and smells better and they reap numerous benefits from food.

34.3 Classes of Common Phytochemicals in Food and Drinks. Their Beneficial Effects

Through diet we get many important biochemicals. Some belong to the following classes : *phenolic acids*, *mono-* and *polyhydroxycinnamic acids*, *flavonoids*, their *glycosides*, *esters of polyhydroxyaromatic acids*, *phytoestrogens* (*isoflavonoids*), *dihydrochalcones*, *hydroxylated stilbene derivatives*, *vitamins*, *provitamins*, *carotenoids* and their precursors, *omega-3-* and *omega-6- long chain polyunsaturated*

fatty acids (LCPUFA), *indole derivatives*, *purine derivatives*, *sulfur containing compounds*, *unsaturated* as well as *saturated carbonyl compounds*, *terpenoids, steroids*, etc. Structures of some are shown in figures included in the sequel. Some beneficial functions of these molecules in our metabolic systems have been understood. Most of the compounds act as health protective as well as health-promoting phytochemicals with various biological activities especially as antioxidants.

34.4 Some Phytochemicals as Radical Scavengers (Antioxidants)

Normal cellular metabolism constantly generates extremely *reactive oxygen and nitrogen intermediates/species* (ROI/ROS/RNS), examples of ROS—superoxide, hydrogen peroxide, hydroxyl radical, etc.

Fenton reported [1] the following reactions as early as 1894: $Fe^{+2} + H_2O_2 + H^+ \rightarrow Fe^{+3} + [^{\cdot}OH] + H_2O$. Much later it has been shown [2] that Fenton reaction-mediated DNA damage is endogenous in our metabolic processes. In the biological system ·OH radical is generated from H_2O_2 by the transition metal (TM)-catalyzed Fenton reaction: $TM^{+2} + H_2O_2 + H^+ \rightarrow TM^{+3} + [^{\cdot}OH] + H_2O$. NADH reduces the metal ion from the higher oxidation state to lower oxidation state and the lower oxidation state of the metal is reused in Fenton reaction to generate the radical. But, there are multiple endogenous defenses, which give inherent protection by some specific enzymes to detoxify these radicals. These oxidative defense mechanisms are overpowered by reactive species with age and the accompanying inability to destroy them, resulting in an abnormal accumulation of ROI/ROS/RNS in the system. Interactions of these ROI/ROS with biomacromolecules like DNA, lipids, proteins, etc., result in their oxidative modifications—an irreversible damage which is manifested in various diseases, especially in neurodegenerative diseases. Exogenous nonenzymatic defensive antioxidants (a group of externally added compounds that quench the free radicals generated within the body and thereby reduce the damages of cellular biomolecules) are necessary; they are external hydrogen radical donors. Such antioxidants are supplied by food, drinks, and medicines. The efficacy of such antioxidants depends on (i) their bioavailability, i.e., the fraction of the external antioxidant absorbed in the body for functioning in the desired way, (ii) the ease with which they can be dissociated into phenoxy (in cases of phenolic antioxidants) and hydrogen radicals and the use of the latter as the *radical scavenger*, and (iii) nonreactive nature of the aryloxy radical ($ArO^{\bullet}$) with the substrates or biomolecules.

The detailed mechanistic explanation of their antioxidant properties and other beneficial mode of actions, as applied to our body, are out of the scope of this chapter. However, the basic fundamental antioxidant properties based on radical

chemistry will be discussed in brief. *The role of the antioxidants offered by food and drink is to intercept the free radicals before they can react with the substrate biomolecules and cause damage*.

Consumption of fruits and vegetables results in many phenolic compounds (phenolic acids, esters, glycosides, flavonoids) (see the figures in the sequel) entering into human system. The formation of radicals (which act as antioxidants) from phenolic compounds is shown in a generalized way (Fig. 34.1).

Because of the stabilization of phenoxy radicals by delocalization, hydrogen radical is generated from phenols with ease and can scavenge unwanted radicals (Fig. 34.1). However, when an antioxidant scavenges a free radical, it itself becomes oxidized and cannot further scavenge any other ROS or RNS. An endogenous potent reducing agent *dihydrolipoic acid* (DHLA) and *thiopeptide glutathione* (*γ-glutamyl cysteinyl glycine*) can reduce their oxidized forms, e.g., α-tocopheryloxy radical to *α-tocopherol* (**vitamin E**) and dehydroascorbate to *ascorbic acid* (**vitamin C**) (see Fig. 34.5). The latter can also reduce α-tocopheryloxy radical to α-tocopherol for further scavenging the ROS and RNS.

One electron reduction of oxygen forms the superoxide radical O_2^{-}. A reaction of O_2^{-} in aqueous media will be in competition with its spontaneous conversion to H_2O_2. Protonation of O_2^{-} gives the $HO_2^{\bullet}$ (hydroperoxyl) radical, which being less polar than O_2^{-} is more reactive. The $HO_2^{\bullet}$ and $HO^{\bullet}$ radicals cause more damage to cells. Most of the $^{\bullet}OH$ radicals are generated in vivo from the metal ion catalyzed breakdown of H_2O_2 (Fig. 34.1).

Polyphenol-rich diets are thus repeatedly advocated to reduce the risk of developing cardiovascular diseases and cancer. Flavonoid glycosides are currently used for the treatment of vascular diseases.

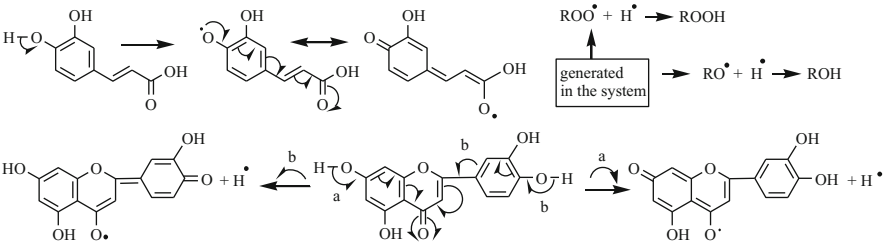


Fig. 34.1 Dietary phenolic compounds acting as antioxidants

34.5 Resveratrol, an Important Antioxidant Present in Red Wine and Blueberries. Constituents of Red Wine and Other Drinks: Polyhydroxystilbene Derivatives and Flavonoids

Extensive studies of red wine polyhydroxystilbenes, specially resveratrol, showed NO (nitric oxide)-mediated vasorelaxation, production of vasodilators, inhibitions of human LDL oxidation, cancerous growth of tumor (animal experiment) and vasoconstriction, and reduction of the risk of rectal, colon, and breast cancers. It can also act as a strong neuroprotective agent and improve Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD) by radical scavenging mechanism. Blueberries, which being a good source for resveratrol, are advised to be given as juice to neurodegenerative patients. However, the bioavailability of resveratrol is comparatively low. Resveratrol is also present in nuts (peanut) and a variety of plants. Flavonoids and other polyhydroxystilbene derivatives of red wine (Fig. 34.2) are also antioxidants.

Some dietary phytonutrients of several drinks and foods are included in Fig. 34.3. Studies on the ***bioavailability*** of polyphenols suggested that *gallic acid* and *isoflavones* have best bioavailability, followed by flavonoids, *catechins, quercetin,* and their glycosides. Recently, it has been shown that microtubule polymerization is perturbed by quercetin through *tubulin* binding, and hence it showed antiproliferative activity against several types of tumor cells.

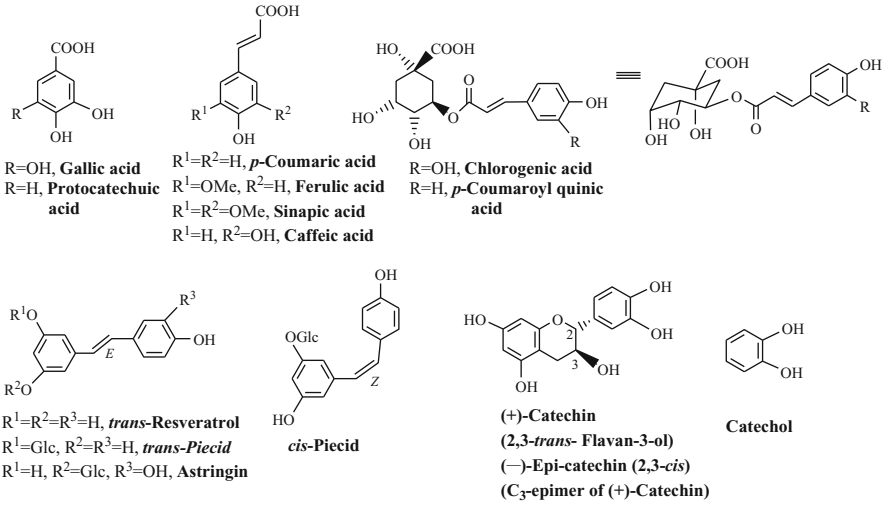


Fig. 34.2 Red wine constituents

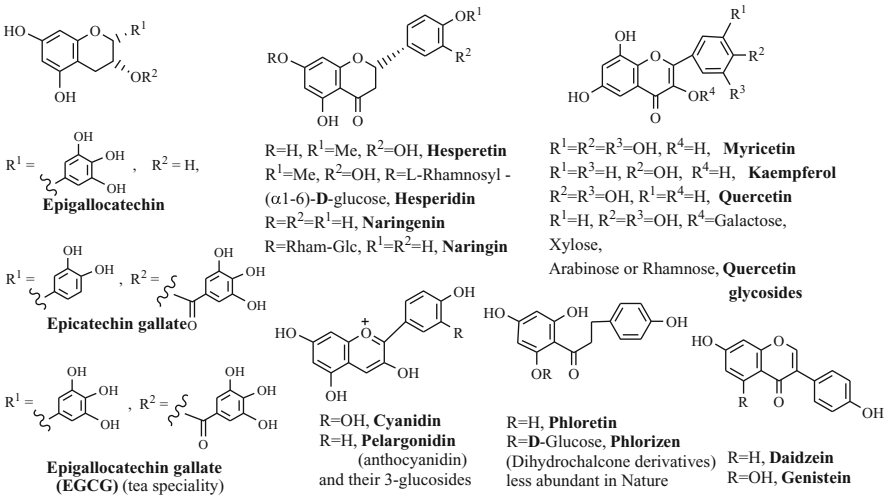


Fig. 34.3 Dietary phytonutrients of some drinks and foods

34.6 Tea, The Most Popular and Wonder Drink of the World. Its Rival Coffee (Fig. 34.4)

Among the beverages, tea is the most popular one. Its refreshing aroma (linalool and geraniol and other volatile terpenoids) subscribes to the common saying, "anytime is teatime" and in fact a cup of tea cheers a person anytime anywhere. As a drink it is next to water. The unusual amino acid **theanine** is largely responsible for its umami taste.

Tea was serendipitously discovered during 2737 BC by the Emperor Sheng Nung (Shan Nong). One day while he was in the garden a few leaves from *Camellia sinensis* (syn. *Thea sinensis*, Fam. Theaceae, Order Ericles) plant fell into his cup of boiling water and gave a rich alluring flavor. The tea plants are grown in hills and foothills and the major cultivators are China, India, Japan, Sri Lanka, and South Africa. Camellia is the largest genus in the family Theaceae. Good Darjeeling (India) tea is highly priced for its exquisite aroma, especially when two tender leaves and a bud are collected during May and June. This period is known as the second flush period. Leaves collected in March–April are first flush tea with bright liquor and thin body, but the flavor matures during second flush. Other plants like *Diospyros kaki* and *Vitellana paradoxa* (the Shea tea of Africa)—both belonging to the order Ericles, some *Cistus* species, *Theobroma cacao,* the honey bush tea of South Africa (*Stryphnodendron adstringens*), and *Salix aegyptiaca* are known to contain EGCG—the speciality of tea. However, the composition of the phenolic components, the whole community of chemicals, their composition, and concentration (both absolute and relative) are responsible for the quality of tea.

Caffeine (*1,3,7-trimethylxanthine*), *theobromine* (*3,7-dimethylxanthine*) and *theophylline* (1,3-*dimethylxanthine*), *theanine,* and *flavonols* are present in tea

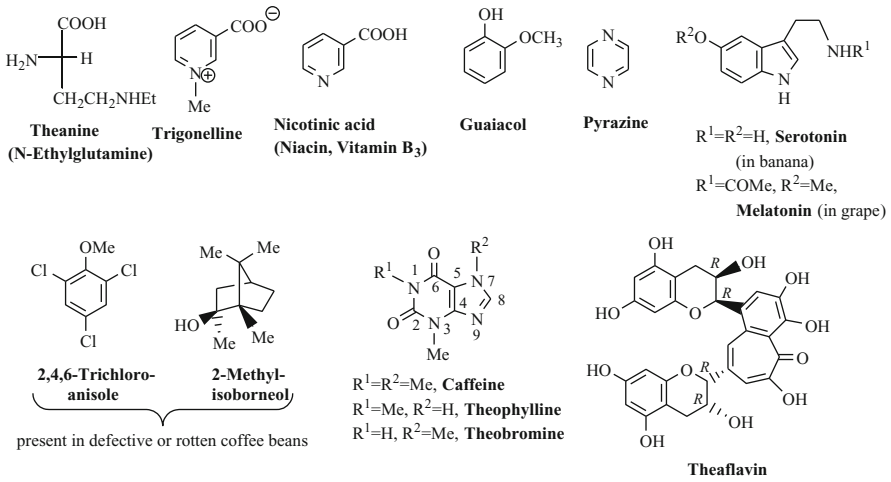


Fig. 34.4 Phytochemicals from tea, coffee, banana, and grape

(see Fig. 34.4). Caffeine acts as a central nervous system stimulant, theobromine—a diuretic and muscles relaxant, and theophylline—a reliever of bronchospasm. The potential benefit of caffeine must be weighed against its adverse effect (see Sect. 34.2). Flavonoids in recent years have emerged as important chain-breaking antioxidants. Polyphenolic compounds are powerful antioxidants which may reduce the risk of a variety of illnesses, cancer, and coronary heart disease. Other chemical compounds of tea include *catechin* and *epicatechin* esterified with *gallic acid*. The most important of the esters are *3-galloylepicatechin*, and *3-galloylepigallocatechin* (Fig. 34.3). *Chlorogenic acid*, *catechol*, *5-hydroxycatechol* (Fig. 34.2), and *theaflavin* (Fig. 34.4) are also present in tea. All these constituents possess antioxidant properties. Theaflavin is most effective in abrogating reactive nitrogen species (RNS) production and protects from cardiovascular diseases, cancer, and aging. These green tea catechins are shown to be effective in Huntington disease (animal experiment). Sometimes in stomach upset liquor tea is prescribed even to the children. The nomenclature black tea and green tea are given to tea based on processing.

> "Ideally the discerning tea drinker likes large leaf aromatic tea grown in the hills with a kippery flavour and very little colour, the exact opposite of what the common imbiber prefers, the crushed leaf of lesser altitudes that yields a stronger taste and brings with it a rich shade of mahogany. Indian rail vendors add further condiments like ginger, cardamom and *kali mirch,* and the more adventurous drinker fancies herbal additives like lemon grass, *tulsi* and rosemary" [3].

Incidentally, it may be mentioned that "Thomas Cook, the world's first travel agent, was an ardent teetotaller who began his cheap railway excursions to wean the working class away from the bottle to the cup that cheers" [3].

Other plant extract drinks which do not contain conventional tea are referred to as herbal tea. They are used as health drinks. They suppress the bacteria that are unfavorable and maintain a healthy intestinal bacterial flora.

Coffee: Like tea, coffee (*Coffea arabica, C. canephora*, Fam. Rubiaceae) is also enjoyed all over the globe. Its popularity is close to that of tea. More than two-third of world's production of coffee comes from *C. arabica,* indigenous to Ethiopian highlands and cooler mountain regions of tropical belt. The difference in the chemical composition (especially percentage wise) depends on the *Coffea* species and techniques of roasting and brewing.

Coffee beans contain *caffeine*, *chlorogenic acid*, *trigonelline*, *catechols*, *guaiacol*, etc. Of these chlorogenic acid represents 13 % of soluble matter of coffee. Around 260 mg of chlorogenic acid is present in a cup of instant or percolated coffee. Caffeine is not addictive—perhaps it does not act on limbic system of our brain. However, caffeine has some side effects if taken in excess through drinks. Hence the beverage industry has developed processes of decaffeination of coffee, keeping the flavor and taste intact by using different solvents to dissolve caffeine, and to remove it, and instead, use it as a source of caffeine for pharmaceutical need. However, the process of decaffeination is complex. *Trigonelline* forms *nicotinic acid* during roasting (*niacin*), often called *vitamin PP* (*Pellagra Preventing*) (**vitamin B_3**), being an *antipellagra* agent. Around 50 % of **vitamin B_3** required by human body is supplied through coffee drinks to moderate coffee consumers. This is important from the nutritional point of view. A very small amount of *α-tocopherol* has also been detected in coffee. The main aroma of the roasted coffee is mainly due to *guaiacol*, *pyrazine*, *furanone*, and some *mercapto derivatives*. However, some rotten or defective beans, if present, will yield *2,4,6-trichloroanisole* and *2-methylisoborneol* (Fig. 34.3) causing very unpleasant smell even when present in small amount.

The roasted roots of "chicory" (*Cichorium intybus,* Asterceae) have been used as a substitute for coffee.

34.7 Vitamins and Related Compounds (Fig. 34.5)

Human body cannot synthesize **vitamins** and hence we have to get them through diet. They are important as many of them play the role of *coenzymes*. Fruits and green vegetables are good sources of vitamins, especially **vitamin C**, **vitamin E,** and also **vitamin K**. Vitamins are classified as water soluble, e.g., vitamin C, and fat soluble, e.g., vitamin E group. There are four different types of *tocopherols* (*α-, β-, γ-*, and *δ-*) (vitamin E group) (Fig. 34.5), of which *α-tocopherol* is primarily known as vitamin E. The proportion of the tocopherols differs widely in different oils, for example, mainly α- in safflower oil, β- in wheat oil, γ- in soya bean oil and corn oil, and δ- in soya bean oil. α-Tocopherol is present in most of the oils especially in olive oil (*Olea europaea*, Fam. Oleaceae), soya bean (*Glycine max.,* Leguminoceae/Fabaceae), sunflower (*Helianthus annuus*, Compositae), and

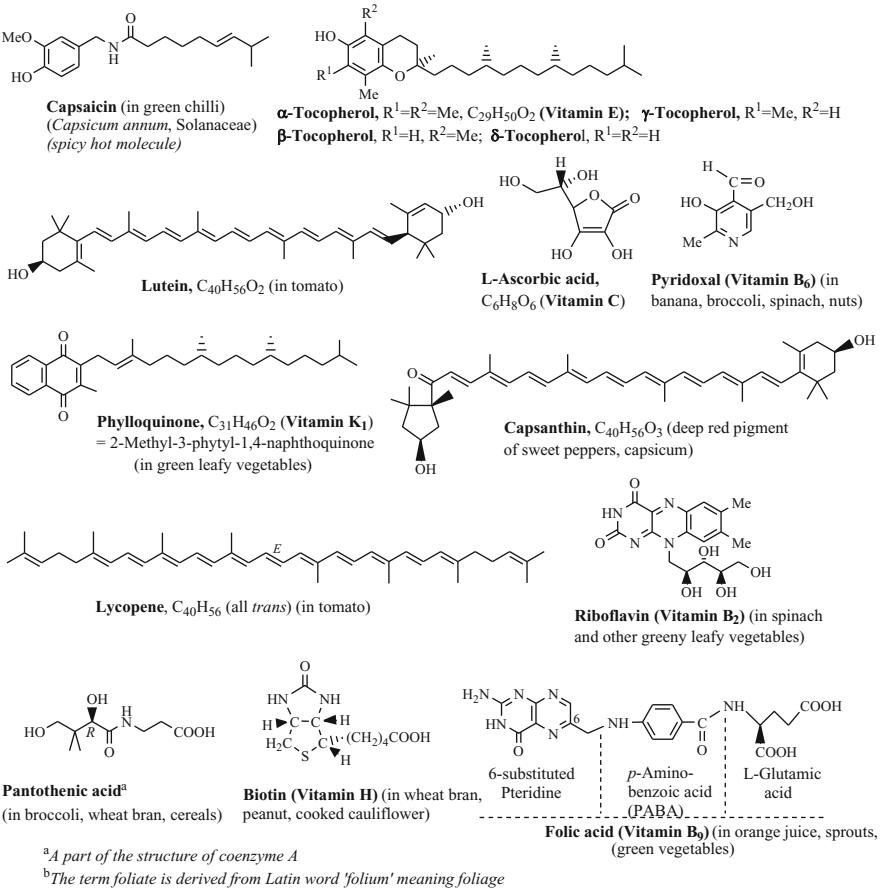


Fig. 34.5 Carotenoids and some vitamins

safflower (*Carthamus tinctorius,* Compositae). It is best known as chain breaking lipophilic antioxidant, and it protects LDL from oxidation by donating hydrogen radical to lipidic radical. *Vitamin E* radical thus formed is reformed to vitamin E (i.e., revitalized) by vitamin C (ascorbate). Vitamin C thus plays an important role in recycling vitamin E. The antioxidant property of vitamin E is very important in lungs, the cells of which are more exposed to oxygen. The high concentration of oxygen affects the cell membranes of lungs and vitamin E protects the cell membranes.

Combination of **vitamin C** and **vitamin E** has been shown to be an efficient inhibitor of lipoprotein oxidation in plasma and cerebrospinal fluid. Vitamin C radical formed by vitamin E radical ($E^{\bullet}$) disproportionates to *ascorbate* and *dihydroascorbic acid*. High concentration of vitamin C in diet shows the higher concentration of vitamin E in the tissues (animal experiment). Vitamin C plays the

role of coenzyme in metabolic pathways. Its reducing property allows it to act as a co-substrate in mono-oxygenase reactions to produce hydroxylated amino acids, e.g., *hydroxyproline, serotonin, noradrenaline, hydroxylysine*, etc., and to protect cells, cell-membranes, and *polyunsaturated fatty acids* (PUFA). Vitamin C protects cellular components in aqueous environment, while **β-carotene** (vitamin A precursor) and vitamin E protect the cellular components in nonaqueous environment. Linus Pauling said, "...nutritional factors have significant value in preventing cancer. In particular, vitamin C (L-ascorbic acid or any of its salts) has great value both in preventing cancer and in treating cancer" [4].

Unsaturated fatty acids undergo auto-oxidation as the allylic C–H bond is prone to radicalization. The abstraction of hydrogen could be effected by $OH^{\bullet}$ radical derived from hydrogen peroxide, by superoxide radical anions, etc. (Fig. 34.6).

The unsaturated fatty acid component is largely protected from such oxidation by **α-tocopherol** (EH). The resonance stabilized α-tocopherol radical ($E^{\bullet}$) is generated by reaction with peroxyl radicals. It cannot undergo self-dimerization because of steric hindrance as either side of the radical is substituted by bulky groups (Fig. 34.7). α-Tocopherol is revitalized by the thiopeptide **glutathione** (GSH) and glutathione disulphide (GSSG) thus formed is reduced by NADPH back to GSH (Fig. 34.7). Butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) is often combined with propyl gallate to use as synthetic antioxidant preservative in food. But because of the mounting pressure against the use of such "chemical additives," α-tocopherol, has been extensively used as a preservative which can arrest oxidation, rancidity of fats, etc. It is also claimed that it can reduce the effects of aging and can prevent heart disease.

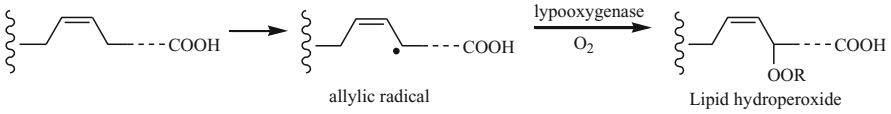


Fig. 34.6 Lipid oxidation

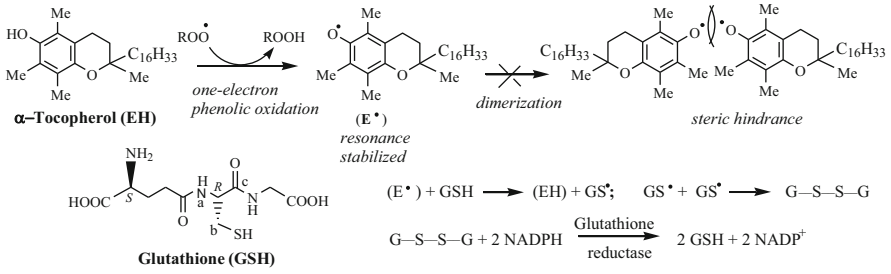


Fig. 34.7 α-Tocopherol (vitamin E) as a radical scavenger and its revitalization

34.7.1 *Vitamin A_1 Formation. The Stereochemistry of Vision (Fig. 34.8) [5–8]*

Vitamin A_1 (**retinol**) is found only in animal products, particularly dairy products, eggs, animal livers, and kidneys. It as such does not occur in plants. However some green vegetables and fruits contain carotenes—considered to be provitamin A_1, which are converted to *vitamin A_1 and vitamin A_2* (Δ^3-vitamin A_1) in the body. The precursor in the diet is **β-carotene** (the orange/yellow pigment of carrots (*Daucus carota*; Umbelliferae/Apiaceae), which is converted to vitamin A_1 via the aldehyde **retinal** (*all-trans*) (Fig. 34.8). In the liver/intestine β-carotene-15,15-′-monooxygenase effects the regiospecific cleavage of the central 15,15′ double bond of β-carotene at "a," probably via a 15,15′-diol formed through a 15,15-′-epoxide. Thus theoretically two molecules of *all-trans-retinal* are expected to be generated from one molecule of β-carotene, but actually only one molecule of it is formed. Evidence shows that cleavage at "b" gives the higher aldehyde, **β-apo-8′-carotenal** (8′ carbon becoming CHO), which subsequently also undergoes cleavages at other double bonds to form shorter aldehydes—ultimately producing retinal. β-Cyclocitral (Fig. 34.8) is also formed along with β-apo-8′-carotenal.

The present knowledge about the chemistry of vision is mostly revealed from the elegant work of George Wald (NL 1967, Physiology). The chemistry of vision is briefly illustrated in Fig. 34.8 for easy visualization.

The *retina* is made up of two types of *light-sensitive receptor cells*, **rods** and **cones**. About three million rod cells are responsible for vision in dim light, and

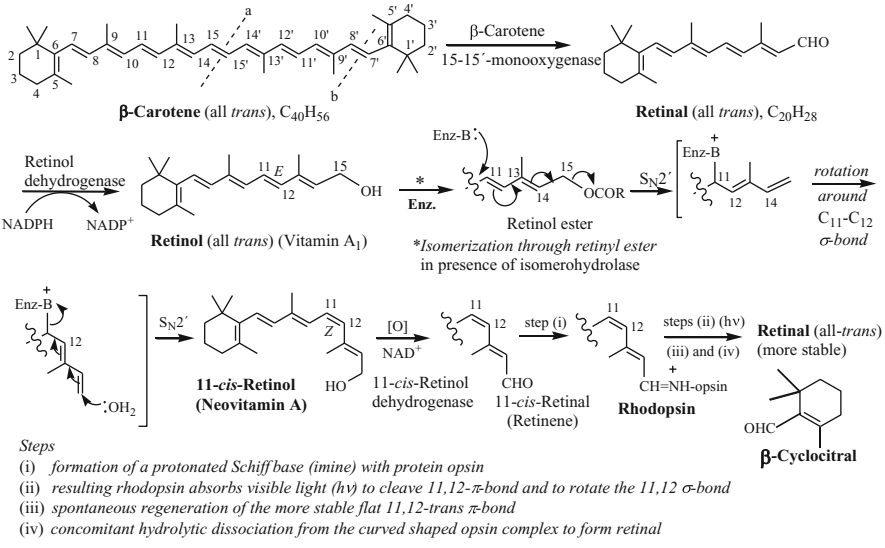


Fig. 34.8 Formation of vitamin A_1 from β-carotene, and the chemistry of vision

hundred million cone cells permit color perception and vision in bright light. Their functional details, not yet understood fully, are better understood for the cones than for the rods.

In the human eye there are three types of cone cells, which absorb light at 440, 535, and 575 nm and thus discriminate among the primary colors. The message of full color vision is received in the brain when their combinations are stimulated.

In the liver, **retinal** upon reduction in presence of retinol dehydrogenase and NADPH forms **retinol** (*vitamin A_1*, *all-trans*). Retinol gets esterified with lecithin in presence of the specific enzyme (in the eye) to form a better leaving group, followed sequentially by a likely S_N2'-type addition at C11 with an enzyme base, rotation about the generated 11–12 single bond and an S_N2' type addition of H_2O at the terminal carbon with concomitant elimination of the base (enzyme) to form 11-*cis*-**retinol** (sometimes called neovitamin A), as shown in the figure. c*is-Retinol* undergoes oxidation with NAD^+ in presence of the enzyme 11-*cis*-retinol dehydrogenase to form **11-cis-retinal** (sometimes called **retinene**). The latter gets bound to the terminal amino group of a lysine residue in the protein **opsin** (unknown structure) in the retina to form a protonated Schiff base, $-CH=^+NH$-opsin, the red visual pigment **rhodopsin**. The positively charged imine N of rhodopsin is located near a negatively charged amino acid residue of the protein chain. There are several millions of rhodopsin in **rod cells**. Rhodopsin is light sensitive. The high energy of a quantum of visible light promotes the fission of the 11,12 π-bond. This is followed by free rotation about the 11,12 σ-bond in the resulting radical and spontaneous regeneration of the π-bond resulting in the more stable all-*trans*-retinal. 11-*cis*-Retinal has a fairly curved shape and it associates with opsin in its complex and specific 3-dimensional shape. The elongated conformation of the all-*trans*-retinal leads to its hydrolytic dissociation from opsin and change of conformation of opsin. During the *cis* → *trans* isomerization process an electrical signal is transmitted due to change in molecular geometry, which triggers a nerve impulse that the brain perceives as vision, the mechanism of which still remains unsolved. *Regeneration of retinal repeats the cycle*.

Vitamin A deficiency is manifested in the degeneration of mucous membrane throughout the body, but is evident more in the eye compared to other parts of the body. Vitamin A_1 deficiency causes vision defects, including inability to see in dim light (night blindness, nyctalopia) and a weakening disease of the cornea. Retinoids (vitamin A and analogues) are also known to act as signaling molecules which regulate vision, embryonic development and growth, and causes dryness of skin.

34.7.2 Lycopene

Lycopene, $C_{40}H_{56}$ is the characteristic carotenoid pigment (Fig. 34.5) in ripe tomato fruit (*Lycopersicon esculentum*, Fam. Solanaceae). It occurs also in other vegetables. It is good for prostate protection and inhibiting its enlargement. Statistics suggests that people who regularly eat tomatoes are less prone to suffer from

cancer of esophagus and prostate, and the risk of cervical cancer in cases of women is reduced. It has been found that carotenoids constitute the macular protective yellow pigment. Vitamin A is a versatile vitamin since it plays important roles in gene expression, vision and maintenance of skin, growth of bone and overall normal development of cell. **Lutein** (in tomato) is also beneficial to our health.

34.7.3 Vitamin K_1. Vitamin B_3. Vitamin B_6. Folic Acid (Vitamin B_9). Vitamin C (Fig. 34.5)

Vitamin K_1 is present in green leafy vegetables, lettuce, broccoli, and other members of cabbage family and some fruits. It helps synthesizing the protein **thrombin** that helps blood clotting and also helps in the synthesis of bone proteins, etc. However, it cannot help clotting in cases of patients with inherited disease hemophilia. Other vitamins like **niacin** (*vitamin B_3*) (Fig. 34.4), **folic acid (vitamin B_9)**, **vitamin B_6**—all play important roles in our health promotion, when administered through food. The use of antioxidants as preventive medicines (nutraceuticals) is wide spread and many people supplement their diet with vitamin C, vitamin E, carotenoids, flavonoids, and other natural antioxidants.

The fruit *ananas* (pine apple) of the plant *Ananas comosus and A. sativus* contains *provitamin A*, *vitamin B*, *vitamin C*, and *bromelain*—a protein-splitting enzyme which helps in digestion and also keeps the lungs healthy. Ananas contains low sugar and fat, hence ananas juice is good for obese as well as diabetic people. The fresh fruit juice is a *digestive* and *diuretic* tonic.

34.8 Long Chain Polyunsaturated Fatty Acids (LCPUFA): Omega-3- and Omega-6 Fatty Acids (Fig. 34.9)

The incidence of heart attack or cardiovascular diseases is much less among Eskimos. They use seal fish oil and its meat which are fortified with ω-3- and ω-6-fatty acids. These acids have been linked to improve heart and brain health and reduce depression and development of dementia, especially in cases of Alzheimer's disease. Our body cannot manufacture omega-3-fatty acids. The role of ω-3-fatty acids in brain health, especially for growing children is very important. DHA (*Docosahexaenoic acid*) is the most abundant fatty acid in the brain. Its low intake in the brain is associated with *attention deficit hyperactivity* (ADH), *asthma*, and *multiple allergies*. Omega-3- and Omega-6-fatty acids are routinely added to the formulated milk or alike produces.

The furthest carbon atom from the functional group (COOH) of a long-chain unsaturated fatty acid is designated **omega** (**ω**), the 24th and the last letter in Greek alphabet. When the double bond starts from the 3rd and 6th carbon with respect to

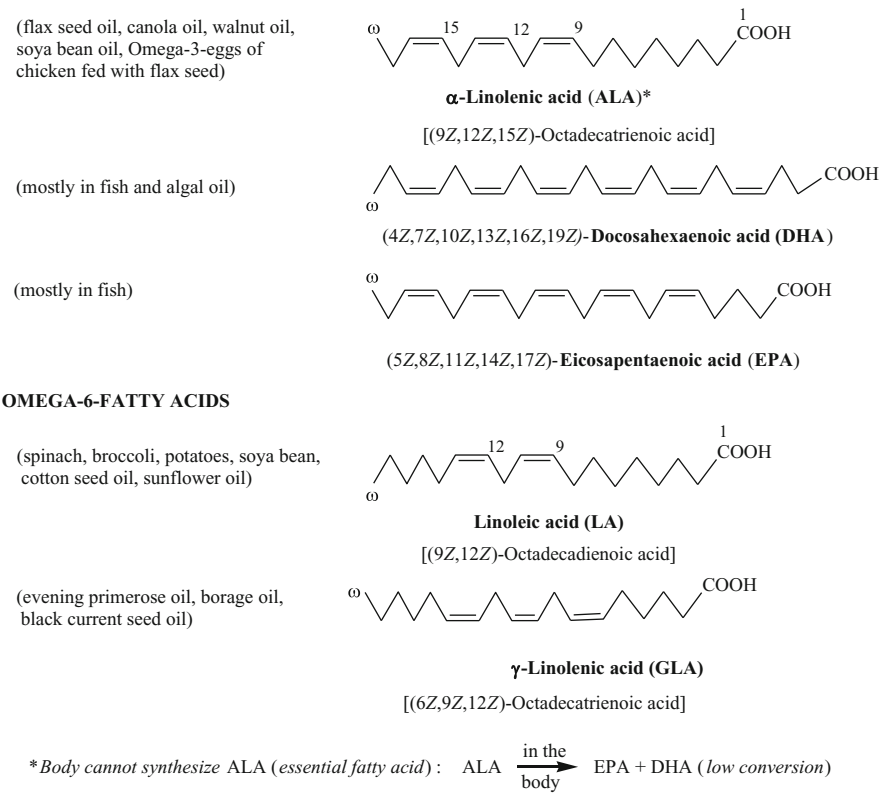


Fig. 34.9 Omega-3 and omega-6 polyunsaturated fatty acids and their sources

the omega carbon atom taken as 1, the respective acids are known as omega-3- and omega-6- fatty acids. They represent the classes of fatty acid with special character. However, the positions of the double bonds are to be counted from the COOH group taken as 1. **α-Linolenic acid** (**ALA**) is a **LCPUFA** with an 18 carbon chain having 3 double bonds at 9, 12, and 15 positions, and the geometry of the doubles bonds are *cis* or *Z* (*Zusammen*, together). Thus the nomenclature of **ALA** is (9*Z*, 12*Z*, 15*Z*)-octadecatrienoic acid. Likewise, the nomenclatures of other acids EPA, DHA, LA, GLA have been given under their structures in the Fig. 34.9.

It may be mentioned here that the natural fats and oils are triglycerides of non-branched fatty acids containing 14–22 carbon atoms having *Z* (*cis*-)-configuration of all the double bonds present in the molecule. It is worthwhile to know that the omega-6 fatty acid to the omega-3 fatty acid ratio in the diet should be at 5:1.

This can be achieved by consuming cooked oily fish like sardines and mackerel once a week. In fact, fish oil has been proven to be effective in reducing asthma attacks.

34.9 Nitrogen Heterocycles (Indole Derivatives)

Serotonin and **melatonin** (Fig. 34.4) are powerful antioxidants, and they play important role as *neurotransmitter* and *neuromodulator*. They occur in some fruits, serotonin especially in ripe banana and melatonin in grapes. With continuous exposure to *reactive oxygen species* (ROS) human system undergoes morphological and biological changes, which may cause *neurodegenerative* diseases and *cancer*. Ripe banana and grapes if taken can help in delaying the onset of such disastrous diseases to some extent. Serotonin also acts as *antidepressant*.

34.10 Some Constituents (Sulfur Compounds) of Garlic and Onion (Fig. 34.10)

Garlic (*Allium sativum*, Fam. Liliaceae/Alliaceae) is rich in sulfur containing compounds. The major flavoring compound of garlic is **allicin**, a thiosulfinate compound (Fig. 34.10). It is formed from **alliin**, a nonodorous compound of garlic,

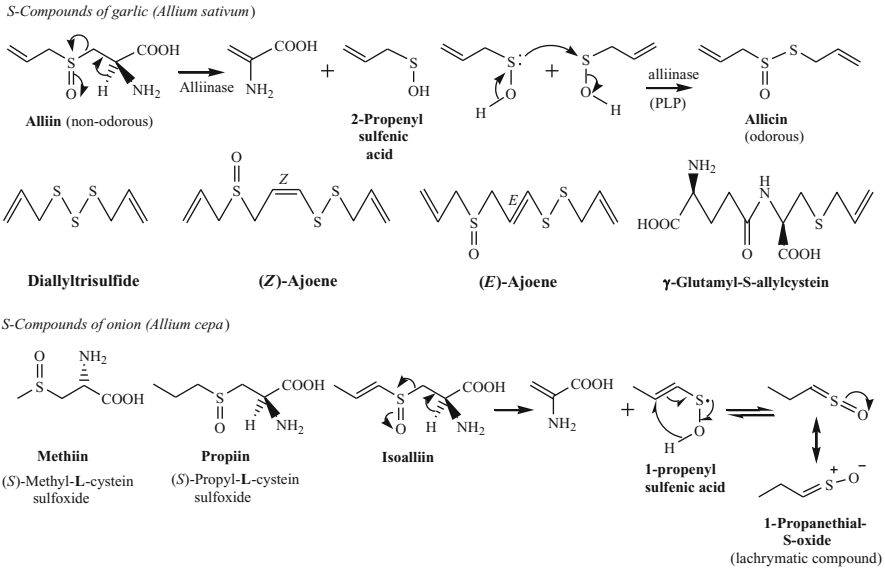


Fig. 34.10 Some sulfur containing compounds of garlic (*Allium sativum*) and onion (*Allium cepa*)

which undergoes breakdown in presence of alliinase when crushed or cut, into **2-propenyl sulfenic acid**. Two such sulfenic acid molecules give allicin through the loss of a molecule of water (Fig. 34.10). The fresh cloves of garlic also contain the precursor (**γ-glutamyl-S-allylcysteine**) of alliin. Additionally, various other sulfur containing compounds, disulfide, trisulfide, etc. along with flavonoids are known to occur in garlic. Disulfide and trisulfide compounds are helpful in reducing *atherosclerosis*, *coronary thrombosis*, *cholesterol level*, and thus garlic takes care of many serious as well as fatal diseases. Of all the sulfur-containing compounds, ajoene is the most beneficial one. It shows *antiaggregatory* properties on human blood *platelets* and is effective in reducing heart attack. The medicinal use of garlic has been known for centuries.

Onion (*Allium cepa*, Fam. Liliaceae/Alliaceae). *S*-Methylcysteine sulfoxide is present in all *Allium* species. Like garlic, onion also contains a number of sulfur containing compounds (Fig. 34.10). One such compound, **isoalliin** suffers breakdown when onion is cut or crushed, to generate the lachrymatic compound **1-propanethial-S-oxide**. Onion also contains *flavonoids* (e.g., *quercetin)*, *phenolic acids*, *sterols*, and *their esters* and *glycosides*. Onion possesses *hypoglycemic* and *antibacterial properties*. Raw or boiled onion reduces the serum cholesterol, serum lipids, triglycerides, and blood-clotting time. Like garlic, onion is also quite beneficial to our health.

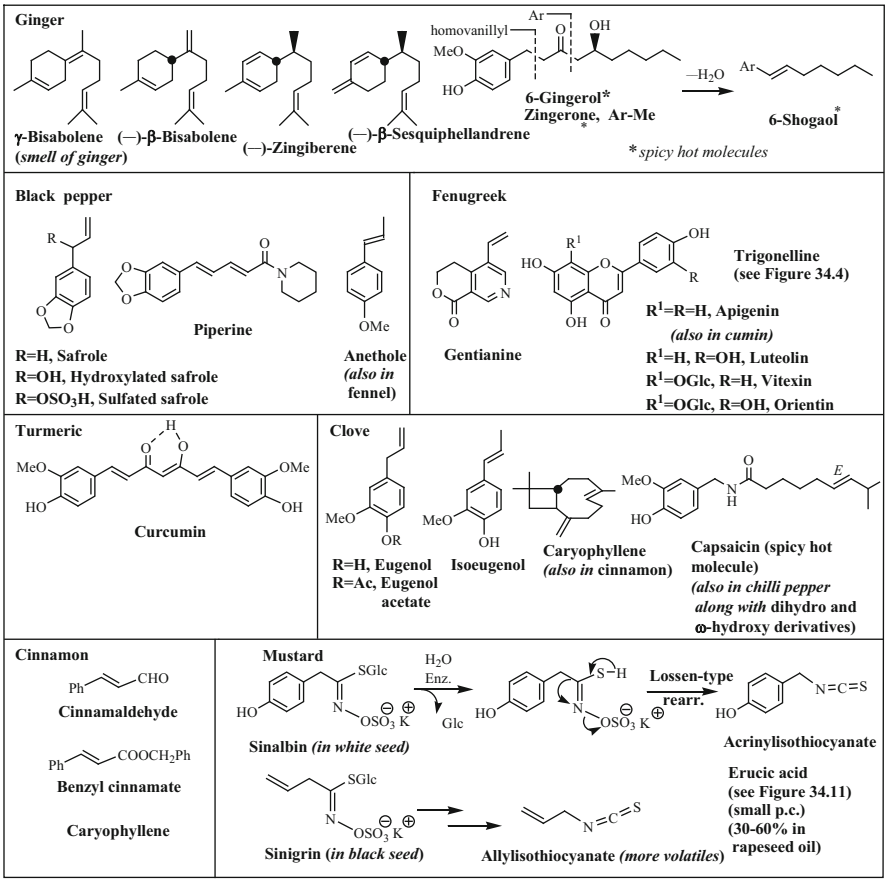
Prior to antibiotic era, trisulfide and disulfide compounds were being used for antifungal and antibacterial protection.

34.11 Some Active Principles of Several Commonly Used Spices (Table 34.2)

Ginger (*Zingiber officinale*, Fam. Zingiberaceae) contains small amount of vitamins and quite a good amount of sesquiterpenes (Table 34.2). *Zingerone, 6-gingerol*, *and 6-shogaol* are hot molecules; the 4-hydroxy-3-methoxybenzyl (homovanillyl) moiety present in these and some other molecules is responsible for hotness. Ginger has been shown to reduce serum and hepatic cholesterol significantly. It inhibits diarrhea, exerts antispasmodic effect, stimulates the heart, and contains antihistaminic compounds. It is good for cold and throat irritation. Recently, 6-gingerol has been shown to be effective in promoting proapotopsis gene in cancer cells (animal experiment) and thus holds promise as an anticancer agent.

Turmeric (*Curcuma domestica, C. longa,* Fam. Zingiberaceae) is an essential spice ingredient in curries. The characteristic constituents of *Curcuma* species are a group of *curcuminoids* (*diarylheptanoids*), responsible for the yellow color. The principal component is *curcumin* (Table 34.2). It is a well-known *antiseptic* and *anti-inflammatory* compound; it prevents *cholelithiasis* and is used in acute chronic *inflammation* of *gall bladder*. It takes care of various oxidative damages of

Table 34.2 Some active principles of several commonly used spices



biological systems. It also possesses antiulcer, antitumor, and cancer-preventive properties. Because of its multifarious biological activities it is now drawing global attention.

Clove (*Eugenia aromatica*, *Syzygium aromaticum*, Fam. Myrtaceae). Clove (dried flowers) contains *eugenol*, *isoeugenol*, *eugenyl acetate*, *caryophyllene*, *capsaicin*, *flavonoids* (Table 34.2), and other *terpenoid* compounds. It has some antiseptic and anesthetic properties; its flavor is agreeable when used in food, and hence it is a good appetizer. It helps in hyperacidity and cough. The large dose especially the oil is not good as it causes *edema* and *necrosis* (animal test).

Black Pepper (*Piper nigrum*, Fam. Piperaceae). The fruits contain *piperine* responsible for its pungent but agreeable smell, *phenolic amides*, and other *piperidine* derivatives. It contains flavonoids, carotenoid pigments, and vitamin C (ascorbic acid). Piperine is *anti-inflammatory*, *antioxidant*, and *hepatoprotective*.

Safrole, a constituent of black pepper might cause *growth retardation*, if incorporated in higher level, gets hydroxylated in the side chain followed by sulfation, and becomes toxic. Black pepper gives an agreeable taste to many food preparations and helps in throat congestion.

Capsicum (**Red Pepper, Chilli**) (*Capsicum annuum*, Fam. Solanaceae). Dried fruit contains *capsaicin* (Fig. 34.5 and Table 34.2), a powerful heart stimulant. It influences the blood circulation. Along with lemon juice and honey it is used in throat gargle. It stimulates digestive system and can act against enteric infections, reduces atherosclerosis, blood cholesterol, and platelet aggregation. Capsaicin and *dihydrocapsaicin* are the major constituents of capsainoids. They are very pungent and lachrymatic. Capsanthin (Fig. 34.5) is the deep red coloring matter of capsicum.

The fruits of *Capsicum* plants have different names depending on the species and country, e.g., red or green pepper, chilli, chilli pepper, capsicum, paprika, and bell pepper, the less pungent (mild) one. Incidentally, it may be mentioned that a Hungarian chemist Albert Szent-Györgyi (pronounced as sent Jurje, 1893–1986, NL 1937) isolated vitamin C from paprika, hence capsicum is also a good source of vitamin C.

Cinnamon [*Cinnamomum verum*, *C. zeylancium* (India and Sri Lanka)], *C. tamala* (Himalayas: Kashmir to Bhutan and Khasi Hills), *C. cassia* (China, Indonesia), *C. aromatica*] (Fam. Lauraceae). The barks of these plants commonly known as cinnamon with different vernacular names are used in various food preparations. The active principle of *C. verum* and *C. cassia* is *cinnamaldehyde* (responsible for the aroma). Some species contain *eugenol*, *coumarin*, *α-pinene*, *β-pinene*, *limonene*, *β-caryophyllene*, *linalool*, *benzyl cinnamate* (also responsible for the identifying aroma), *eugenyl acetate*, and some other terpenoids (Table 34.2). Cinnamon helps in our enjoyment of food and gaining appetite, reduces cough/bronchitis, fever, cold, and inflammation of mouth.

Cardamom (*Elettaria cardamomum,* Fam. Zingiberaceae) contains *2-terpinyl acetate*, *cineole*, and *linalool*. These terpenoids impart a likeable flavor and is used as condiment in various sweet preparations and cooking.

Fennel (*Foeniculum vulgare*, Fam. Umbelliferae/Apiaceae). The fennel seeds contain *anethole*, *fenchones*, *flavonoids*, *coumarin*, *bergapten*, *tannin*, and *stigmasterol*. The aqueous extract showed mucociliary activity of the ciliary epithelium. *Anethole*, *dianethole*, and *photoanethole* are shown to have estrogenic activity.

Fenugreek (*Trigonella foenum-graecum*, Fam. Leguminoceae/Fabaceae) grown widely in India. The seeds are used in curries as spice. They contain *trigonelline* (Fig. 34.4), *choline*, *gentianine*, flavonoids, *apigenin*, *luteolin*, *orientin*, *quercetin*, *vitexin*, and *steroidal saponins*. The whole seed extract is *hypoglycemic*, *hypocholesterolaemic*, and showed lipid lowering effect—an overall health protective activity.

Mustard: White mustard (*Brassica alba*, syn. *Sinapis alba*, Fam. Brassicaceae/Cruciferae) seeds contains *sinalbin*, an s-glycoside (known as glucosinolate). Addition of water to the crushed seeds leads to the enzymatic hydrolysis of the S-glucoside bond followed by a Lossen-type rearrangement to produce *acrinylisothiocyanate*, a pungent-tasting material (mustard oil) (Table 34.2). Black mustard

(*Brassica nigra*) seeds contain *sinigrin*, which by a similar sequence is hydrolyzed via an enzyme to *allylisothiocyanate*, which is more volatile than *acrinyl-isocyanate*, and hence has a pungent aroma as well as taste. Mustard is used in internal congestion. It stimulates gastric mucosa and increases pancreatic secretion and thus helps in digestion.

Cumin (*Cuminum cyminum*, Fam. Apiaceae) seeds contain volatile monoterpenoids, some aldehydes, *pinenes*, *alphaterpeneol*, *flavonoids*, and *apigenin*. The seeds help in digestion, useful in diarrhea, uterine infection, cough, and anorexia.

Black cumin (*Kalajira*) (*Nigella sativa*, Fam. Ranunculaceae) seeds contain *nigellone* and *2-methyl-4-isopropyl-p-quinone*, *palmitic acid*, *myristic acid*, *oleic acid*, *linolic acid*, *linolenic acid*, and *sitosterol*. The seed shows galactagogue activity, proliferation of acne, and secretory activity in breast tissues (animal experiment). After delivery of the new-born baby, the mothers (in India) are advised to take black cumin seeds with fatty materials and in various preparations which help in lactation.

Coriander (*Coriandrum sativum*, Fam. Umbelliferae) contains *α-pinene*, *α-terpinene*, *(+) linalool* and is used in cooking as flavoring ingredients.

34.12 Edible Sources of Some Beneficial Phytonutrients

These are summarized in Table 34.3.

Table 34.3 Edible sources of some beneficial phytonutrients

Carotenoids: Carrot, wheat, corn, apricot, peach, soybean, orange, grape fruit, squash, red pepper, chili, mango, spinach, bokchoy, pumpkin, cantaloupe, sweet potato, broccoli, red cabbage, yellow corn, tomato, paprika, etc.
Isoflavonoids*: Phytoestrogens containing food (present mostly in soybean products) may be given to women to combat some unwanted symptoms during and after menopause*
Vitamin C: All citrus fruits, sweet red pepper, grape fruit, sweet potato, brussels sprout, broccoli, green pepper, orange, guava, carrot, apple, palm, etc. Being an excellent antioxidant vitamin C is used in dehydrated potato products. It eliminates traces of oxygen from the package of dehydrated potatoes and keeps their color and natural flavor intact
Vitamin E: Sunflower oil, wheat germ, safflower, almonds, lettuce, sweet potato, peanut oil, soybean oil, corn oil, various fruits
Vitamin K: Dark green vegetables
Organosulfur compounds: Garlic, onion, mustard
Phenolic acids: Tea, coffee, beans, apples, blueberries, cherries, grapes, oranges, various fruits of Rosaceae family, green vegetables, potatoes, soybeans, etc.
Acids (like dihydroxycinnamic acids, gallic acid, chlorogenic acid, and quinic acid, etc., as such or in esterified form): various vegetables and fruits
Flavonoids: *Citrus* fruits, grapes, soybean, tea, berries, wheat, wines, various green vegetables, onion

34.13 Nutraceuticals

"Nutraceuticals" [9, 10] play a major role in health enhancement. The term "nutraceutical," a combination of the terms "nutrition" and "pharmaceutical," was coined in 1989 by Dr. Stephen DeFelice, Chairman of the Foundation for Innovation in Medicine, and is subsequently accepted globally. Nutraceutical is a marketing term developed for nutritional supplement which is sold with the aim of treating and preventing diseases. It is considered as a food or a part of food offering health benefits. The nutraceuticals may range from isolated nutrients, dietary supplements, and genetically engineered "designer" foods, herbal products, processed foods such as cereals, beverages, soups, etc. Currently 470 nutraceutical and functional food products are available. For details of nutraceuticals as therapeutic agents and their availability from various sources recent reviews [9, 10] may be consulted. *All the phytonutrients discussed in this chapter are thus natural terrestrial nutraceuticals.* They enter our body through foods and drinks and are released in vivo and function to keep us healthy. The deficiency in natural nutraceuticals taken through foods may be replenished by marketed nutraceuticals.

34.14 Some Natural Toxins

A common belief is that if a small amount of a thing does good better will be the effect if taken more, especially for a long time. This concept is not always true. In cases of vitamins there are dangers if taken in excess—an effect of hypervitaminosis is observed. Prolonged use of vitamin A by way of its precursor β-carotene might cause liver enlargement, skeletal abnormalities, psychiatric problems, and skin and hair problems. Excess intake of vitamin D might promote calcification of soft tissues. Other vitamins, if taken in excess, will be eliminated in urine and will not do further improvement beyond the dose.

The well-known plant toxins are **α-solanine** and **α-chaconine** usually present in potatoes (*Solanum tuberosum*, Fam. Solanaceae) as glycosides of the steroidal alkaloid **solanidine** (Fig. 34.11). *Solanine remains under the skin of potatoes and when exposed to light becomes green. Such potatoes should not be eaten*. Further, in sprouted potatoes the concentration of solanine is higher. Tomatoes contain the toxin **α-tomatine**, a glycoside of a steroidal alkaloid **tomatidine** (Fig. 34.11). The damaged tomatoes or their preparation if not preserved properly, might lead to the formation of the toxin, harmful to the health.

Caffeine stimulates the release of hormones *epinephrine* and *norepinephrine* that affect the blood sugar, fat and cholesterol level. If these effects lie within the limit it is harmless, and if not, cause damage. In view of these side effects the addition of the stimulant caffeine in many soft drinks has been restricted to certain limits, and coffee is sometimes decaffeinated.

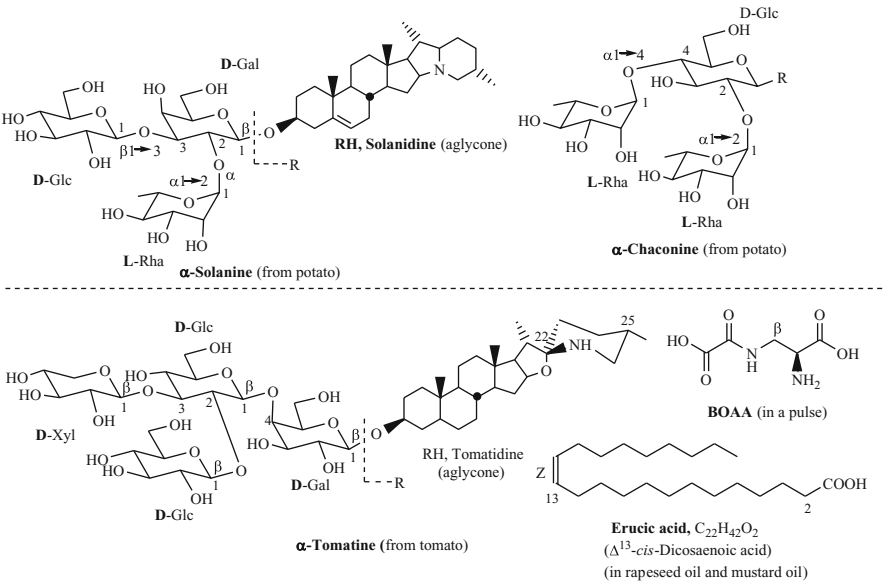


Fig. 34.11 Some plant toxins from potato, tomato, a pulse, rapeseed oil, and mustard oil

Chocolate is well known for causing migraine-headache to some susceptible individuals. This is due to the presence of a vasopressor amine, phenylethylamine. The latter is also present in cheese, red wine, etc. Only some individuals are affected, otherwise our body is equipped to eliminate this effect. However, dark chocolates are beneficial since they contain health promoting phytochemicals like flavonoids.

An African staple food *cassava* contains *β-hydroxyisobutyronitrile*, $(Me)_2C(OH)CH_2CN$, which is capable of generating HCN during damage in harvesting and working; for this purpose much time should, therefore, be allowed to disperse cyanide prior to its use as food.

Erucic acid (Fig. 34.11) present in mustard oil in small amount is the main constituent (30–60 %) of rapeseed oil. Erucic acid gets deposited in tissues of heart and causes swelling and lesions in heart muscles. Sometimes mustard seeds are adulterated with A*rgemone* seeds which look like mustard seeds and yield carcinogenic compounds. Other vegetable oils like *sunflower oil*, *soya bean oil*, *groundnut oil*, *olive oil*—all of them contain *mono-* and *polyunsaturated fatty acids* and have different nutritional values—hence blended oil may preferably be used.

Corn, *wheat*, *rye*, etc., if stored for a long time, sometimes are infested by fungus and the latter liberates toxins known as *ergotoxins*. They are *peptide alkaloids*. The fungi and the produced toxins may be removed by washing with saline.

Aram, *Corn*, and other vegetables, which grow underground, contain oxalic acid and oxalates. The latter react with metal nutrient like Ca, Mg, Fe, Cu to form insoluble oxalates and the body is deprived of such metals if such food is taken regularly. Incidence of kidney stone formation is high from such food consumption.

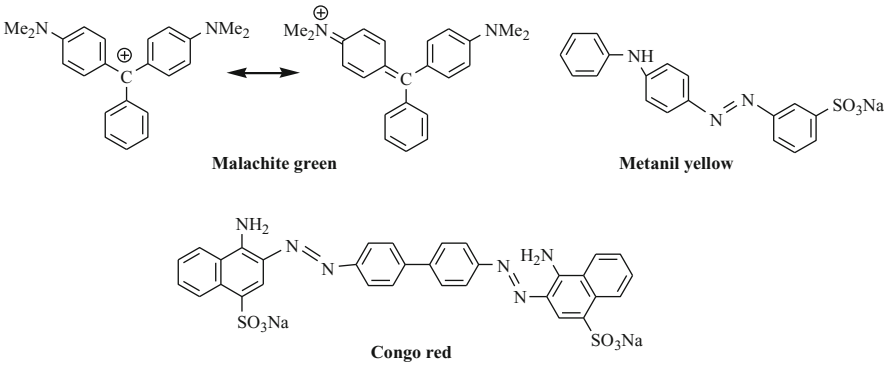


Fig. 34.12 Structures of some color additives

Lathyrus sativus (*khesari*, a kind of pulse used in India), a cheap source of nutrition contains a nonprotein amino acid, *Beta-N-oxalylaminoalanine* (**BOAA**) (see Fig. 34.11). It is a toxin that affects the nerve system and makes people cripple because of neuronal degeneration (neutrolathyrism), if taken for a long time. The toxin could be removed by boiling the pulse in water, throwing away the water and washing before cooking. This powdered pulse is often used for adulteration in powdered pea (*besan*).

Mushrooms are rich in protein content, but many mushrooms are not edible. The nonedible mushrooms generate toxins and hence mushrooms should be carefully used.

Long-stored cereals and ground nuts (red skin) release the toxins, *aflatoxins* (produced by *Aspergillus* fungus), which are highly *carcinogenic*.

Color additives (Fig. 34.12). Green vegetables are sometimes colored with *malachite green* in water. *Congo red* is used to color *chili powder*; *turmeric* is colored yellow with *metanil yellow*—and sometimes with *lead chromate*. All these are highly harmful to human systems.

In addition to these food hazards, foods and crops may remain contaminated with *residual fertilizers* and *pesticides*.

34.15 Some Useful Remarks

Anyone who is interested to know the organic phytonutrients present in some common fruits, nuts, beans, vegetables, and spices that are used or consumed by human beings may find this Chapter quite handy. The biosynthesis of most compounds of various classes has been already discussed in the relevant chapters, and it is beyond the scope of this book to study the biosynthesis of some other interesting active principles.

One should always emphasize on the good quality of food one consumes. Restriction of the quantity of food should begin at least from around forty years

of age and should be increased with age. Generally, an ideal diet should consist of fruits, slowly digestible carbohydrates, high fiber food—both soluble and insoluble, whole grains, nuts, red wine, green vegetables, fishes, and berries and should be rich in antioxidants.

Foods that can raise good HDL cholesterol: Polyphenol-rich diets, β-carotene-rich foods (carrots, spinach, broccoli), vitamin C-rich foods (sweet peppers, broccoli, oranges, lemons), olive oil, grape seed oil, sea food especially fatty fishes (salmon, tuna, mackerel, sardines and other fatty fishes), beans (different kinds), garlic, ginger, onion (raw), almonds, avocados, walnuts, apples, mangoes, wine, beer, alcohol in moderation, oysters, mussels, and grains high in soluble fiber (oat).

Foods that can increase harmful LDL cholesterol: high saturated fat, high cholesterol foods. *Cautions*: very low-fat diets (10 % or less of calories from fat) depress HDL cholesterol.

Total calories of diet should be low. Fruits and red wine lower the risk of various diseases—as already mentioned. Foods that one should not take in excess but is tempted to take, e.g., sugar, sweet preparations, munching items, etc., should not be kept in front of eyes while taking food or watching TV or relaxing. Finally, the present state of knowledge of the biological metabolic systems and the chemical composition of various foods and their relationship may give guidance to select proper healthy and beneficial food and drink. This will ensure a really enjoyable long life to a great extent.

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Erratum to: Chemistry of Plant Natural Products

Erratum to: Appendix A

S.K. Talapatra and B. Talapatra, Chemistry of Plant Natural Products,
DOI 10.1007/978-3-642-45410-3, © Springer-Verlag Berlin Heidelberg 2015

Dear Reader,

In spite of careful checking by the authors and the editorial team a mistake has occurred. In the former print edition of this book on page 1048 the picture did not show Arthur John Birch, but instead Stephen Pyne, Professor at the University of Wollongong, Australia, and recipient of the A.J. Birch Medal for excellence in Organic Chemistry Research in 2012. We apologize to our readers, Stephen Pyne and the family of Arthur John Birch.

Source of photo A.J. Birch: Research School of Chemistry, The Australian National University.

The online version of the original book can be found under
DOI 10.1007/978-3-642-45410-3

Appendix A

Brief Life Sketches of Some Pioneers Who Significantly Contributed, Directly or Indirectly, to Natural Products Chemistry

The sciences are not abstract constructions, but rather the results of human endeavor; they are closely connected with the personalities and the fates of the dedicated researchers who developed them[1]

Emil Fischer

This Appendix contains brief life sketches of 31 illustrious chemists who have given us the privilege of seeing much of the unfolding of the chemical panorama, especially in the area of organic and bioorganic chemistry in general and natural products chemistry in particular. Life sketches of such key personalities are not available in one book. Small fragments of their valuable contributions have been mentioned in their life sketches and also in different chapters of the text. The information regarding the life sketches of the scientists has been collected from websites, review articles, literature, university news bulletins, biographies, and autobiographies that we could procure and from personal contact in a few cases. Students of chemistry and history of science may find this account interesting and encouraging. Readers would gain inspiration from the bits of the personal data presented here on each of them and from the life lived by the man behind his contribution. Inclusion of the lives of a few other intellectually equals would have made this Appendix more comprehensive, but space constraint did not allow us to do so. We deeply regret our inability. However, we are also aware of the fact that ideas and concepts often do not have a singular discoverer; many more exponents are often involved in the same intellectual exploration.

[1] Rolf Huisgen, Adolf von Baeyer's Scientific Achievements—A Legacy, *Angew. Chem. Int. Ed Engl.,* **1986,** *25,* 297-311, pertinent page 297.

S.K. Talapatra and B. Talapatra, *Chemistry of Plant Natural Products*,
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A.1 Duilio Arigoni (1928–)

One of the founders of bio-organic stereochemistry, a major contributor in the biosynthesis of terpenes, alkaloids and cofactors, and in the stereochemistry of enzyme-catalyzed reactions

Duilio Arigoni was born in Lugano, Switzerland on December 6, 1928. He did his PhD with Professor Dr. O. Jeger in 1955 from ETH (Eidgenössische Technische Hochschule), Zürich. During 1961–1962, he was privatdozent with Professor Dr. L. Ruzicka at ETH and has been there with the Organic Chemistry Department ever since. He climbed the academic ladder in a conventional way: Associate Professor of Organic Chemistry (1962–1967), Full Professor of Organic Chemistry (1967–1996), Head of the Chemistry Faculty (1976–1977), Head of Organic Chemistry Laboratory, (1971, 1988–1990, 1993), and Professor Emeritus, ETH (1996-present). He is mainly interested in two main areas: the chemistry of natural products including structural elucidation, mechanistic studies, and biosynthesis particularly of terpenoids and bioorganic investigations with major emphasis on the stereochemistry of enzyme-catalyzed reactions.

Simultaneously with Rohmer, he made a major contribution in our understanding to non-mevalonate pathway of terpenoid biosynthesis and showed that the anomalies in the incorporation of data of the labeled substrates into the products during the biosynthetic studies of diterpenoids, like *Ginkgo* diterpenoids, could be well explained by non-mevalonate pathway [1]. This becomes a major pathway during the biosynthesis of monoterpenoids and diterpenoids. His pioneering contribution includes a masterly elegant synthesis of chiral acetic acid (1969, 1975) simultaneously done by Cornforth (1969), which becomes instrumental in understanding methylene–methyl conversion and vice versa in the study of the biosynthesis of natural products, more specially of terpenoids. All the hydrogen atoms of the methyl group are different, being isotopic to each other (H, D, T), and serve as the reference for the chiral acetic acid formed during specific hydrogen addition to the differently labeled methylene containing substrates (Chap. 5). This experiment

widened our knowledge of the dynamics of enzymatic reactions. He also contributed significantly to the explanation of biosynthesis and the manner of functioning of Vitamin B_{12}. He has been decorated with many awards, invited Professorships, and medals, of which a few are mentioned below:

He received Ruzicka Award, ETH (1961); Guenther Award, American Chemical Society (1970); Cannizzaro Award, Accademia dei Lincei (1971); Davy Medal, Royal Society, London (1983); Robert Robinson Medal, Royal Society of Chemistry, London (1984); R. A. Welch Award, Robert A. Welch Foundation, USA (1985); Arthur C. Cope Award, American Chemical Society (1986); Wolf Award (1989); Paul Karrer Award, University of Zürich (1989); Marcel Benoist Prize, Switzerland (1992); and George Kenner Award, University of Liverpool UK (2002).

He was A. D. White-Professor-at-Large at Cornell University (USA), Alexander Todd Visiting Professor at the University of Cambridge (1981), and R.B. Woodward Visiting Professor at Harvard (1983). About him, Professor Barton wrote in his memoir [2], "Duilio is brilliant at any time, on any subject and in any language. His expositions are crystal clear and he makes complicated biosynthesis simple."

The present authors (SKT and BT) had the opportunity of listening to his outstanding lecture on the biosynthesis of deterpenoids ginkgolides at a Symposium in Kolkata, explaining the biosynthesis in terms of non-mevalonate pathway. He delivered the lecture not with power point projection, but with transparencies through overhead projection.

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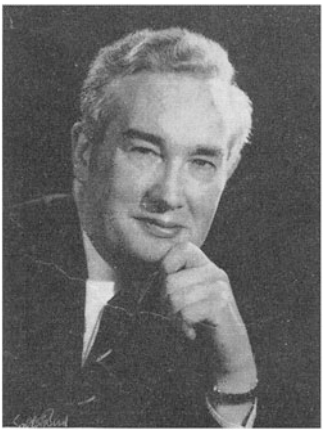
A.2 Sir Derek Harold Richard Barton (D.H.R Barton) (1918–1998) [NL 1969]

—The lights came on with conformational analysis
W.S. Johnson, Advanced Topics in Organic Chemistry, fall quarter, Stanford University, 1963



While paying tribute to D. H. R. Barton on the occasion of his 80th birthday [1] K. B. Sharpless remembered how much he was influenced by the course work with the above title offered by W. S. Johnson at Stanford where he was a graduate student. The title of the course reflects the impact of conformational analysis on organic chemistry. Further in his Nobel lecture [2], Sharpless said, "...After getting a double dose of him in the classroom, Derek Barton became my model. ...Being wet behind the ears I took conformational analysis for granted; it was Sir Derek's search for new reactivity that electrified me."

The man who could see the three-dimensional shapes of organic molecules and realized their importance in biological systems and chemical reactions was born to William Thomas and Maude Henrietta in Gravesend, Kent, UK, on September 8, 1918, in a family of carpenters. He studied in Tonbridge School and graduated from Imperial College, London, in 1940, and within only 2 years he received the PhD degree from the same institute.

Barton thereafter had been associated with Birkbeck College (London), Harvard University, MIT, Illinois University, and Wisconsin University. On his return to UK he held the Chairs of Chemistry at Birkbeck College and Glasgow University before returning to Imperial College, London, in 1957, where he developed a remarkable synthesis of the steroid hormone aldosterone [3], by a high yielding nitrite photolysis which came to be known as **Barton reaction**.

During a sabbatical leave at Harvard (1949–1950) he published an epoch-making paper [4] entitled "Conformation of the Steroid Nucleus." This four-page paper clarified the concept of the chemists about the shape and reactivity of steroid

molecules. It had a tremendous impact on the development of organic chemistry. It showed the dependence of the reactivity of the functional groups in steroids on their axial and equatorial orientations in their rigid or biased conformations and on their shape and environment. The discovery of the pioneering conformational analysis concept brought him the Nobel Prize in Chemistry in 1969, shared with the Norwegian chemist, Odd Hassel (1897–1981), a retired Professor of Oslo University. A conceptual understanding of the molecular reactivity in terms of shape, ring inversion or flipping of flexible molecules, bond angle and orientation that developed through the subsequent works of Sir Derek, and many others constitutes the various aspects of conformational analysis. Establishment of the configuration of the chiral centers of a meso-tricarboxylic acid of a cyclohexane derivative obtained as an oxidation product of diterpene abietic acid (Sect. 8.3.2, Fig. 8.12) published by Barton during 1948–1950 was an early example of conformational analysis [5]. His work on conformational analysis proved useful in synthetic organic chemistry, medicinal chemistry, and drug design and helped the discovery of the double helix structure of DNA. A few serial publications/monographs have come out on conformational analysis within the twentieth century.

Barton retired from Imperial College in 1978 and became an Emeritus Professor at London University for a while and then the Director of the Natural Products Institute (CNRS) at Gif-sur-Yvette, France, where he studied new chemical reactions of radicals. In 1986 he moved to the A & M University, Texas, as the Distinguished Professor of Chemistry where he continued to work on novel reactions involving radical chemistry and the oxidation of hydrocarbons; this work acquired great industrial importance.

During a research career spanning more than five decades, Barton made many useful contributions to organic chemistry including major discoveries of new reactions and methodologies, in addition to conformational analysis and Barton Reaction. Some of these are cited below:

(1) The breadth and influences of his work illustrated in "Biogenetic Aspects of Phenol Oxidation" [6] led to many experiments on alkaloid biosynthesis and to a set of rules for *ortho-para*-phenolic oxidative couplings which allowed the prediction of new natural products and correction of several erroneous structures. (2) Invention of new reactions, in addition to Barton reaction, which have been patented and are used worldwide in the pharmaceutical industry:

(i) "Invention of a New Radical Chain Reaction" generating carbon radicals from carboxylic acids [7]—a method of great synthetic utility—which has proved to be one of the most important methods of the twenty-first century.

(ii) "The Deoxygenation of Secondary Alcohols," yet another "Barton Reaction," was introduced [8] which has been very widely applied in carbohydrates and nucleosides having complex local environments.

Barton's more recent work is summarized in a few articles [6, 7]. His creativity and stamina (working 12 h a day) remained undiminished to the day he expired. He authored more than 1,000 papers and held many successful patents. He trained over 300 students and postdoctoral fellows in his long career—many of whom now hold major positions throughout the world. In addition to the Nobel Prize many

invited Professorships, medals, honors, and awards were showered on Barton—to mention a few—the Davy, Copley, and Royal Medals of the Royal Society of London and the Roger Adams and Priestly Medal of the American Chemistry Society. He became the President of the Chemical Society, London. He was knighted in 1972; he received honorary degrees from 34 universities. He belonged to many national and international academies and societies. He received the recognition "Order of the Rising Sun" from the Emperor of Japan. At the age of 71 he received the 1989 ACS Award for creative work in synthetic organic chemistry published in the preceding 5 years. Sir Derek had been the lifelong Chairman of the Editorial Board, *Tetrahedron* and *Tetrahedron Letters* since 1979.

His students and coworkers used to organize symposia on the occasion of his every 5th birthday beginning with the 60th. In honor of Sir Derek and to celebrate his 80th birthday a symposium was organized by K. C. Nicolaou on February 6, 1998, in the Chemistry Department, the Scripps Research Institute, San Diego. A souvenir entitled "Barton Memorabilia" containing good wish letters, comments, and memoirs of some of his students, postdoctoral fellows, friends, and colleagues from different parts of the world was released on this occasion, and we quote here comments from two most celebrated organic chemists. Eschenmoser wrote, "And the impact you had on organic chemistry in our time is incomparable. Molecular Constitution, Configuration and Conformation is a triad of concepts which, in essence, embraces the entire history of organic chemistry. . . . Life itself depends intrinsically on the existence of conformational diversity; as constitution relate to conformation in organic chemistry so does the genotype relate to phenotype in biology. . . . Organic chemistry of our time is very fortunate in having a Derek Barton. You personify one ideal and. . . an example to be followed by the young." Barry Sharpless wrote, ". . .He is my nominee for the single most responsible and most reliable human being. In actual truth, at 80 Sir Derek Barton is in all likelihood, the most tireless servant to chemistry who has ever lived. He is every chemist's conscience, as well as a dog lover of epic proportion."

From the cover page of "Barton memorabilia" [1] we quote, "The Elucidation of Universal Principles is a Celestial Endeavour—**The BARTON LEGACY.** Chairs and Boats . Thoughts and Inventions . Radical and C-H Bonds . Timeless Impact"—very aptly written. The souvenir starts with a lovely photograph (provided by his colleague A. I. Scott) of 4-year-old Derek standing against a bench and wearing a frock. Only 38 days after his 80th birthday celebration symposium Sir Derek died of sudden heart failure in College Station, Texas, on March 16, 1998. "With the death of Sir Derek Barton, the world of Science has lost a major figure, who together with Sir Robert Robinson and Robert B Woodward, the cofounders of *Tetrahedron*, changed the face of organic chemistry in the twentieth century" [9].

To commemorate the centenary of the Royal Institute of Chemistry a set of four postal stamps [10] was released in 1977 to recognize the British achievement in Chemistry. The theme of the centenary was *Chemistry in our lives*. One 8.5 penny stamp carries on it the conformation of cortisone nucleus against the background of some pharmaceutical products in honor of Barton and his work. The other three stamps contain (i) a model of ascorbic acid (to honor W. N. Haworth, NL

1937), (ii) chromatographic separation curves (in honor of A. T. P. Martin and R. L. M. Synge, joint NL 1952), and (iii) crystal structure of NaCl (in honor of W. H. Brag and W. L. Brag, joint NL 1915).

An award, D. H. R. Barton Medal, has been instituted by Royal Institute of Chemistry (London) in honor of Sir Derek Barton. As per his desire the award is given to the achievers attaining the age of 60 or above. Gilbert Stork is the first recipient of this award.

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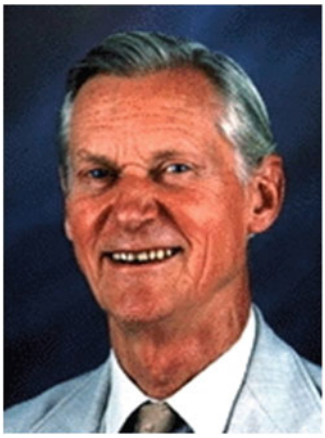
A.3 Sir Allan Rushton Battersby (A. R. Battersby, 1925–)

A scientist of rare originality and masterfulness. Through his work—the biosynthesis of natural products—he reveals Nature's strategies for the synthesis of natural products



A.R. Battersby was born to William and Hilda Battersby on 4 March, 1925, in Leigh, UK. He studied in Grammar School at Leigh and did his MSc from the University of Manchester, PhD from the University of Andrews, DSc from Bristol University, and ScD from Cantab. Battersby married Margaret Ruth in 1949 and she died in 1997.

Battersby joined the University of Andrews as an Assistant Professor (1948–1953) and went to the University of Illinois for a year (1951–1952) as a postdoctoral fellow. On his return he joined the University of Bristol (1954–1962) as a lecturer. He then held a Chair at the University of Liverpool (1962–1969). In 1969 he joined the Faculty of Organic Chemistry at the University of Cambridge where he became Professor in 1988 and retired in 1992. Since then he remained associated with Cambridge as an Emeritus Professor. His outstanding as well as pioneering work on the biosynthesis of natural products with special reference to alkaloids of different classes profusely showered on him awards and honors from home as well as outside.

In 1966 he has been made the Fellow of the Royal Society (FRS). In 1992 he received a knighthood for his lifetime contributions to science.

He demonstrated and elucidated the special role of enzymes in the biosynthetic processes and shared the 1989 Wolf Prize in Chemistry Laureates jointly with Duilio Arigoni (see Sect. A.1) for "their fundamental contributions to the elucidation of the mechanism of enzymic reactions and the biosynthesis of natural products, in particular the pigments of life."

Battersby extensively used labeled compounds as the precursors in the steps that lead to the final products. Of all his works, the most outstanding one is the work on decoding the genetic blueprint, structure, and synthetic pathway of the formation of

Vitamin B_{12}. In this work we find the multifaceted experiences of Battersby (isolation, structure elucidation, spectroscopy, synthesis, isotopic labeling biosynthetic analysis, etc.) in a combined way resulting in an elegant entry to the biosynthetic pathway of vitamin B_{12}. For complementary work of Battersby and A. I. Scott, Director of the Centre of Biological NMR at Texas A & M University on the decoding of the genetic blueprint, structure, and synthetic pathway for vitamin B_{12}, they were jointly awarded Welch award, 2000. This award is meant for "important basic chemical research contributions that have had a significant, positive influence on mankind." Norman Hackerrman, Chairman of the Welch Scientific Advisory Board, said, "Working separately, these two chemists have significantly increased our understanding of how nature makes products essential to human life."

About this work on "Vitamin B_{12}," whose structure has been described as "frighteningly complex" by one of his colleagues, Battersby himself observed that this structure "presented just a challenge I like. And the elucidation of the pathway to it, in collaboration of an outstanding group of French Biologists, is one of the major highlights of my scientific career." Battersby received "Honoris Causa" from six universities, Hony. Membership/Fellowship of a number of foreign academics. He traveled extensively and lectured in all the major universities and institutes of the globe; some are Karl Folkers, Wisconsin; Tishler, Harvard; August Wilhelm von Hoffmann, Ges. Deutscher Chem; Pedler, Chem. Soc.; Kharasch, Chicago; Baker, Cornell; Seshadri Memorial. India; Dauben, Berkeley; Linus Pauling, Oregon; Robert Robinson, U.K.; Paul Karrer Lecture, Zurich; R.B. Woodward Award, USA. He is recipient of almost all the medals, associated with the names of great chemists that are given to the distinguished scientists for their achievements.

Battersby loves music, fishing, and gardening. He is adventurous enough for hiking, camping, and sailing.

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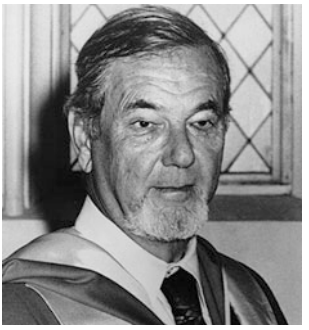
A.4 Arthur John Birch (1915–1995)

—The inventor of Birch reduction and a propounder of polyketide pathway



Arthur John Birch was born on August 3, 1915, to a skeptical Englishman father Arthur Spencer Birch and an Irish mother Lily Bailey at Sydney, New South Wales, Australia. He acquired some attitudes of his father. One such was not to accept anything granted and another one was to be curious about the world around him. His school days were eventful. Arthur was a boy of strong personality and independent mind. His objectives and viewpoints were dominant which sometimes irked the teachers and acted against marks, ranking, etc., awarded by the teachers. He was greatly influenced by an unselfish and strong-willed minister, Harold King, with a keen sense of humor. Interactions with this minister made him a person with principle which he followed in his actions all through his life.

Arthur inherited 100 pounds (quite considerable in those days) from his beloved aunt Maude. With that money and the help from his father he bought a Liebig condenser, a Kipps apparatus, a few chemicals, and books. He did some experiments by himself and taught himself organic chemistry at the age of 12 from Cohen's textbook which he kept with him all through. The ferment of his chemical curiosity started with his love for the beautiful bright colors and odors of various flowers and leaves of Australian plants and that sowed the seed for his future chemical activities. In fact, at the age of 12, he isolated limonene, a lime-smelling terpenoid, by steam distillation of some leaves and resins. He had to be financially independent at the age of 17 because of his father's illness and did odd jobs to support him.

Birch then won a scholarship to attend the University of Sydney and completed BSc (1937) and MSc (1938). From 1938 to 1948 he was an 1851 Exhibition Scholar and an ICI Fellow at the University of Oxford, UK. He received the D.Phil. degree (1940) from Oxford University and remained there working under Sir Robert Robinson (NL 1947) until 1948 when he became the Smithsonian Fellow at the University of Cambridge and worked under Lord Todd until 1952. The same year he accepted the Professorship in Organic Chemistry at the University of Sydney. He became a Fellow of the Australian Academy of Science in 1954. He joined

Manchester University in 1955 as Professor and was made a Fellow of the Royal Society in 1958. He was showered with many awards, medals, and lectureships.

Birch returned to Australia again in 1967 and established the Royal School of Chemistry at the Australian National University (ANU) in Canberra, became its Founding Dean, and remained attached to the School until 1980. Thus, his career path involved (Sydney (1933–1938), Oxford (1938–1948), Cambridge (1949–1952), Sydney (1952–1955), Manchester (1955–1967), and Canberra (1967–1980) and he retired in 1980 at his mandatory retirement age of 65.

He then took up a Visiting Professorship at Oxford and returned to the ANU in 1982 as a University Fellow in the Chemistry Department. He was awarded the Tetrahedron Prize for creativity in Organic Chemistry. He served the Royal Australian Chemical Institute (RACI) as its President (1977–1978) and became the President (1982–1986) of the Australian Academy of Science. He was made a Companion of the Order of Australia in 1987 for his contributions to science in Australia. In 1989 he was made Foreign Fellow of the Indian National Science Academy. In 1994 the RACI made him one of their few Honorary Fellows. The main building of the Research School of Chemistry at ANU was named the "Birch Building" before his death in 1995. The RACI named their premier award in his honor in 1996.

Birch had outstanding friends. Cornforth (NL 1975) (A-9) was one of them. Cornforth has been a great achiever in chess, sports, languages, and literature. As an example of his wit Birch quoted one of Cornforth's famous [1] limericks (which Cornforth used to compose as an undergraduate during lectures since he was deaf) that Cornforth wrote about Birch when Birch had wantonly offended Cornforth.

That Outpost of Empire, Australia
Produces some Curious Mammalia;
The Kangaroo Rat
The Blood-sucking Bat
And Arthur J. Birch, inter alia. [1]

Contributions to Organic Chemistry

To achieve a partial reduction of aromatic system needed for the synthesis of a steroidal hormone, Birch invented a novel way to introduce an angular methyl (alkylation) and partial reduction of an aromatic system using Na/Li/K-ethanol/*t*-butanol in liquid ammonia, later came to be known as Birch reduction [2, 3, 4, 5]. This valuable synthetic methodology was first reported [2] in 1944 and finds wide application in various fields [3]. Its reaction mechanisms under different conditions have also been studied [4]. A review [5] on Birch reduction has appeared in 1991. Birch's polyketide hypothesis [6, 7] for the formation of aromatic compounds was proved with labeled precursors (Chap. 14). Biomimetic synthesis of several compounds using polyketide chain cyclization has been reported in the literature [8]. Additionally, he elucidated the structures of a number of natural products of diverse skeletons, synthesized many of them, and studied their biosynthesis. He published more than 460 scientific papers. The American Chemical Society published his

scientific autobiography, incisively entitled *"To See the Obvious"* in 1995, which was written over the last 10 years of his life.

As a Human Being

Birch acknowledged immensely his mother—the kindest and strongest person he has ever seen, for her contribution, especially during hard days toward his career continuation. A beautiful picture of little Arthur sitting on his mother's lap appeared in his autobiography [9]. His would-be-wife Jessie Williams nursed his sick mother, a patient of Parkinson disease. This act of her relieved him of his strain to a great extent which allowed him to work peacefully. They were married in 1948. She followed him to all his working places. Both his mother and wife were happy to see him achieving his goal.

Birch loved music, arts, and traveling. As a man of diverse interests inside and outside science his definition of achievement differs from many. Once being told that he was a rich man Birch replied, "That is true in many other ways also, although as the world counts wealth I am not rich. I abhor those scientists who sit like an oyster on a rock, without a pearl, gradually swelling scientifically and financially" [10]. Birch was extremely humorous. He wrote about Francis Lions, a great teacher in Sydney University and over enthusiastic about sports, that the broken nose he sustained in consequence of playing football was used in his lectures on chemical asymmetry [11].

Birch's family and his fighting spirit and humor sustained him through long illness and successive operations. He died in Canberra on December 8, 1995. He disliked pomp and ceremony, and had said that there should be neither service nor eulogy at his funeral; the occasion was to be more in the spirit of an Irish wake (watch by corpse before burial). His wishes were essentially met at his cremation and the subsequent gathering at the Australian Academy of Sciences on 11 December 1995.

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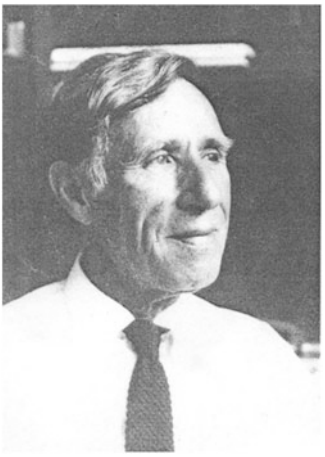
A.5 Konrad Emil Bloch (1912–2000) (NL 1964)

A remarkable biochemist who brilliantly deciphered how cholesterol is biosynthesized in living animal tissues and one of the very few biochemists who first used stable isotopes to label the potential precursors in the study of biosynthesis of complex molecules



Bloch was born on January 12, 1912, in Neisse (now Nysa in Poland), an attractive town in the eastern German province of Silesia. He was the second child of Fritz Bloch and his wife Hedwig (née Striemer) belonging to an upper middle-class Jewish family. Bloch attended the elementary school and Real gymnasium in Neisse and went to study at the Technische Hochschule, Munich, in 1930. He got interested in and fascinated by organic chemistry, specially the natural products chemistry, when he took courses from Hans Fischer (NL 1930), a nephew of Emil Fischer, and also listening to the lectures delivered by leading chemists of the time

like Adolf Windaus (NL 1928), Richard Willstater (NL 1915), and Heinrich O. Wieland (NL 1927). He earned a degree but could not continue there because of the Nazi regime. He moved to Switzerland in 1934, and 2 years later, he joined Hans Clark's Department of Biochemistry at Columbia University, New York, from where he obtained his PhD degree. The most interesting part of the interview with Clark was a question that he asked young Bloch, "Do you play any musical instrument?" Fortunately when Bloch was young, one of his great uncles wanted him to choose one of the two items, a cello and a canoe, as a gift from him. He was initially for a canoe, but on his parents' insistence he accepted a cello—and learnt playing the same. Clark delighted in chamber music and welcomed another cellist. Bloch, though initially resistant, now thanked his parents for the decision. He began to do research on lipids and cholesterol at Columbia University along with Rudolf Shoenheimer, an outstanding biochemist who died prematurely in 1941 at the age of 43 only. Shoenheimer used tagged molecules for the study of metabolism in the living systems. After his death Bloch carried out the work on cholesterol biosynthesis independently. He moved first to Chicago University where he rose to Professorship (1940–1946), then moved to Harvard along with his cholesterol problem, and identified acetic acid as the precursor of cholesterol in rats. He also found that acetic acid is converted to a symmetrical highly branched hydrocarbon squalene which is subsequently converted to lanosterol and then to cholesterol through sequential loss of three methyl groups (Chap. 11). Bloch used deuterium as well as ^{13}C-labeled acetic acid for this study and found cholesterol with the isotopic signature at the expected locations. He concluded that acetic acid is the source of all carbons and hydrogen atoms of sterols excepting an extra methyl group on the side chain of sterol. Bloch became the Higgins Professor in 1954 at the Department of Biochemistry, Harvard University, a position he held till his retirement. He became the Chairman of the Department. He was also the Professor of Science in Harvard's School of Public Health from 1979 to 1984.

For his outstanding work on lipid metabolism and biosynthesis of cholesterol one of the most important molecules of life playing a dual role, both beneficial and harmful, he was awarded almost all the treasured awards (medals, prizes, professorships, and Honoris Causa, etc.) that are open to a biochemist. He shared the 1964 Nobel Prize in Physiology/Medicine jointly with his formidable competitor, F. Lynen who discovered acetyl coenzyme A, the active form of acetic acid. A group of drugs called statins, which lower the cholesterol content of the living system, has been invented; they function as the enzyme inhibitor of the essential step of cholesterol biosynthesis. However, Bloch commented on cholesterol as a Janus-faced substance, a villain causing vascular disease on the one hand, yet at the same time a molecule essential for bodily functions.

Bloch used to enjoy skiing and tennis. He was a man of wide cultural interests. He loved books, music, and art. At old age he wrote a wiry couplet

Alter werden ist nicht schwer
Alter sein, dagengen seht
(To grow older is not difficult but to be older
On the contrary, is very much so).

He wrote a popular book entitled "*Blondes in Venetian Paintings, the Nine-handed Armadillo, and other essays in Biochemistry*." He was a soft-spoken man with strong ethical principles. His joyous family life was centered on Lore Teutsch whom he met at Munich and married in 1941 in the USA. Leaving two children, a daughter Susan and a son Peter, Konrad Bloch died in 2000 at 88.

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A.6 Herbert Charles Brown (1912–2004) (NL 1979)

His exceptional talent overpowered all of his early academic years and the compounds he discovered (the main elements of which are synonymous with the initials of his name HCB) led him to discover a "new continent" which requires settlers to develop its riches to contribute to mankind. [1]

This is the story of a determined boy who had to do odd jobs to support his family and his study, and who finally won the Nobel Prize in Chemistry. Herbert C. Brown was born Herbert Brovarnik on May 22, 1912, in London to Ukranian Jewish immigrant parents Charles Brovarnik and Pearl Gorinstein. They moved to Chicago in 1914 to join the family of his paternal grandfather where the grandfather's name was anglicized to Brown and that became his family name. He had three sisters—Ann senior to him and two younger sisters Sophie and Riva.

His early *academic career* has been punctuated from time to time. He attended the Haven school in Chicago with predominantly black classmates. After graduation he went to Englewood High School on south side of Chicago [2]. He left the school to look after the small hardware store of his father after his death in 1926. Brown was more interested in reading than the business. So his mother started looking after the store and he reentered the school in 1929 and graduated in 1930. He then entered the college and thought of becoming an engineer so as to live a financially comfortable life. But he took chemistry as he was fascinated by the subject and joined Crane Junior College. Because of lack of funds, the Crane Junior College closed down when he had just completed one semester. He went to a night school and supported himself financially working as a *part-time shoe clerk*. He joined a laboratory opened for a handful of students by Dr. Nicholas Cheronis, an Instructor at Crane, so that the students could continue their study on their own. There he met Sarah Baylen, the brightest student in chemistry at Crane prior to his arrival. She initially "hated" his "guts," but since she could not beat him, she later decided to join him to his everlasting delight. They graduated from Wright Junior College in 1935 and Sarah predicted in the year book that Herbert would be a Nobel Laureate. Perhaps she could realize his potential more than him.

He entered the University of Chicago in the Fall of 1935, accompanied by his girlfriend Sarah. At that time it did not involve more payment to register for ten courses than that for the usual three; he did so and completed his junior and senior years in three quarters and received his BS in 1936. On his graduation Sarah presented him with a gift, a book by Alfred Stock entitled "The Hydrides of Boron and Silicon"—the cheapest ($2) available book in the Chicago University Book Store. The book interested him so much that hydrides of boron became his lifelong love. They got married in 1937 and remained partners for nearly six decades until his death in 2004. Sarah became very much a part of him in all his success and everlasting delight.

Initially he worked with Professor H. I. Schlesinger who was active in that area. Brown received his PhD in 1938. He did not get any job in the industry and joined Professor M. S. Kharasch as a postdoctoral fellow. This was a turning point in his life. He fully entered into the academic world. He spent 4 years as an Instructor in Chicago University. In 1943 he moved to Wayne University, Detroit, as an Assistant Professor, became an Associate Professor there, and then in 1947 joined Purdue University where he became Wetherill Distinguished Professor in 1959 and Wetherill Research Professor in 1960, a position he held till retirement in 1978. He then became Emeritus and continued to work with a large group of postdoctoral fellows.

Originally his research covered physical, inorganic, and organic chemistry and he took students from all the three areas. However, when the Department became more organized into divisions, he had to make a choice and he elected to work primarily with coworkers in organic chemistry. He discovered diborane (B_2H_6), the simplest compound of boron and hydrogen which exhibits facile addition of boron–hydrogen bond to carbon–carbon multiple bonds to form organoboranes [2, 3]. These compounds find wide application in synthetic organic chemistry. Chiral organoboranes [4, 5] led to the development of the first general asymmetric synthesis of pure enantiomers. This is an extraordinarily rich area. Its utilization lies in the synthesis of complex molecules such as natural products, pharmaceuticals, fine chemical, and petrochemicals. Brown said "A new continent has been discovered—it requires settlers to develop its riches to contribute to mankind" [1]. In addition to borane–organoborane area, his research program involved "the study of steric effects, the development of quantitative methods to determine steric strains, the examination of the chemical effects of steric strains, the non-classical ion problem, the basic properties of aromatic hydrocarbons, a quantitative theory of aromatic substitution, and the development of a set of electrophilic substitution constants, σ^+, which correlate aromatic substitution data and a wide variety of electrophilic rections" [1].

His awards and recognitions were numerous, to mention a few—Fellow of the National Academy of Sciences (1957) and of the American Academy of Arts and Sciences (1966), Hony. Fellow of the Chemical Society and Foreign Member of the Indian National Academy of Sciences (1978), the recipient of The Linus Pauling Medal (1968), Ingold Memorial Lecturer and Medal (1978), and Nobel Prize in 1979 which he shared with George Wittig (University of Heidelberg, Germany).

The Herbert C Brown Laboratory of Chemistry was named after him in Purdue University Campus.

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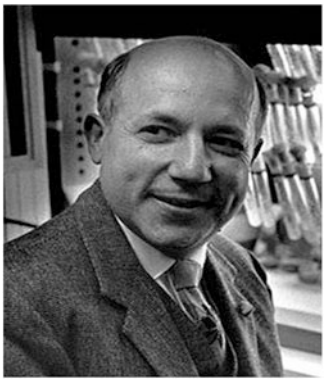
A.7 Melvin Calvin (1911–1997) (NL 1961)

Decipherer of the single most biological event—photosynthesis



Melvin Calvin [1, 2] was born on April 8, 1911, to Russian immigrant parents, Elias Calvin and Rose Hertviz, in St Paul, Minnesota. He graduated in 1931 from Michigan College of Mining and Technology and obtained his PhD degree in 1935 from the University of Michigan. After spending two postdoctoral years as the Rockefeller Foundation Fellow in Manchester, UK, he returned to USA and joined the University of California, Berkeley, where he found his chemically spiritual home. He steadily climbed up the academic ladder, and eventually became the full Professor in 1947.

Melvin studied extensively the dark reaction part of photosynthesis during which CO_2 and H_2O combine to form sugar. He used $^{14}CO_2$ and observed the distribution of the label in the intermediates and could map the conversions. The key step has been shown to be carboxylation of a C_5-sugar, ribulose-1,5- diphosphate to form 3-phosphoglycerate acid (3-PGA), which is reduced to 3-phosphoglyceraldehyde. NADPH and ATP generated in the light reaction of photosynthesis (cf. Fig. 3.3) are utilized during the reduction and phosphorylation. Ribulose monophosphate generated by another enzyme is phosphorylated and then the above process is repeated. The cycle is known as *Calvin cycle* (see Fig. 3.4). In 1961 he received the Nobel Prize when he said, "...an honor conferred to mankind through an individual" [2]. He also received many other major prizes. He was offered prestigious lectureships and was made Honorary Member/Fellow of many academies.

In his young days, he used to dismantle all toys to understand how they are built and how do they work. After satisfying his curiosity he used to reassemble the toys. He had been a wonderful observant and a determined boy. At the age of 3 he watched his uncle studying in the University and he decided to study. He was in love with the *Periodic Table*. When he realized its construction he said, "...it seemed to me a personal discovery."

His parents never went to college. The father was a talented auto mechanic, and his mother was talented with her needles. Because of financial constraint, his sister did not have the benefit of higher education and Melvin lamented that.

In the autobiography of Melvin, "*Following the Trail of Light* [3] appeared a picture of the strongest human relationship:—a mother and her son, and the son is Melvin Calvin, wearing a tie with a motive of photosynthesis on it, made by his mother who was exceptionally talented with the needle. His wife Geneviene Jemtegaard, also a scientist, died of cancer in 1987. She inspired him in his research and he said, "My personal life was built around her presence." His collaborators fondly remember his leadership in research, and friendly, congenial, as well as exciting atmosphere of the laboratory. In the Department of Chemistry, UC Berkeley, a building has been named after him. Later he worked on the values of plants as alternative source of energy. He found arid zone plants like *Euphorbia* species to be a good source of oil. He also worked on synthetic sensitizers and catalysts involved in photochemically produced oxygen from water and reduction of CO_2 to useful chemicals.

Melvin Calvin died in 1997 at the age of 86 years.

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A.8 Elias James Corey (1928–) (NL 1990)

20th Century folk hero [1]

Elias J. Corey was born as William in a small town *Methuen*, 30 miles north of Boston, on July 12, 1928. He lost his father Elias, a successful businessman, when he was a baby of 18 months. His mother changed his name from William to Elias shortly after his father's death. He did not remember his father but from whatever he heard about him from his friends and associates, he could realize that his father was a remarkably gifted man, and young Elias wanted to be a worthy son of his father. His mother raised her four children, of whom Elias was the youngest, during the depression of World War II. His grandparents of both sides emigrated from Lebanon and were capable of coping with hardship. His mother's sister and her husband lived with them. They had no child of their own and they were like second parents to Elias and his sister and brothers. As instructed by his strict aunt he had to do some domestic chores seriously. From her he learnt to be efficient and to derive pleasure in a job well done, no matter how mundane. Corey was fond of playing football and basketball and of hiking.

Corey attended Saint Laurence O'Toole Elementary School in Lawrence, a city next to Methuen and graduated at the age of sixteen from Lawrence Public High School where he did not study science except mathematics. At the age of seventeen Corey went to MIT and was very fond of mathematics. However, he was advised to go for some scientific work, and not mathematics, that would provide him productive years for future. Inspired by self-enthusiasm, he attended many classes on extra subjects and every class of chemistry to find out which subject he liked most. He picked up organic chemistry as his subject of choice thinking that with his knowledge in organic chemistry he will be able to solve many health problems and some other problems related to human welfare.

He was offered a lectureship at the University of Illinois and was asked to join within six weeks. He completed his thesis writing within four weeks with full involvement and joined the University of Illinois at Urbana-Champaign in 1951. He was interested in the *stereochemistry of natural products and mechanism of organic reactions including orbital overlap effect in transition states*. Corey and his students showed that *α-amyrin*, a natural triterpene, is not the mirror image of *β-amyrin*, as it was thought at that time. His interest in *triterpenes* remained for several years. He found that *friedelin* also has the same molecular formula as β-amyrin (Chap. 10), but is formed by a set of 1,2-migrations beyond β-amyrin forming a new skeleton, which is entirely different from that of β-amyrin (Chap. 10). His work on the synthesis of *sesquiterpenes* is remarkable (Chap. 7).

In 1959 he was happy to accept an offer of a Professorship at Harvard University and said, "I was really going home." "I accepted the offer with alacrity since I wanted to be near my family and since the Chemistry Department at Harvard was unsurpassed. The Harvard faculty in 1959 included Paul D. Bartlett, Konrad Bloch, Louis F. Fieser, George B. Kistiakowski, E. G. Rochow, Frank H. Westheimer, E. B. Wilson and R. B. Woodward—all giants in the field of Chemistry. . . .I have always regarded the offer of a Professorship at Harvard as the most gratifying of my professional honors" [2].

He had a number of projects in his hand and worked on the use of transition metals in organic synthesis, *enantioselective synthesis of sesquiterpenes*. He gave the *concept of*

retrosynthesis, i.e., moving backwards stepwise from the product, identifying probable intermediate precursors, and leading finally to the starting materials. Bond formation and bond making manipulations are there. His book *"The Logic of Chemical Synthesis"* [3], coauthored by X-M Chang, became very popular. With the help of his student Todd Wilke he developed the computer-generated "retrosynthetic" chemical synthesis and was quick to realize its promise. This concept of retrosynthetic methodology has been inspirational to many chemists involved in total synthesis. Photochemical dimerization was long known in the literature. However, Corey was the first chemist to realize the potential of this method of intermolecular addition using different substrates toward the synthesis of natural products [4]. An elegant synthesis of (±)-*caryophyllene* and (±)-*isocaryophyllene* will remain classical examples of such a potential (Sect. 7.5). His first synthesis of *prostaglandin* was completed in 1968. He synthesized complicated natural products of plant origin like *longifolene*, *gibberellic acid*, *ginkgolide B, bilobalide*, etc., in addition to many others of marine or fungal origin. His group was very much involved in the synthetic work of prostaglandins because they are, biologically, a very important class of compounds. He has more than 1,000 publications. Corey has developed number of reagents (e.g., PCC, PDC, Corey-Bakshi reagent, etc.), some alcohol blocking reagents (e.g., TBDMS, TIPS, MEM, etc.), and new methodologies widely used in organic synthesis all over the globe. About his research associates he said "I am very proud of my graduate students and postdoctoral fellows from all over the world who have worked in my research group. Their discoveries in my laboratory and their subsequent achievements in science have been a source of enormous satisfaction" [2].

As a recognition of his outstanding contributions to organic synthesis and methodology he received almost all the top national (US) and international awards and honors open to an organic chemist, and above all he won the 1990 Nobel Prize in Chemistry "*for his development of the theory and methodology of organic synthesis*" [2]. In 2002 The American Chemical Society declared him as the "Most Cited Author in Chemistry." He is the first recipient of "Cycle of Excellence High Impact Contributor Award" from ACS Publication Division in 2007. He ranked *second* among all living organic chemists and *third* among all living chemists by the Hirsch Index[2] (based on both productivity and impact of the published research work, December, 2011).

Corey said [2], "I've enjoyed working on a wide variety of projects as well as educational part of the university work. I have been driven by in-built individualism and independence, and the concentration of new ideas and ways of looking at problems. . . . I want to know as much about the world of chemistry as possible." The Corey research family now includes about one hundred fifty university professors and an even larger number of research scientists in the pharmaceutical and chemical industry and research institutes. About his teachers[3] he expressed his

[2] Hirsch Index has been devised by Jorge Hirsch, a Physicist, in 2005. Corey's h-index is 140 which means he has published 140 papers, each of which has received at least 140 citations.

[3] At MIT: Arthur C. Cope, John C. Sheehan, John D. Roberts, and Charles Gardner Swain; at University of Illinois at Urbana-Champaign: Roger Adams and Carl S. Marvel.

gratitude by saying, "I am forever grateful to them for giving such a splendid opportunity, as well as for their help and friendship over many years."

In 1961 Corey married Claire Higham, a graduate from the University of Illinois. They live near the Harvard Campus in Cambridge for over the last 50 years. They have three children; all of them graduated from Harvard and are successful in their subsequent careers and undertakings. Corey's leisure interests include outdoor activities and music.

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A.9 Sir John Warcup Cornforth (1917–) (NL 1975)

The unsurpassed master of stereochemistry. The major biosynthetic paths where prochiral hydrogens are involved (removed or added) in delivering the stereochemical information of the processes were extremely elegantly studied by Cornforth.

John W. Cornforth, the second of the four children of his parents, was born on September 7, 1917, in Sydney. His father was English born and a graduate from Oxford and his mother was descended from a German minister of religion, settled in

New South Wales. His first signs of deafness (from otosclerosis) became noticeable when he was about 10 year old. The total deafness was a slow process and took nearly a decade. In the mean time he studied in Sydney Boys' High School where a young teacher, Leonard Basser, influenced him in studying chemistry in which deafness might not be an insuperable handicap [1]. He spent his childhood in Sydney and rural part of New South Wales. He then entered the University of Sydney, the oldest in Australia (founded in 1851) having an honorable tradition in organic chemistry. The student quality in those days (1935–1939) was unique and the stimulating group in chemistry included A.J. Birch, Alan MacColl, Ronald Nyholm, and Rita Harradence. Cornforth is known as "*Kappa*" amongst his friends and later in his chemical community as he used to sign his initials in Greek (iota omega kappa) [2]. In 1937 he graduated with first-class honors and a University medal. In those days no PhD degree was awarded in Australia. After a year of postgraduate research Cornforth won an 1851 Exhibition Scholarship (1939) to work in Oxford with Robert Robinson (NL 1947). Two such scholarships were awarded each year, and the other was won by Rita Harradence also an organic chemist of Sydney University. Thus, an association started and continued. They were married in 1941 and have three children and two grandchildren.

Cornforth was a self-starter, highly intelligent and creative, and gained his PhD degree in 1941 working with Robinson. During his stay at Oxford his hearing power began to deteriorate, and he became totally deaf soon. He served on various academic assignments. He was in British Medical Research Council (1946–1962) and became the Director of Milstead Laboratory of Chemical Enzymology, Shell Research Limited. For the period 1965–1971 he was the Associate Professor at the University of Sussex and then became full Professor (1971–1975). In 1977 he was knighted.

In the biosynthesis of natural products with special reference to terpenoids he had shown very painstakingly, at the same time most elegantly, which of the two enantiotopic or diastereotopic hydrogens is stereospecifically selected by the pertinent enzyme from the substrate or from the coenzyme (Chap. 5) for their delivery to the active location onto the coenzyme/substrate. In doing so he labeled the key hydrogen atom with deuterium and/or tritium. This work is indeed a landmark in the biosynthesis of terpenoids and, for that matter, in natural products chemistry. The entire biosynthetic path of terpenoids and steroids are governed by the prochirality of hydrogens involved in the process during isomerization and chain elongation (Chap. 5). Such concept of prochirality was first identified by Alexander Ogston [2] (see Appendix B) for a system like $Cabd_2$; Cornforth succeeded brilliantly in realizing its full implication in the biosynthetic pathways. This concept is equally applicable in the biosynthesis of alkaloids and other identified metabolic pathways like shikimic acid pathway. For his outstanding work on the stereochemistry of the biosynthetic pathways he was awarded Nobel Prize in 1975 which he shared with V. Prelog. In his Nobel Prize address Cornforth said—"For him truth is seldom the sudden light that shows new order and beauty; more often truth is the unchartered rock that sinks the ship in the dark" [3]. With the progress of his work he received ample recognition, even prior to his becoming a Nobel Laureate. He was elected to

the Royal Society in 1953; awarded the Corday-Morgan Medal of the Chemical Society (1953), Flintoff Medal (1968), Pedlar (1968), and Robert Robinson (1971) lectureships; and received Ernest Guenther Award (1968, from ACS), CIBA Medal (1965, Biochemical Society) jointly with Popjak, the Stouffer prize (1967), and the Royal Society's Davy Medal (1968), etc.

Bloch (NL 1964) in the acknowledgment part of an article [4] wrote "I want to mention specially the beautiful work of John W. Cornforth and George Popjak that has supplemented, complemented and stimulated our own." Popjak coauthored with Cornforth in many of his papers contributing to the enzymological part of the work.

Birch wrote about Cornforth, "His high literary ability in the presentation of his work is very unusual, set against the accepted style of scientific papers, which is often characterized by flatness or by pretentiousness confused with profundity" [5].

He is excellent in chess, in sports, in language, and in writing limericks [2, 5] "which he started to compose as an undergraduate during lectures which he could not hear" [3]. His wife Rita is a talented outstanding student who shared the University graduation medal with Birch and coauthored with John Cornforth in many of his papers. About her Cornforth wrote "Throughout my scientific career my wife has been my most constant collaborator. Her experimental skill made major contributions to the work; she has eased for me beyond measure the difficulties of communication that accompany deafness; her encouragement and fortitude have been my strongest support" [3].

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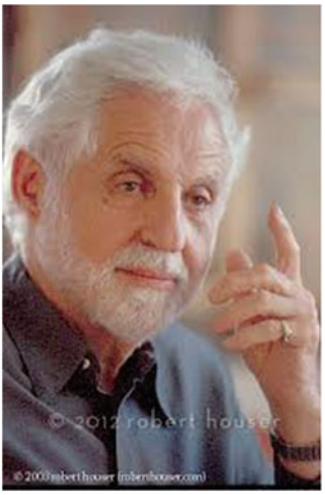
A.10 Carl Djerassi (1923–)

An Outstanding Organic Chemist, Father of the Pill, Art collector, Prolific Novelist—and now Playwright



Carl Djerassi [1] was born on October 29, 1923, in Vienna, Austria, the sole child of an Austrian mother and a Bulgarian father, both physicians. He spent most of his first 5 years with his parents in Bulgaria. Upon their divorce, he moved to Vienna with his mother for his schooling (which included the same Realgymnasium that Sigmund Freud attended) while spending summers in Bulgaria with his father. In 1938, shortly after the Nazi Anschluss (political union of Austria with Germany) his father briefly remarried his mother to acquire a Bulgarian passport and to enable her and Carl to leave Vienna without delay. Carl stayed with his father for a year in Sofia to attend the "American College" where he learned English, while his mother went to England to procure a visa for the USA to escape from the Nazi regime. In December 1939 Carl, a Jewish teen with a German-Viennese accent, and a damaged left knee from a skiing accident and without any high school diploma, arrived almost penniless in the USA with his mother.

After attending Newark Junior College in Newark, NJ, and then for one semester Tarkio College in Tarkio, MO (the school from which Wallace Carothers, the inventor of nylon, had graduated), he graduated summa cum laude from Kenyon College (BA in Chemistry, 1942). Following a year at CIBA Pharm. Co. (now Novartis). where he developed one of the first antihistamines, pyribenzamine, Carl left for the University of Wisconsin-Madison, where he received his PhD in 1945. He returned to CIBA as a research chemist for another 4 years before joining Syntex, S.A., in Mexico City as associate director of chemical research (1950–1951), where he and his team achieved the first synthesis of a steroid oral contraceptive as well as the first synthesis of cortisone from a plant raw material (diosgenin). In 1952, he started his academic

career at Wayne State University (1952–1957) and then returned to Mexico City as vice president for research of Syntex. In 1960, he moved to Stanford University as Professor where he was as of 2002, Professor Emeritus. In the 1960s, Syntex moved its research headquarters to the Stanford Industrial Park with Djerassi serving concurrently with his academic position as President of Syntex Research (1968–1972) and from 1968 to 1988 as CEO and chairman of the newly founded Zoecon Corporation on the Stanford Industrial Park—a company pioneering novel approaches to insect control through hormonal insect growth regulators. His association with Syntex afforded him the means to purchase 1,200 acres of land in the Santa Cruz Mountains near Stanford which included redwood forests, deep canyons, and undulating meadows with sweeping views of the Pacific Ocean. In 1982, in memory of the suicide of his artist daughter, Pamela, Djerassi converted much of this property into the Djerassi Resident Artists Program, which provides residencies and studio space for artists in the visual arts, literature, choreography and performing arts, and music. Over 2,000 artists have passed through that program since its inception. Djerassi is also a great art collector and he bequeathed his large Paul Klee collection [2] in equal parts to the San Francisco Museum of Modern Art and the Albertina Museum in Vienna. He has also donated large sculptures to the cities of San Francisco and Vienna and to the British Library in London.

Djerassi published more than 1,200 articles and seven monographs dealing with natural products chemistry (alkaloids, terpenoids, steroids, antibiotics, lipids, etc.), as well as applications of physical measurements [optical rotatory dispersion (ORD), circular dichroism (CD), magnetic circular dichroism (MCD), and mass spectrometry] for the elucidation of the structure and stereochemistry of natural products and to computer artificial intelligence technique applied to organic chemistry problems. He is the only American chemist to have been awarded both the National Medal of Science (in 1973, for the first synthesis of a steroid oral contraceptive—"the Pill") and the National Medal of Technology (in 1991, for promoting new approaches to insect control). A member of the US National Academy of Sciences and the American Academy of Arts and Sciences as well as the Royal Society (London), the Leopoldina (Germany), and many other foreign academies, Djerassi has received 28 honorary doctorates together with numerous other honors, such as the first Wolf Prize in Chemistry, the first Award for the Industrial Application of Science from the National Academy of Sciences, the American Chemical Society's highest award, the Priestley Medal, and more recently, the Erasmus Medal of the Academia Europaea (2003), the Great Merit Cross of Germany (2003), the Gold Medal of the American Institute of Chemists (2004), the Serono Prize in Literature (Rome, 2005), the Great Silver Decoration for Services to the Republic of Austria (2008), and the Edinburgh Medal (2011). In 2005, the Austrian Post Office issued a stamp in his honor.

During the past 23 years, he has published a collection of short stories, two poetry collections (*The Clock runs backwards, A Diary of Pique*), and five novels (*Cantor's Dilemma; The Bourbaki Gambit; Marx, deceased; Menachem's Seed; NO*)—which illustrate as "science-in-fiction" the human side of science and the personal conflicts faced by scientists—as well as an autobiography (*The Pill, Pygmy*

Chimps and Degas' Horse), a memoir (*THIS MAN'S PILL: Reflections on the 50th birthday of the Pill*), a biography in dialogic form (*Four Jews on Parnassus—a Conversation: Benjamin, Adorno, Scholem, Schönberg)*, and nine plays: *An Immaculate Misconception, Oxygen* (written with Roald Hoffmann), *Calculus, EGO, Phallacy, Taboos, Verrechnet* (with Isabella Gregor), *Foreplay,* and *Insufficiency*. His most recent book (2012) is *Chemistry-in-Theatre*.

His autobiography "The Pill, Pigmy Chimps, and Degas' Horse" [3] is presented in such a different and interesting way that Linus Pauling (NL 1954) wrote, "I found the first few pages so interesting that for two days I neglected my work in order to read the book from beginning to end" [4]. Incidentally it may be mentioned that Linus Pauling (b.1901) read this book (published in 1992) when he was around 91. In one chapter [5] where Djerassi described the search for his missing daughter and finally how he found her lovely face "terribly misshapen and bloated" [5] through the windshield of her Opel car in the wood, a feeling of personal loss holds the readers. Joshua Lederberg (NL) [4] rightly wrote, "With many doses of humor, some of pathos, the book creates a bond of reader to author that would seem improbable for a specialist in the physical chemistry of steroids." A truly versatile, verbal, and flamboyant person, Djerassi has also published a scientific autobiography "*Steroids Made It Possible"* in addition to the above-cited literary works.

The drama "Oxygen," which he coauthored with Roald Hoffmann (NL 1981), is based on the theme of a retro Nobel award with an emphasis on what is discovery, and its importance to be first. The recreation of three discoverers of oxygen and the conversation of their wives in a Stockholm sauna revealed the controversy, priority, and hype in such awards when more than one contender could claim the same. Djerassi, who lives in San Francisco, London, and Vienna, is thus the possessor of two outstanding parallel careers, in science as well as in the literature, and leads a very eventful life.

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A.11 Albert Eschenmoser (1925–)

An outstanding creative Organic chemist with original thinking and insightfulness, searching reasons for Nature's selection of RNA, DNA rather than other related molecules as repository of genetic information

Albert Eschenmoser was born on August 5, 1925, in Estfeld, Switzerland. He attended gymnasium and met an extremely popular teacher who made him interested in chemistry. He entered ETH Swiss Federal Institute of Technology and did his PhD working with Professor L. Ruzicka (NL 1939), the acknowledged master of terpene chemistry. Eschenmoser coauthored with Ruzicka and Heusser the most vital paper on isoprene rule and terpene biosynthesis. This concept remains as the cornerstone of terpene biosynthesis [1]. During his PhD days, Ruzicka quite often traveled outside the country for collecting Dutch paintings of which he was a great admirer. Absence of supervisor allowed Eschenmoser to grow academically of his own and he himself used to design experiments and implement his ideas. His results pleased Ruzicka. After his PhD in 1951, he stayed with Ruzicka and was allowed to start his own research group with students. He became "privatdozent" a post equivalent to Assistant Professor. He then got an offer of Professorship from the USA. Though ETH thought him too young to become a Professor, they did not want to lose such a brilliant researcher and outstanding teacher and made him Professor, and Eschenmoser stayed at ETH. The biomimetic polyene cyclization to cyclic hydrocarbons is now known as Stork–Eschenmoser polyene cyclization. This concept suggests that the polyene reacts in defined conformations (preformed chair/boat) and the antiperiplanar addition to double bonds accounts for the relative stereochemistry of the product. This inspired Professor W.S. Johnson who refined it to effect steroid and terpenoid synthesis (A.14, Chap. 10). He synthesized complex natural products like colchicine in 1959 (Chap. 24). However, his work on the synthesis of Vitamin B_{12}, one of the complex

molecules known, in collaboration with R. B. Woodward and the fact that it took 12 years to complete all remain a landmark in the history of the synthesis of organic molecules and for the genesis of Woodward–Hoffmann rule.

The cause of Natural selection, namely, the fundamental molecules of life, the genetic code bearers, stirred him most in recent decades. He synthesized alternative backbone structures for DNA and RNA with base pairing different from those of Crick and Watson that bind more strongly than those in our genetic information carrier. Though it was thought by biochemists that Nature uses the best type of pairing of bases in choosing DNA and RNA, Eschenmoser's work showed that it is not correct. Had his synthetic type of DNA and RNA with stronger base pairing be present in the primordial soup even then Nature would not have picked up the alternatives—as they might not be able to replicate and thus cannot be the evolutionary competitor of the nucleic acids as we find today.

Eschenmoser's name has been associated with reactions, techniques, and compounds, e.g., Stork–Eschenmoser polyene cyclization, Eschenmoser sulfide contraction, Eschenmoser salt, etc.

Some of his numerous academic awards are Royal Society's Davy Medal; the Tetrahedron Prize for Creativity in Organic Chemistry; ACS Arthur C. Cope Award; 2002 Oparin Medal, International Society for the Study of the Origin of Life; Royal Society of Chemistry Sir Derek Barton Gold Medal; Ruzicka Prize, Swiss Federal Institute of Technology; Robert A.Welch Award, Houston; 2008 Benjamin Franklin Medal in Chemistry; and Honoris Causa from several universities.

He retired in 1992 and remains as Professor Emeritus. He is also a Professor at Skaggs Institute for Chemical Biology, San Diego, since 1996.

Regarding his remarkable sense of humor we are tempted to narrate an incident told by a lady Professor of Education, Chicago University, and a native of Zurich. In a cafeteria in Zurich Prelog, Arigoni and Eschenmoser were having some discussions and they were in a very jovial mood. Then the question arose who was the best amongst the three. By that time Prelog got the Nobel Prize so it was decided that Prelog was the best. But Arigoni said "no I am the best because God told me that." Eschenmoser then asked Arigoni, "Did I tell you that"?

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A.12 Emil Hermann Fischer (1852–1919) (NL 1902)

Unparalleled contributor in the history of organic chemistry and responsible for the stereochemical foundation of biochemistry and enzyme chemistry

Emil Hermann Fischer who led a very eventful life chemically was born to a merchant family on October 9, 1852, in Euskirchen, near Bonn, Germany.

After finishing school in Bonn as head of the class he joined the family business for a while but left it to enter the University of Bonn to study under Friedrich Kekulé in 1871. However, he moved to Strasbourg next year and got his PhD degree in 1874 working on phthalein dyes under Adolph von Baeyer (NL 1905). He passed his "habilitation" (1878), served as an unpaid lecturer for a year (1878) and as a paid Assistant Professor (1879) at Strasbourg, and became a full Professor at the Universities of Erlangen (1882), Wurzburg (1885), and finally he succeeded von Hofmann as Professor of Chemistry in the Chemical Institute of the University of Berlin in 1892. During his tenure, his laboratory at Berlin was one of the topmost laboratories of the world, and he attracted brilliant students from different countries. Before he moved to Berlin, in 1888 he got married to Agnes Gerlach, daughter of J. von Gerlach, Professor of Anatomy, University of Erlangen. Sadly, she died young in 1895. They had three sons: Two sons were killed in World War I. The eldest son, Hermann Otto Laurenz Fischer, became an outstanding chemist and the Professor of Chemistry at the University of California, Berkeley, and died in 1960.

Emil Fischer published his landmark work on the hydrolysis of α- and β-glucosides. His deep insight allowed him to postulate in 1899 the *lock and key hypothesis* to explain the stereospecificity of enzymes—a hypothesis which is still valid after more than a century. His work on sugars unveiled some important concepts and features of organic chemistry like representing the absolute configuration of three-dimensional chiral centers by Fischer projection formula on a plane following a specified convention (Chap. 2). The Fisher projection formulae [1, 2], used by him to express the absolute configuration of carbohydrates very conveniently, have been adhered to ever since.

Using van't Hoff's theory he showed that there are 16 possible isomers [2] (see Sect. 2.76, Fig. 2.55) of aldohexoses, all of which were represented by his projection formulae. This extensive as well as valuable work made the name Emil Fischer synonymous with sugar chemistry. His idea of writing three-dimensional formulas on a plane was published in 1891.

In 1875 he discovered phenylhydrazine, an indispensable reagent for sugars and for aldehydes and ketones in general. By the formation of a common osazone he established the structural relationship between glucose, fructose, and mannose. During 1891–1894 he established the stereoconfiguration of all the known sugars. He succeeded in synthesizing glucose, fructose, and mannose starting from glycerol. His monumental work on sugar chemistry done during 1884–1894 is considered to be the pioneering contributions, which paved the way for researches on biochemistry and biomolecules. He synthesized indole in 1886 and uric acid in 1897. During 1881–1914 he intensively worked on purines named by him along with other works. Purines later were shown to be the constituents of nucleic acid—one of the master molecules of life. The theme of his work was always the application of organic chemistry to biochemistry and living organisms. His other works on tannins, peptides, and proteins are also brilliant.

In recognition of his outstanding chemical research on sugar and purine syntheses, Fischer was awarded the Nobel Prize in Chemistry in 1902 and the Davy Medal of the Royal Society. He was made a foreign member of the Royal Society in 1899. He received the Nobel Prize 3 years before his teacher Adolph von Baeyer (NL 1905). He held honorary doctorates of the Universities of Cambridge, Manchester, Brussels, and Chistiania He was among the first to devise safety measures and exhaust systems.

Emil Fischer suffered from mercury poisoning while working in inorganic chemistry, and from severe phenylhydrazine poisoning while working with sugars, and contacted cancer. This physical agony along with the deep pain of losing two sons in World War I led him to commit suicide on July 18, 1919. Thus, a great life came to a pathetic end.

"Emil Fischer is one of the greatest natural product chemists of all time" said Prelog (NL 1975). In his honor, Emil Fischer Memorial Medal is awarded every alternate year by the Gesellschaft Deutscher Chemikar (GD Ch, the German Chemical Society).

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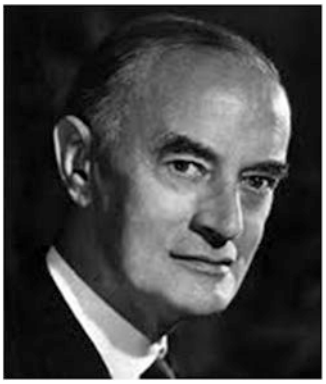
A.13 Sir Christopher Kelk Ingold (C.K. Ingold) (1893–1970)

Father of the mechanistic organic chemistry, one of the pioneers of R/S nomenclature of chiral molecules, an outstanding teacher with deep interest in students, a great lover of music and a humble lovable person



Christopher K. Ingold was never a direct participant in the work of natural products chemistry, but the inclusion of his brief biographical sketch is not out of place here. Nature uses organic molecules as building blocks for natural products' frame work, i.e., the C–C, C–N, C–S, C–O, etc., bond formations and substitutions at the periphery of the frame work—explainable in terms of mechanistic approach as applied to organic chemistry, and C.K. Ingold is regarded as the pioneering man in the mechanistic approach to organic reactions. In connection with the classification of enzyme-catalyzed reactions in vivo, Ronald Kluger [1] mentioned that "... it is useful if the reactions can be systematically divided into mechanistic types such as the Ingold formulation (Ingold 1953)" [2]. Further, the descriptors of the chiral centers, chiral axes, and chiral planes in terms of *R* and *S* were the outcome of his collaboration with Prelog and Cahn, now known as Cahn–Ingold–Prelog (CIP) nomenclature [3, 4]—which is absolutely necessary to describe natural or synthetic chiral molecules. The chirality of a drug molecule dictates its biological properties in the living organisms which quite often differ in case of its enantiomer (Chap. 33). Thus, the absolute configuration of the biologically active chiral drug can be specified in terms of (*R, S*)-nomenclature (Sects. 2.6.2, 2.16, 2.17.2).

Christopher was born to fairly well-off middle-class parents—William Kelk Ingold, a silk dealer, and Harriet Walker Newcomb—on October 28, 1893, in Forest Gate, East London. The year 1893 is politically and scientifically remarkable—Labor Party was born, and Davy Medal was awarded to van't Hoff and Le Bel in this year for their independent proposal of enantiomerism in 1874.

In his school years Christopher was fascinated by physics and mathematics, but when he came to Hartley University College at Southampton, he was much influenced by the lectures of his chemistry teacher Professor D.B. Boyd. In Boyd's lectures he found the art of judgment, reasoning, scientific appraisal, and

the significance of scientific insight and outlook, and he discovered chemistry as the subject of his choice. He went to Imperial College (London), then spent nearly 2 years at Cassel Cyanide Company, and moved to Leeds in 1924. He spent six academically very fruitful years at Leeds. The idea of electronic theory of orientation in electrophilic aromatic substitution, the mesomeric effect of delocalized electrons in alternative valency structures, the stability of aromatic radicals, theories of synchronous substitution (S_N2), a prior dissociation (S_N1), and a bimolecular elimination (E2), etc., were all conceptualized while he was at Leeds. He married his collaborator, Edith Hilda Usherwood in 1924 who remained very much a part of Christopher in all his achievements. She had been a remarkably brilliant wife. His work on the electrophilic aliphatic substitution spreads light over the traditional boundaries of organic and inorganic chemistry and occupies a central role in organometallic chemistry, "The philosophy of chemistry as unified science in a department dedicated to that principle is given in Ingold's words: "Today, and for the future, if one is an organic chemist every metal is of potential interest, and if one is an inorganic chemist so is every type of organic structure" [5]. The tremendous development of organometallic chemistry during the last few decades of the twentieth century bears testimony to the validity of this statement.

The progress of his career was rapid. Many awards and decorations were showered on him. He was elected FRS in 1924 at a very young age of 31. He was awarded the first Meldola Medal (instituted for the outstanding researcher of the year) in 1922 and also in the consecutive year 1923—a very rare distinction. He was knighted in 1958. He received Faraday Medal (1962) and many other distinctions from within and outside the country. He was the President of the Chemical Society (London) in 1952 and 1953. He was recognized as "unquestionably one of the founders of mechanistic organic chemistry and was selected by the Sunday Times of London in 1969 as one of the one thousand "*Makers of the Twentieth Century*."

He was a great teacher and lecturer. He traveled extensively and delivered lectures. Barton wrote in his memoir ". . .Sir Christopher's lectures were impeccable. It was as if he was preaching the gospel. The introduction was followed by the logical presentation of theory, and results blended into a harmonious whole, neatly summed up in the conclusion. One felt that one understood perfectly and that no better, or more qualified theory could follow" [6].

He was a competent piano player and kept this interest alive throughout his life. He loved to play cricket in his young days and while at Leeds. He was very fond of adventurous journeys. In a hand-written letter dated 10 July, 1965, to the present authors (SKT and BT) Professor Ingold wrote, "I am taking August for a holiday. While I am still not too old to drive a car, I plan to drive South through France and Spain, and (with the aid of a ferry) into Morocco, over the Atlas Mountains, and into the beginning of Sahara. Ever since I slept out in the California desert in 1932, I have told myself that I would sleep in the Sahara, but up to now had never been there. It is a theoretical plan, to the extent that if I cannot stand the long hours of driving, or the desert heat, I can always turn back at any point." He completed the trip in August 1965 [7]. His spirit of adventure was so high that being invited he

tried water-skiing for the first time at the age of 72, on the Rideau River at the foot of his son Keith's garden [8].

Ingold was kind, charming, humble, and immeasurably helping. He wrote in a letter to the Provost of the University of London after retirement "I love this college, and will do a chore of any kind at any time for it as long as I stay vigorous" [9]. One of his collaborators wrote of him, "There are few people in the future who will be capable of rivalling his knowledge of the subject, …. his ability for achieving the integration of chemistry with physics and mathematics in the way that he was able to do, . . .Sir Christopher will forever remain immortal in the eyes of chemists in general and those at the University College in particular" [9]. The new chemistry building named *Christopher Ingold Laboratories* was inaugurated by Lady Ingold on 28th September, 1970. Christopher died on December 8, 1970, in full domestic and foreign affluence of fame.

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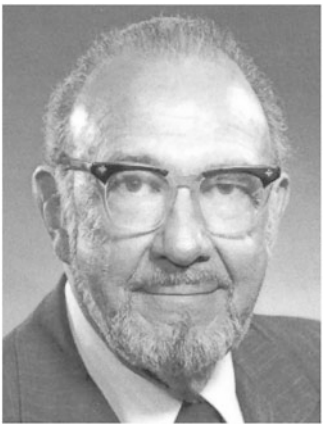
A.14 William Summer Johnson (W. S. Johnson) (1913–1995)

Highly respected for his creative, insightful and thorough research, a confluence of command of the material, passionate enthusiasm and deep interest in students—an outstanding teacher, a great lover of music and a humble lovable person



On February 24, 1913, W.S. Johnson, the second child, was born to Roy Wilder Johnson and Josephine Summer in New Rochelle, New York. He studied at the Governor Dummer Academy, Massachusetts, of which his father was an alumnus. He did his undergraduate studies in Amherst College with special focus on organic chemistry (elected to Phi Beta Kappa in his Junior year and to graduate magna cum laude) and doctorate at Harvard University with Professor Louis Fieser in late 1939 and developed a lifelong fascination for steroids and related polycyclic systems. He briefly worked as a postdoctoral assistant at Harvard with Professor R. P. Linstead. Since 1940 his independent *academic career started at the University of Wisconsin*. By the end of the year he married Barbara Allen whom he met at Cambridge, and the happy partnership lasted for 55 years until Johnson's death in 1995. They valued friendship and personal relationship very much and used to entertain friends, guests, and students in their house. Bill Johnson rose through the academic ranks and became full professor in 1944 and Homer Adkms Professor of Chemistry in 1950 in Wisconsin. He was already known as a great chemist and his administrative acumen was recognized. Ireland wrote [1] "'Wisconsin era' was devoted to a classical approach to the total synthesis of the steroid skeleton and resulted in the development of the benzylidene blocking group for the angular methylation of α-decalone type molecules, the use of Stobbe reaction for the synthesis of aromatic steroids equilenin and estrone, and the 'hydrochrysene approach' to the total synthesis of non-aromatic steroids." He published remarkable papers in this area. In 1960 Johnson moved to Stanford as the Executive Head of the Department of Chemistry. Following 9 years he not only did vigorous remarkable research but developed and expanded the Department in such a way that Stanford

Chemistry Department became one of the top-ranked chemistry schools in the world. Within 4 years stars like Carl Djerassi, E. E.van Tamelen, John Brauman, Paul Flory, Henry Toube, and Harden McConnell were included in the Faculty [1, 2]. He retired from the Executive Head's position in 1969 and was appointed Jackson-Wood Professor of Chemistry.

Johnson, from the very start of his research career, was focused on problems of regio- and stereochemistry. The huddle of regiochemistry of angular methylation was solved by him by introducing a temporary controlling group [2]. Stimulated by Stork–Eschenmoser cation–olefin cyclization of squalene to polynuclear hydrocarbon Johnson developed and refined the polyene approach for steroid synthesis. This beautiful piece of work is a reflection of the genius and mechanistic insight of Johnson. He used carbocation-based chemistry as a powerful tool for the construction of polynuclear hydrocarbons. He used fluorine atom which in spite of its high electronegativity is very effective in stabilizing adjacent carbocation. A total synthesis of dl β-amyrin is reported [3] (see Sect. 10.3) utilizing as the key step a cyclization of a polyolefin having a fluorine atom as the cation stabilizing (C-S) auxiliary. The entire field of controlled synthesis based on cation–olefin cyclization is one of the finest contributions in the area of biomimetic synthesis.

On the other side of his life we find him as a great lover of music and a competent saxophone player. He could even arrange to pay his way for a round-trip to Europe on a transatlantic liner by playing in one of the ship orchestras [2]. The abiding love of this instrument since his boyhood came to the surface when he spent a lot of time in Paris, while attending a conference, in search of a rare highly priced saxophone.

His command of the subject, enthusiastic passion, and deep interest in students all contributed to his outstanding teaching. His titles of the course work were very inspiring, e.g., "the light came on with conformational analysis" [4]. Most of his students have become leaders in their own field.

Awards and recognitions were lavishly showered on him. To mention only a few: he was elected to the Board of Editors, Organic Synthesis (1948), Fellow of the National Academy of Sciences (1954), ACS award for creative work in Synthetic Organic Chemistry, the National Medal of Science, and many many more.

His graduate student Robert E. Ireland wrote ". . .as a Johnson graduate student, I am allowed a personal note of farewell to a mentor, a colleague and most of all, a close and dearly loved friend. Were there a way to have it otherwise we would never have had to part" [1].

Johnson's colleagues started annual highly successful Johnson Symposium at Stanford since 1986. This gesture touched Johnson highly—a man the title of whose autobiographical memoir reads "A fifty year Love Affair with Chemistry" [1, 2].

The tenth Johnson Symposium, 1995, at Stanford, in which one of the authors (SKT) was present, turned out to be his memorial symposium as he died a few weeks before the symposium. Several Nobel Laureates and top stalwart organic chemists used to deliver lectures in each Johnson Symposium.

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A.15 Paul Karrer (1889–1971) [NL 1937]

—A major contributor in the chemistry and synthesis of vitamins, carotenoids, and many natural pigments and the first to establish the structure of a vitamin/provitamin

Paul Karrer, the son of a Swiss dentist, was born on 21 April, 1889, in Moscow and returned in 1892 with his parents (Paul Karrer and Julie Lerch) to the Swiss countryside when he was only three. He received his early education at Wildegg and at the grammar school in Lenzburg, Aarace, and matriculated in 1908. The same year he started studying chemistry at the University of Zurich under Professor A. Werner (NL 1913) and received his doctorate in 1911 in Inorganic Chemistry. After spending a year at the Chemical Institute at Zurich he moved in 1912 to Frankfurt-am-Main to work under a brilliant medical researcher Paul Ehrlich (NL 1908 Physiology/Medicine) on organoarsenic compounds for their use in chemotherapy at the Georg Speyer-Haus, a research institute. He worked there

for 6 years and then returned to Zurich and joined the university as the Reader where he subsequently became the Professor and succeeded Werner as the Director. He headed the Department until he retired in 1959.

He established the structures of vitamin A_1, vitamin B_2 (riboflavin) (1935), and vitamin E (tocopherol) (1935) and synthesized these molecules. He also confirmed the structure of vitamin C (ascorbic acid) as proposed by Albert von Szent-Györgyi (NL 1937 Physiology/Medicine). His masterly research on vitamins offered a better understanding of vitamin metabolism.

In 1926, he started working on natural pigments like carotenoids containing many double bonds. This also includes lycopene from tomatoes. He worked extensively on the coloring matters of various substances including lobster shells and human skin. Karrer showed the existence of several isomers of carotenes and proved that vitamin A_1 is equivalent to half of a molecule of its precursor β-carotene. The deficiency of vitamin A_1 causes night blindness.

Karrer did extensive research on flavones. For his time, the synthesis of these and flavones was remarkable. He published a textbook on organic chemistry entitled "Lehrbuch der Organischen Chemie," which was translated in different languages, passed through several editions, and remained a standard textbook of organic chemistry for many years. He published nearly 1,000 papers.

For his remarkable work on vitamins and carotenoids, he was awarded Nobel Prize in 1937 which he shared with a British Chemist Norman Haworth who synthesized ascorbic acid and contributed greatly on carbohydrate chemistry.

Karrer was the President of the 14th International Congress of Pure and Applied Chemistry in 1955 where he gave his visionary comments on the potential and application of chromatography (Chap. 31). He received honoris causa from several European and American universities and various prestigious prizes like Cannizzaro Prize, Marcel Benoist Prize, etc. He was made Honorary fellow of a number of Academies and chemical societies of different countries.

He was married to Helena Froelich in 1914 and they had two sons.

On 18th June, 1971, Professor Paul Karrer died in Zürich.

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A.16 Feodor Lynen (1911–1979) [NL 1961]

Discoverer of acetyl coenzyme A, isopentenyl pyrophosphate, cholesterol biosynthetic pathway, and fatty acid metabolism

Feodor Felix Konrad Lynen was born on April 6, 1911, in Munich, Germany. His father, Wilheim, was the Professor of Engineering at the Technische Hochschule and mother Frieda came from a manufacturer family. Feodor had his primary and secondary schooling in Munich. He matriculated in 1930 from the Chemistry Department of Munich University. He received scientific training from outstanding chemists like Heirich Wieland, Otto Hönigschmidt, Kasimir Fajans, and others when he studied at the University of Munich and received doctorate working under Heinrich Otto Wieland (NL 1927) (A.27) in 1937. On May 14, 1937, he married Eva, the daughter of his teacher, Wieland. They had five children including a pair of twins. He was in the faculty of the University of Munich from 1942 to 1953 and served as Lecturer, Assistant Professor, and full Professor of Biochemistry. From 1954 to 1972 he was the Director of the Max Planck Institute for Biochemistry where he had outstanding opportunities for scientific research.

Lynen and Konrad Bloch (USA) studied independently the complete and complicated mechanism of the cholesterol formation. Bloch suggested simple acetate, a two-carbon unit, as the basic unit for cholesterol formation. Fritz Albert Lipmann (NL 1953), the discoverer of coenzyme A, suggested it to be a carrier of acetate unit. In 1951 Lynen isolated active acetate which is nothing but acetyl coenzyme A. The existence of γ,γ-dimethylallyl pyrophosphate, an intermediate in the bioconversion of mevalonic acid to cholesterol, was also first recognized by Lynen. He showed the conversion of 5-phosphomevalonate to farnesyl pyrophosphate and then by reincubation of farnesyl pyrophosphate in the presence of NADPH to squalene. He discovered the long-sought biological isoprene unit, the isopentenyl pyrophosphate (IPP), which is capable of getting converted into squalene and then to cholesterol. This supported the conjecture of Ruzicka's isoprene rule.

Lynen and Bloch corresponded with each other and deciphered the biosynthesis of squalene, terpenoids, and cholesterol. They shared the 1964 Nobel Prize. Lynen gave his Nobel Lecture titled *The Pathway from Activated Acetic Acid to the Terpenes and Fatty Acids* on 11 December, 1964.

The repeated condensation of acetate unit via acetyl coenzyme A is responsible for the biogenesis of a large number of natural products. Lynen also studied extensively the fatty acid metabolism; various activated carboxylic acids, acetic acid degradation in yeasts, degradation of tartaric acid, formation of acetoacetic acid, and biosynthesis of cysteine, terpenes, rubber, etc.

Lynen received a number of national and international awards, medals, and prestigious lectureships: the Neuberg Medal (1954) of the American Society of European Chemists and Pharmacists, the Liebig Commemorative Medal (1955) of the Gesellschaft Deutscher Chemiker, Carus Medal of the Deutsche Akademic der Naturforscher, and Olto Warburg Medal of the Gesellschaft für Physiologische Chemie in 1963.

He was made the member/fellow of many national and international academies. He was an honorary member of the Harvey Society in New York and of the American Society of Biological Chemists in Washington, a foreign member of the National Academy of Sciences of USA in Washington and the American Academy of Arts and Science in Boston. On January 1, 1972, he became the President of the Gesellschaft Deutscher Chemiker (GDCh). He received an honorary doctorate from the Faculty of Medicine of the University of Freiburg.

Lynen died on August 6, 1979. Alexander von Humboldt Foundation has instituted a fellowship named in his honor.

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A.17 Koji Nakanishi (1925–)

"... a pioneer and a towering figure in natural products research, a major contributor at the crossroads of bioorganic chemistry." [1], a man with perpetual young heart, and an amateur magician with unbelievable skill

Koji Nakanishi was born in Hong Kong on May 11, 1925. Because of his father's posting abroad for business Nakanishi was raised in Lyon (France), London, and Alexandria (Egypt) before the family came to Osaka (Japan) giving birth to and nurturing his unique world vision. Nakanishi is truly a man of the world since his young days, a statement supported by his subsequent extensive academic involvement in different scientific institutions, committees, meetings, etc., located at different places of the world.

He graduated in Chemistry from Nagoya University in 1947 followed by two profitable postgraduate years (1950–1952) in the laboratory of Louis Fieser at Harvard. He came back to Nagoya to complete his PhD degree (1954) under Professors Fujio Egami and Yoshimasa Hirata. He served for 14 years in the Faculty of three universities in Japan [Nagoya (1955–1958), Tokyo Kyoiku (1958–1963), and Tohoku (1963–1969)] and finally came to USA to join the Faculty of Columbia University in 1969 where he (subsequently) became the Centennial Professor (and the Chairman of the Department) in 1980.

His research interest includes isolation and structural elucidation of bioactive natural compounds of plant as well as animal origin, retinal proteins, the macular degeneration, the mechanism of vision, interactions between ligands and neuroreceptors, and development of various spectroscopic methods especially circular dichroism and exciton chirality. He made significant contribution to the chemical understanding of chirality. In late 60s with his graduate student Nobuyuki Harada he developed the exciton-coupled circular dichroic method for the determination of absolute configuration of complex organic molecules. The method turned out

to be a versatile one. He authored and coauthored papers (*~800*), books including his autobiography "A Wandering Natural Products Chemist," from the *Am. Chem. Soc*. He served as the Editor-in-Chief along with D. H. R. Barton in *Comprehensive Natural Products Chemistry* (9 volumes), published by Elsevier. His book on IR spectroscopy [2] is one of the first very few books on the application of spectroscopy on the structural elucidation with adequate discussions. Carl Djerassi wrote in the Foreword [2] (2nd edition), "The large collection of problems with associated solutions in the appendix greatly added to the 'self teaching' utility of the book." He further wrote, "present day organic chemists, especially those who entered graduate school during the past 10 years, have a tendency to use NMR and mass spectrometry almost indiscriminately without realizing that much useful and sometimes unique information can be gathered from older established methods, such as infrared spectroscopy" [2].

Nakanishi's work on diterpenoids, ginkgolides isolated from ancient ginkgo tree (*Ginkgo biloba*) generally used in temple construction, is remarkable. The compounds are highly complex in structural framework and are polyoxygenated. He was the first scientist to use Nuclear Overhauser Effect (NOE) in the structure elucidation of ginkgolides and discovered the first molting hormones from plants. Recently, it has been shown by Arigoni that ginkolides are biosynthesized in the plant tissues by the non-mevalonate pathway.

Nakanishi is the recipient of innumerable awards (medals, fellowships, professorships, Honoris Causa, Directorships) from all over the world in recognition of his outstanding contributions. In 1999 he was awarded one of the highest honors of Japan—"*Person of Cultural Merit*." He also received the prestigious *King Faisal International Award in Science* from Saudi Arabia. In 1973 Professor Nakanishi was elected a Member of the American Academy of Arts and Sciences.

He is a founder member and one of the directors of research at the International Center of Insect Physiology and Ecology in Kenya and the first director of Suntory Institute for Bioorganic Research, Osaka. He is also involved in setting up a center of excellence, Institute of Medicinal and Ecological Chemistry in the Amazons, Brazil with Sao Paolo as the headquarters.

Nakanishi's students and postdoctoral fellows are holding responsible positions all over the globe, of which ~ 200 hold academic positions at universities. "He is a visionary, and in essence the investigatory seeds planted by Koji are now in full bloom in research gardens headed by those he taught" [3].

Most of his invited lectures are concluded with a spectacular treat—his fantastic magic show. A small write-up came out in the "Columbia University News," regarding Nakanishi's magic. The News started with the following, "The wiry magician with wispy white hair stood before a conference of Polish Chemists and tore an issue of Pravda in half, to audible gasps in those days before Gdansk, only to produce it whole again a moment later. He has reversed the stripes on handkerchiefs and join pieces of rope for, among others, the Crown Prince of Japan, the Lord Mayor of Sheffield and cardinals at the Vatican" [4]. Fortunately, the present authors (SKT/BT) had the opportunity to see the same trick in the Mohendra Lal Sircar Hall, Indian Association for the Cultivation of Science, Calcutta, India, at the

end of his delivering Mohendra Lal Sircar Memorial Lecture in the 1970s. One author (SKT, when he was associated with Professor Nakanishi as a UNESCO Senior Visiting Scientist in 1982) also saw his enchanting tricks with cards at the house of Professor T. J. Katz of Columbia University after dinner, where Professors E. J. Corey, K. Nakanishi, and the author were invited. Incidentally, it may be mentioned that "Nakanishi began doing card tricks in his late teens, mainly at wedding receptions in Japan, to avoid singing duties at such ceremonies. He moved on to more complicated tricks, usually trying out first on his wife. "Her reaction, that's my barometer," Professor Nakanishi said" [4].

Nakanishi is a soft-spoken man with a happy family life. He complimented his wife Yasuko as a "strong Japanese wife over the years" in his acknowledgment of the book on IR spectroscopy [2]. They have two children Kay (Keiko) and Jun. In a tribute letter A. I. Scott wrote to Koji, "I can guess the secret of your success in chemistry and life—that you are fortunate like myself, to have such a long and happy marriage" [5, 6].

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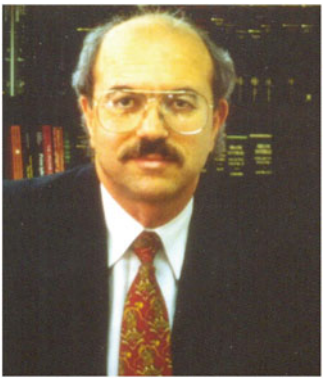
A.18 Kyriacos C. Nicolaou (K. C. Nicolaou) (1946–)

An outstanding creative Synthetic Organic Chemist who amalgamates natural products chemistry, organic synthesis and chemical biology [1] and is well-known for tackling alarmingly difficult structures.



The life of K. C. Nicolaou is an inspiring tale of blossoming of a small 7-year-old village boy from a distant Mediterranean island, who delivered bottles of milk to neighbors in the early hours of each morning [2], into one of the greatest synthetic organic chemists of all time and a prolific scientific writer. In a 55-page article [2] published in 2003 Nicolaou reflects back on his career highlighting a number of lucrative campaigns which shape the art, science, and technology of chemical synthesis and their impact on chemistry, biology, and medicine.

K. C. Nicolaou was born on July 5, 1946, in the panoramic village of Karavas on the northern coast of Cyprus, with the idyllic coast of the Mediterranean Sea on the one side and the sharply ascending pine-covered mountains on the other. The village was green with profuse lemon groves and has thereby been known as the "Lemon Capital" of Cyprus. The village offers plenty of fruits, vegetables, and fish for its inhabitants, and Kyriacos spent his childhood in the enchanting beauties of Nature, away from the material life. His present home in San Diego offers him a similar view of ocean and mountains and perhaps acts as a constant reminder of his village and childhood.

His father, who had a meager schooling up to the third grade, could only become a builder. He had great respect for architects and wanted Kyriacos to become one. Nicolaou had one younger brother and four younger sisters. The family purse was not sufficient to have an easygoing life; rather it lay on the wrong side of income. Being the eldest sibling, he had to contribute to the family's income. Thus, from the tender age of seven, rising early he had to collect the bottles filled with milk from their only cow and then ride in his secondhand bicycle (that made him the envy of his peers) to deliver them to the local wealthy people and come back before the school started. He spent his "first 13 years in this humble environment beside the Mediterranean Sea attending primary school, farming in the fields, delivering

goods, and playing in the most natural and placid surroundings" [2]. He was then sent to his uncle in Nicosia, the capital of Cyprus, to attend the "Pancyprian Gymnasium," a most prestigious and highly disciplined high school. Along with his schooling he was also baptized in cake-making for his uncle's confectionary shop. The cake-making place was his first "laboratory"; he quickly learned the art of making pastries and excelled in synthesizing confectionary products, especially the pastries and birthday cakes which were adored by customers appreciating his creativity. Perhaps the seed of synthesis was sowed in his brain at that young age. The local professionals thought that he was so creative in this profession that after completion of high school he should stay in Cyprus where he had a great future as a confectioner, practicing the art. Nicolaou, of course, turned down the offer and traveled abroad to further his studies. At Nicosia Gymnasium he was greatly influenced by his chemistry teacher. Nicolaou said, "It was at that high school I had the good fortune to encounter my first chemistry teacher Dr. Telemachos Charalambous, who immediately and decisively inspired me and instilled in me the passion for chemistry. For his guidance and enthusiasm and for showing me the way, I will be eternally grateful."

After graduation from the Gymnasium in 1964 at the age of 18 he moved to England, spent 2 years learning English, and prepared himself for admission in London University. He graduated (BSc, 1969) from Bedford College and obtained PhD degree (1972, University College of London) working with Professors F. Sondheimer and P.J. Garratt. He joined Professor T.J. Katz at Columbia University as a postdoctoral fellow (1972–1973) and then moved to Professor E.J. Corey at Harvard University (1973–1976). His association with them instilled in him a passion for natural products. In regard to his stay at Corey's laboratory, he said "The Harvard years were delightful both scientifically and socially. Corey was a marvelous teacher–mentor and I learned from his incredible genius, methodical application to science and admirable discipline." Following the postdoctoral career, in August 1976, he joined the Faculty at the University of Pennsylvania, Philadelphia, where he subsequently became Rhodes-Thompson Professor of Chemistry. In 1989, he accepted the joint appointments of Professor in the Department of Chemistry, University of California, San Diego, and Chairman and Darlene Shiley Professor, Department of Chemistry, The Scripps Research Institute. In 1996, he was offered Aline W. & L.S. Skaggs Professorship of Chemical Biology in the Skaggs Institute of Chemical Biology, San Diego. From 2004 to 2010 Nicolaou directed a research program at the A* STAR Chemical Synthesis Laboratory at Biopolis, Singapore. In 2013, he moved to Rice University in Houston, Texas, where he is the Harry C. and Olga K.Wiess Professor of Chemistry.

K.C. Nicolaou's research interest includes synthetic organic chemistry, biology, molecular design, and medicinal values of natural and synthetic products "Nicolaou is well known for tackling alarmingly difficult structures with Greek heroic spirit." He admits that he enjoys the challenge. "The most important ingredient is courage, supplemented with flashes of ingenuity and a little serendipity," he says . . . adding that the physical attraction of a molecule is important in choosing a target "like falling in love" [3]. Nicolaou's work led to the discovery and invention of novel

synthetic strategies and methodologies. “Synthetic design is an art as well as a science,” says Nicolaou [1]. “It is almost like a ballet where you move from one step to another—a cyclization, a ring-opening or a cascade reaction—to give an aesthetically pleasing sequence” [3].

Among the molecules that he has synthesized are included the endiandric acids, amphotericin B, calicheamicin $\gamma_1{}^1$, Taxol, brevetoxins A and B, vancomycin, the CP molecules, diazonamide A, azaspiracid, and thiostrepton. Taxol being a successful anticancer agent, its synthesis constituted a hotly contested race of around 30 research groups. Nicolaou was the first to report the total synthesis of taxol [4] within a period of less than 3 years. He has demonstrated how cascade of reactions could be employed in building rings from the linear precursors in a satisfyingly stereocontrolled fashion. His natural products are both of plant and marine origin. In drug design he has employed combinatorial chemistry extensively.

In recognition of his outstanding contributions in the field of organic synthetic chemistry and its applied areas he has been lavishly showered with awards and medals both domestic and international [3, 4]. He is a Fellow or Member of several prestigious academies and societies including the American Academy of Arts and Sciences, National Academy of Sciences (USA), German Academy of Sciences Leopoldina, American Philosophical Society, and Royal Society of London, UK. He holds numerous honorary doctorate (*Honoris Causa*) degrees.

The impact of his work in Chemistry, Biology, and Medicine flows from his contributions to chemical synthesis, as described in over 740 publications. His dedication to chemical education is reflected in his book series *Classics in Total Synthesis* I (1996) [5], II (2003) [6], and III (2011) [7] with his students Erik Sorensen, Scott Snyder, and J. S. Chen, respectively, and *Molecules that Changed the World* (2008) [8] with his research associate T. Montagnon and in his training of hundreds of graduate students and postdoctoral fellows.

In 2012 he published several insightful review articles [9–12] and in 2014 an illuminating article [13] on the current state of affairs in the drug discovery and development process.

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A.19 Ryoji Noyori (1938–) (NL 2001)

An outstanding contributor in the field of chiral catalytic hydrogenations which found tremendous industrial applications.

Ryoji Noyori was born as the first son of Kaneki and Suzuko Noyori on September 3, 1938, in a beautiful suburb of Kobe (now Ashiya), Japan. Soon afterwards his family moved to Kobe, a pleasant city blessed with attractive natural surroundings. At the very end of World War II in 1945, US Air Force B29s heavily bombed Kobe reducing the central city to ashes. The Noyori family took refuge for a while in a nearby countryside. Even after the war they had difficult times as the country was suffering from severe food shortage. Noyori grew up with two younger brothers and a sister. Except for a short period he attended an elementary school affiliated to Kobe University from ages 6 to

12 and then moved on to Nada Middle and High School from ages 12 to 18. He enjoyed many outdoor activities in his youth.

His father was a gifted research director of a chemical company; his profession strongly influenced the path of his life and he wanted to be a scientist ever since he was a small child. At home he was surrounded by his father's scientific journals and books and various samples of plastics and synthetic fiber products, the quality of which he was frequently asked to test. At 12 when he was in middle school, his father took him to a public conference on the miracle fiber "nylon." The speaker, the President of the Toray Company, proudly explained, "This new fiber can be synthesized from coal, air, and water and it is thinner than a spider's thread, yet stronger than a steel wire." Even at that tender age he was deeply impressed by the power of chemistry which could create important things from almost nothing! The first Japanese to receive Nobel Prize was Professor Hideki Yukawa who was known to his parents. This great event of 1949 extremely delighted the 11-year-old Noyori. His childhood was still difficult. After World War II, in 1951 Japan became so poor, terribly short of food and supply. Thus, the Noyori family of six lived frugally. At this point it became his dream to be a leading chemist to contribute to the society by inventing beneficial products.

His middle/high school enthusiastic teachers that include his first chemistry teacher Dr. Kazuo Nakamoto (a product of Osaka University who later moved to USA) influenced him greatly. Together with regular school work, "judo" a traditional sport of Japan was a major passion at that time. He has always been very humble; he acknowledges with reverence the influence of his classmates and teachers on the formation of his personal character.

In 1957, at 18 he entered Kyoto University, being inspired by Professor Ichiro Sakurada of Kyoto University, who invented Vinylon, the first Japan-made synthetic fiber. It was the most active institution in polymer chemistry research. That year USSR launched into space the first artificial satellite, the "Sputnik." Young Ryoji, like young science students in Japan, was astounded at the power of science-based technology. Soon he started to study organic chemistry under Professor Keiiti Sisido and obtained BSc degree in 1961 and MSc degree in 1963. Immediately he was appointed an Instructor of Professor Hitosi Nozaki's laboratories. Prof Nozaki strongly encouraged him to pursue new, original chemistry. In 1966 Noyori discovered an interesting asymmetric catalysis while studying transition metal effects in carbene reactions. Styrene reacted with ethyl diazoacetate in the presence of a small amount of chiral Schiff base–Cu(II) complex to give optically active cyclopropane derivatives with <10 % *ee*. Although not synthetically useful, *this reaction is the first example of asymmetric catalysis using a well-defined organometallic complex*.

In 1967 he received his Doctorate (Deng) degree, was appointed Associate Professor of Chemistry at Nagoya University, chaired a newly created organic chemistry laboratory, soon launched his own research group, and created a new stream of organic chemistry—different from that of Professor Y Hirata, an outstanding natural products chemist in the department, who consistently helped Noyori in many respects.

His stay at Professor Corey's laboratories (1969–1970) as a postdoctoral fellow was academically extremely profitable, mostly through his personal interactions with Professor Corey, and also with K Barry Sharpless working with Professor Konrad Bloch. Synthesis of prostaglandins (PGs) was his research theme in Corey's group. After the completion of several undertakings, he was asked to selectively hydrogenate a PGF_{2a} derivative having two C=C bonds to a PGF_{1a} compound possessing a single C=C bond. His interest in this area was significantly enhanced through personal interactions with the Assistant Professor John A. Osborn at Harvard, an authority of Rh-catalyzed homogeneous hydrogenation. The fruitful Harvard experience along with his asymmetric cyclopropanation in 1966 led to his three-decade-long research on asymmetric hydrogenation *via* organometallic chemistry. In August 1972 at 33, 2 years after his return to Nagoya, Noyori was promoted to Full Professorship.

Noyori was University Professor of Nagoya University till 2003 when he retired and then became the President of RIKEN, Saitama, Japan, the position he is still holding. He is also the Director of the Research Center for Material Science, Nagoya. He served as Dean of the Graduate School of Science, Director of the Institute for Advanced Research at Nagoya University, and President of the Chemical Society of Japan (2002–2003). Since 2005 he has chaired the Science and Technology Council of the Japanese Ministry of Education, Culture, Sports, Science and Technology.

Noyori's outstanding scientific contributions:

(i) Preparation of optically pure BINAP [2,2′-bis(diphenylphosphino)-1,1′-binaphthyl] a novel $\mathbf{C_2}$-chiral disphosphine.

(ii) Asymmetric hydrogenation using as catalysts complexes of Rh and Ru, particularly those based on the BINAP ligand [*see* (iii) to (x)]. Ru behaves differently from conventional Rh.

(iii) Development of BINAP-Ru(II) decarboxylate complexes in 1986 causing a major breakthrough in *asymmetric hydrogenation of olefinic substrates*.

(iv) Asymmetric hydrogenation of an alkene in the presence of ((*S*)-BINAP)Ru $(OAc)_2$ for the commercial production of enantiomerically pure (97 % *ee*) *naproxen*, used as an anti-inflammatory drug.

(v) Development in 1987–1988 of a versatile general *asymmetric hydrogenation of functionalized ketones* with BINAP-Ru(II) dihalide complexes. The scope of this method is far-reaching—allowing for the synthesis of terpenes, alkaloids, vitamins, β-lactam antibiotics, α- and β-amino acids, prostaglandins, and other compounds of biological and physiological interest.

(vi) Large-scale production of synthetic intermediates of antibiotic *carbapenems* (Takasago International Co.) and *levofloxacin*, a quinolone antibacterial agent (Takasago International Co./Daiichi Pharmaceutical Co.) by application of BINAP chemistry. *The efficiency of BINAP chemistry rivals or in certain cases even exceeds that of enzymes*.

(vii) Discovery of catalysts of type $RuCl_2$ (diphosphine) (diamine) for the preferential *reaction of the C=O function of unsaturated ketones leaving the olefinic linkage intact*—another major breakthrough.

(viii) Asymmetric hydrogenation of a range of aromatic, heteroaromatic, and *olefinic ketones* combined use of the BINAP ligand and a chiral diamine for their conversion *to chiral secondary alcohols*—a very rapid, productive, and stereoselective reaction.

(ix) *Asymmetric isomerization of allylic amines to enamines of high enantiomeric purity* by use of BINAP-Rh(I) complexes, realizing the industrial production of (-)-menthol (Sect. 6.4) (Takasago International Co.), and some other optically active terpenes.

(x) *Asymmetric transfer hydrogenation of ketones and imines* using 2-propanol or formic acid as hydrogen donors—effected by a range of Ru(II) catalysts modified with a chiral β-amino alcohol or a 1,2-diamine derivative (invented by Noyori group during 1995–1996).

(xi) Highly enantioselective addition of dialkylazincs to aldehydes using a small quantity of a camphor-derived chiral amino alcohol where the alkylation products with high enantiomeric excesses are accessible.

(xii) Development of binaphthol-modified $LiAlH_4$ reagent (1979) used for the commercial Corey PG synthesis (Ono Pharmaceutical Co.) and realization of the long-sought three-component PG synthesis in 1985, which now plays an important role in biochemical and physiological studies of PGs.

(xiii) Discovery of the utility of supercritical CO_2 as a medium of homogeneous catalysis. Thus, Ru-catalyzed hydrogen produces formic acid, methyl formate, and dimethylformamide with extremely high turnover.

(xiv) Devising practical, environmentally sound methods for olefin epoxidation and alcohol oxidation using aqueous H_2O_2, e.g., conversion of cyclohexene to adipic acid (1996–1998).

Impact of BINAP chemistry: BINAP chemistry is now utilized worldwide in research laboratories and also at the industrial level. In fact, the selective synthesis of single enantiomers using well-designed chiral molecular catalysts has now become common practice, opening tremendous potential for chemical synthesis. Long before Green Chemistry (see Sect. 4.2.11) received proper appreciation, the Noyori group consistently focused on molecular catalysis and contributed in many ways in the progress of Green Chemistry, every step needing ~100 % stereo- and regioselectivity, ~100 % yield, no unwanted waste products, and hence atom economy with a low ecological (E) factor.

Noyori has over 400 publications in top scientific journals.

For his outstanding contribution in the field of asymmetric hydrogenation he received Nobel Prize (2001) which he shared with K. Barry Sharpless and William S. Knowles. He has been showered by almost all prestigious awards from within and outside the country. A few of them are as follows: The Chemical Society of Japan Award for Young Scientists, **1972**; The Chemical Society of Japan Award, **1985**; The Centenary Medal (The Royal Society of Chemistry, UK), **1990**;

Tetrahedron Prize for Creativity in Organic Chemistry, UK, **1992;** The Japan Academy Prize, **1995;** The Arthur C. Cope Award (American Chemical Society), **1997**; Person of Cultural Merit (Japanese Govt.), **1998**; and The Wolf Prize in Chemistry (Wolf Foundation, Israel), 2001 (incidentally it may be mentioned that his paper on asymmetric cyclopropanation rejected by *J. Am. Chem. Soc*. was published in *Tetrahedron Lett*. This work was partially responsible for receiving the Wolf Prize which he shared with Kagan and Sharpless) and for the Roger Adams Award in Organic Chemistry (American Chemical Society), **2001**.

Noyori has been a great believer in the importance of fundamental research and industry, and his life is an expression of that belief. Noyori believes strongly in the power of catalysis and of "green chemistry." In a recent article[4] he argues for the pursuit of "practical elegance in synthesis" and further states that "our ability to devise straightforward and practical chemical synthesis is indispensable to the survival of our species."

In 1972 Noyori married Hiroko Oshima, daughter of a Professor of Medicine at Tokyo University, who was studying the immunology of cancer at a research institute in Tokyo. According to Noyori she has played the most important part in their private life at Nagoya. They have two sons. The elder son Eiji (born in 1973) is working in a newspaper company, and the younger one, Koji (born in 1978), studied painting at an art university in Tokyo.

The text is based on the article, "The Nobel Prize in Chemistry 2001—Autobiography" available in the Google website.

A.20 Louis Pasteur (1822–1895)

—One of the greatest scientists in the history and a house-hold name who worked to "bestow some of the greatest benefits that science has produced upon mankind"

[4] R. Noyori, Pursuing Practical Elegance in Chemical Synthesis, *Chem. Commun*., **2005** (14), 1807-1811.

A French biologist and chemist, Louis Pasteur, the versatile genius and a humble minded son of a tanner, was born on 27 December, 1882, in Döle in eastern France.

Our day starts with pasteurized milk. Pasteurization named after Pasteur, the inventor of the process, is the partial sterilization of some perishable food by heating. Rabies vaccine, a remarkable invention of Pasteur, saves the lives of so many from rabid dog bites. In 1856 Pasteur was asked to find out a way of preventing the souring of beer and wine. This souring used to cause a great loss in the wine and beer industry. He extensively studied the fermentation process and showed that there are two types of yeast, one producing alcohol and the other producing lactic acid. Fermentation occurs anaerobically, and the microorganisms present in the yeast are responsible for fermentation. He found that prior to setting the beer aside for aging heating the beer and wine would kill the residual yeast including those producing lactic acid. However, accidental discovery by Eduard Büchner (1860–1917, NL, 1907) in 1897 of the occurrence of fermentation with the juice of yeast (first cell-free enzyme extract) opened the door of modern enzyme chemistry. When commissioned by the Government to find out the cause for silkworm disease that threatened the silk industry, Pasteur found that two types of bacilli caused the disease of silkworm and advised them to separate the diseased silkworm from the healthy ones prior to their use. That was how he saved the French silk industry. Pasteur made outstanding contributions in the field of communicable diseases. He established the germ theory for diseases such as anthrax, chicken cholera, and rabies and was able to discover vaccines for the diseases. His work on rabies made him a household name and brought him international fame.

In 1848 Pasteur succeeded in separating crystals of the sodium ammonium salts of (+)- and (−)- tartaric acid from the optically inactive racemic variety found in wine caskets. When the salt was crystallized by slow evaporation of its aqueous solution, large crystals displaying enantiomorphous hemihedric facets [similar to those found in (+)- and (−)-quartz crystals by Boit in 1812 (see Appendix B, **1812**)] formed. By looking with a lens he could separate the two mirror image types of crystals by hand picking with a pair of tweezers. As expected, he found that their aqueous solutions exhibited rotations of opposite sign but of equal magnitude. He then suggested that the molecules must be dissymmetric, an original and fundamental pivotal concept which serves as the seed for the entire future harvest of stereochemistry. Chemists, biochemists, pharmacists—all will remain ever indebted to him for this original observation and conclusion.

Further, he separated (+)- and (−)-tartaric acids from their mixture (racemate), using optically active base cinchonine through the formation of diastereomeric salts, from which active forms of the acids could be regenerated—thus, he was the first person to achieve resolution of a racemate into the enantiomers. *This is indeed a great conceptual experimental rung in the ladder of stereochemistry*.

As a student he was not very good. He showed special aptitude for painting and mathematics and was thinking of becoming a Professor of Fine Arts in future. He obtained his BA (1840) and BSc (1842) degrees from the Royal College of Besancon and his PhD degree from Ecole Normale in Paris. He was inspired by lectures of Jean Baptiste André Dumas (1800–1884). He became the Professor of

Physics for a year at the University of Strasbourg, and next year he became the Professor of Chemistry there. In 1849 he married Marie Laurent, the daughter of the Rector of the University. He then moved to the University of Lille where he was made Professor of Chemistry and Dean of the Science Faculty (1854–1857). In 1857 he became the Director of the Scientific Studies at Ecole Normale and stayed there for nearly 7 years.

His great success in fighting rabies led to the foundation of Pasteur Institute in 1888 for advanced studies on rabies and vaccines. He headed the Institute since its inception to the rest of his life. The Institute was funded by public money. For his extremely valuable contributions in bacteriology and chemistry (stereochemistry) he was recognized during his lifetime. He received Bumford Medal of the Royal Society for his work on stereochemistry. He was elected a Member of the Academy of Sciences in 1862 and a Member of the French Academy in 1881.

A rare combination of unusual talent, deep intuition, high ambition, arrogance, combativeness, and nationalism made him a man who is held in great esteem all over the globe. In 1892, during the celebration of Pasteur's jubilee, Joseph Baron Lister (1827–1912) a great contemporary of Pasteur while paying his homage said "your researches threw a powerful light which has illuminated the dark places of surgery, and have changed the treatment of wounds from an uncertain and too often disastrous business into a scientific and certainly beneficial art." Lister further said not only surgery but medicine, too, would recognize for all time the debt it owed to the name of Pasteur.

The boy called Joseph Meister whom he saved from rabies was the doorkeeper of the Institute, during Nazi invasion of France. When Nazis demanded him to open the tomb of Pasteur, Joseph denied and committed suicide. Perhaps he paid the greatest offering to his savior.

Source: From website and various scientific articles and books on great men and dictionaries

A.21 Piere-Joseph Pelletier (1788–1842)

The Father of Alkaloid Chemistry

Perhaps Piere-Joseph Pelletier is one of the very few who had isolated so many natural products which went directly to human benefit. His attention was drawn to vegetable alkaloids. Of all the compounds the most important one is quinine which he along with his collaborator Jean Bienaime Caventou (1795–1877) isolated in 1820 as the major alkaloid of *Cinchona* bark. This compound has served for centuries as the savior to the people distressed with a mosquito-borne disease called malaria. Quinine is the first pure chemical compound used to combat an infectious disease. The structure of the compound later on helped to design simpler antimalarial drugs. Structurally related other congener alkaloids like cinchonine and others as well as quinine have been chemically manipulated on their chiral frame works to offer the most important natural chiral auxiliaries, phase-transfer catalysts, chiral catalysts, and chiral resolving agents (Chap. 32). The Postal Department of the French Government issued a stamp in 1970 with portraits of the discoverers and the structure of quinine on it to commemorate the 150th year of its discovery and to honor the discoverers.

Pelletier was born on March, 22, 1788, in Paris. His father, Bertrand Pelletier, was a distinguished chemist and pharmacist and a follower of Antoine Laurent Lavoisier (1743–1794). He followed the family profession and studied at the Ecole de Pharmacie and showed precocity in science. After qualifying in 1810 he earned his doctorate degree in 1812. He taught at Ecole as an Assistant Professor of Natural History and Drugs and then became a full Professor in 1825. Pelletier was awarded in 1827 Montyon Prize of 10,000 Francs by Paris Academy of Sciences. He became Assistant Director of the Institute in 1840 and was elected to the Academy of Sciences. The same year he took retirement due to illness from which he never recovered and died on July 19, 1842, in Paris.

His investigation on plant products led to the discoveries of a number of very important bioactive compounds. In 1818 he discovered strychnine and brucine and veratrine in 1819. In 1817 he discovered emetine from *Ipecacuanha* roots, ambrein

from ambergris, and crotonic acid from croton oil. He also isolated colchicine and caffeine.

His collaborative work with Caventou on alkaloids brought him international acclaim. In 1823 in collaboration with Jean-Baptiste Andre Dumas (1800–1884), he showed the presence of nitrogen in alkaloids. Pelletier developed improved methods for the isolation of strychnine from the plant nux-vomica and for the preparation of quinine sulfate which possesses considerable medicinal value. He also discovered narceine, an opium alkaloid, and claimed to isolate for the first time thebaine which he named paramorphine. From the pine resin by-product, used as the source of illuminating gas, he isolated toluene which he named retinaphthine.

Near the Luxembourg Park, Paris Courtesy: Mr. Partha Majumder (France) and Dr. Bhupesh C. Das (France)

His contribution in alkaloids and, for that matter, in natural products chemistry is incomparable, since he could first isolate the compounds in the pure state and thus allowed their uses in the pure form rather than as mixtures, as found in the crude extracts from the respective plants. The green coloring matter of the plants Pelletier and Caventau worked with was named by them as chlorophyll. Two alkaloids, one a piperidine alkaloid—a derivative of coniine, and the other one with a bicyclic system featuring a piperidine ring, were named after him as (−)-pelletierine and ψ-pelletierine (structures **18** and **19** in Fig. 4.17), respectively).

His life span was comparatively short (~54 years), but within that short span, his contribution in alkaloidal chemistry is enormous and he is, therefore, remembered as the *father of the alkaloid chemistry*.

A.22 Vladimir Prelog (1906–1998) [NL 1975]

An outstanding natural product chemist who stands as a firm pillar in the field of stereochemistry

Vladimir Prelog was born in Sarajevo, Bosnia, on July 23, 1906. At that time Sarajevo was the capital of the Austrian-Hungarian province of Bosnia-Herzegovina. He was at Zagreb, the capital of Croatia, during his early years and attended first 3 years of high school. His aunt was a very enlightened teacher who believed in truth, beauty, and goodness, and she could instill these virtues in young Vladimir. In 1918 while he was in a science-based school in Osijek, he met Ivan Kurea, a chemistry teacher. His teaching and encouragement helped him to develop love for chemistry. Prelog wrote "Among the books I read in Zagreb, I specially treasured The School of Chemistry by Wilhelm Ostwald." At the age of 18, he moved away from his family, went to Prague, and got admitted in the Chemical Engineering School of the Institute of Technology. He did his postgraduate research there. Rudolf Lukes was his mentor and friend. In this context, we may quote Prelog ". . ..my view that the best way to study science is as an apprentice to a master who is model both in his field and in his personal character" [1]. Prelog wrote "In addition to these two 'real' teachers I admired Robert Robinson, Christopher Ingold and Leopold Ruzicka, all of whom I consider as my 'imaginary' teachers" [2]. For nearly 5 years (1929–1934) he was involved in the preparation of fine chemicals in a laboratory in Prague. He then joined the technical faculty of the University of Zagreb, first as a Lecturer and then as an Associate Professor.

During German occupation of World War II in 1942, he joined Leopold Ruzicka at the Swiss Federal Institute of Technology (ETH) at Zürich which was considered the Mecca of Natural Products Chemistry. Ten years later he succeeded Leopold Ruzicka as a full Professor and retired in 1976.

He worked extensively on the alkaloids and established the structure of quinine. He corrected Robinson's formula of strychnine and also worked on heterocycles, antibiotics, steroidal alkaloids, and steroids extracted from animal organs and for sometimes collaborated with D. H. R. Barton (NL 1969). He was able to separate the chiral enantiomers of Troger's base on chiral column and showed that tricoordinated nitrogen atom could also be chiral.

He experimentally showed that some microorganisms were capable of stereospecific reductions of carbonyls present in acyclic substrates. His contribution to stereochemistry had added a new dimension to the understanding of organic chemistry. He used asymmetric synthesis as a means for mechanistic elaboration of many reactions. The absolute configuration determination by way of the formation of atrolactic acid or mandelic acid and the identification of the absolute configuration of the acid could be well related to the absolute configuration of the parent chiral alcohol. A generalization of this process in terms of the speculated lowest energy transition state model is termed Prelog's rule/generalization (Sect. 2.11.5). He also contributed significantly in the field of stereochemistry of enzyme reactions.

Prelog, in collaboration with Cahn and Ingold, formulated rules based on some well-defined conventions to specify, in terms of (*R*/*S*), the absolute configurations of the dissymmetric part/s of any chiral molecule possessing chiral center/s, chiral axis, or chiral plane. Such specification is well known as Cahn–Ingold–Prelog (CIP) nomenclature (Sects. 2.6.2, 2.16, 2.17.2). Since chirality of a molecule (natural or synthetic) dictates its biological properties in the living system which differ not only in the diastereomers but also in the enantiomer, the absolute configuration of the chiral molecules must be specified in terms of (*R*,*S*)-nomenclature.

In his memoir [1] a very interesting unusual photograph appeared in which Woodward, well dressed in suit and tie in standing position, rested his right leg on the back of Prelog and the left leg almost on Djerassi, both dressed in swimming trunk and lying ventrally in shallow beach water of Baltic Sea. The photograph was taken by D. E. Koshland Jr. during 1970 IUPAC Symposium on Natural Products Chemistry held at Riga (then in USSR) and the caption reads "we are solid fundaments of his glory," commented Prelog, and "According to Djerassi the chemists were staging the supposed superiority of Harvard over ETH and Stanford." To the present authors it appears that in every man's heart independent of his achievement there resides a child, who sometimes peeps out of the shield.

Prelog received a wide range of awards by way of prestigious lectureships, medals, fellowships, etc. He shared the 1975 Nobel Prize in Chemistry with J. W. Cornforth for his outstanding contributions in stereochemistry and natural products chemistry.

Barton wrote in his memoir [3] about Prelog, "His charm and wit are legendary, as demonstrated by his mirror image signature on the declaration (Buergenstock Declaration) as witness."

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A.23 Robert Robinson (1886–1975) (NL 1947)

A versatile Natural Products Chemist with an insight in Organic and Biochemistry, a pioneer in electronic mechanism in organic chemistry, biomimetic synthesis and multicomponent reaction (MCR) strategies for syntheses

Robinson was born to a local manufacturer family on 13 September, 1886, in Bufford near Chesterfield, Derbyshire, England. He did not intend to enter his father's business and took a glorious academic ride and finally landed in Oxford (1930–1955). He then became the consultant and Director of Shell Company, London.

His lifelong interest resides in natural products though he worked in other important fields too. His major interest was in alkaloids, steroids, and plant pigments. He worked on their structural elucidation and synthesis. He synthesized cholesterol in collaboration with J.W. Cornforth and stilboestrol, a highly active estrogen, with E.C. Dodds. Although the number of synthetic oestrogens was quite large, none of them surpasses stilboestrol in practice [1] He spent quite a number of years on the structure of the alkaloid strychnine, revised the structure of morphine, and arrived at the correct formulation for brazilin. In the absence of spectroscopic support the arrival at the correct structure of morphine is considered to be an outstanding feat. He first gave the idea of biomimetic synthesis when he

synthesized tropinone in the laboratory in the biogenetic way in which alkaloids and other natural compounds are generated in plant cells and thus laid the foundation of biogenesis and biosynthesis [2]. This synthesis of tropinone serves as an early example of multicomponent reaction (MCR) strategies for synthesis of natural products [3]. His ring annulation reaction, known as Robinson's annulation using vinylethyl ketone (or its precursor—the corresponding Mannich's Base) on a ketomethylene group, serves as a very good method for ring formation. He contributed to the electronic mechanism of organic chemistry and introduced the curved arrow as the direction of flow of electrons in organic reactions and their influence in aromatic substitution. He did the structure of penicillin and his methods are followed for determining the structures of many other antibiotics.

Robinson was sometimes irascible in his behavior, but he also liked pleasing people. Birch wrote in his memoir [4], "I was in his office when the secretary rushed in and said, 'Dr. X (a Canadian collaborator from some 25 years before) is here and would like to see you.' Sir Robert responded 'Quick, get me the reprints for 19XX, When the lady was ushered in he said "How nice to see you again. Was it not remarkable that the anthocyanin we worked on turned out to be a mannoside?' She went away happy, thinking that her remarkable result had been in his mind all that time. Robert chuckled happily over his little piece of gamesmanship."

In his later years of research he became interested in the origin of petroleum and its composition.

Robinson in an interview [1] opined that "Organic chemistry is a key science because it is right in the middle of the sciences; it has contacts with physical chemistry on one hand, and through that with physics and mathematics, and contact with biochemistry on the other. Biochemistry is really organic chemistry affecting physiology, pharmacology and all medicine, and also the normal academic subjects of botany and zoology—in fact there is hardly any science which hasn't got some contact with organic chemistry. In that respect I think organic chemistry is rather unique." Robinson believed in a closer relationship between organic chemistry and physiology and argued for the same.

He has been flooded with awards, medals, and lectureships. He was the President of the Royal Society (1945–1950). He was knighted in the year 1939. He received the Nobel Prize in 1947. Coplay and Davy Medals were awarded to him and he was made foreign associate of the US National Academy in 1934.

He founded two important Organic Chemistry leading journals, *Tetrahedron* and *Tetrahedron Letters*, in 1957.

Robinson was a powerful chess player. He loved mountaineering, traveling, fast driving, and music.

He died on 8 February, 1975, at 88+ years.

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A.24 Leopold Stephen Ruzicka (1887–1976) (NL 1939)

A highly acknowledged master of terpene chemistry, and propounder of 'isoprene rule' for the biogenesis of terpenoids, steroids and carotenoids.

Leopold (Lavoslav) Stephen Ruzicka (pronounced as Rougistchka (French transcription), affectionately called "Poldi," was born to Stjepan Ruzicka and Ljubica (Amaliza) Sever on September 13, 1887, in a Eastern Croatian (a part of Austria—Hungary at that time) small town, Vukovar (Vukova) on the river Danube. His father was a cooper. His ancestors were artisans (who used to make and repair casks, barrels, etc.) or farmers who had enjoyed at most a few years of schooling. After the early death of his father in 1891, Ruzicka returned to his mother's birthplace, Osijek, on the Drava, somewhat west of its junction with the Danube. There he attended the primary school and the classical gymnasium where the Croatian language was used. He was a good student and was interested in physics and mathematics. Other descriptive subjects did not interest him. There was no chemistry in the curriculum, but he decided to study chemistry out of his interest in the composition of natural products.

Initially he was determined to enter the Catholic priesthood. As a teenager he changed his mind and wanted to study chemistry and looked for schools in Germany and Switzerland. He studied chemistry and obtained his doctorate in only 4 years under the direction of Herman Staudinger at the Technische Hochschule at Karlsruhe (Germany) in 1910. Next 5 years he continued to work with Staudinger (NL 1953) on the chemical constituents of a *Pyrethrum* species exhibiting insecticidal properties. In 1912 with Staudinger he moved to

Eidgenössische Technische Hochschule (ETH) also known as Swiss Federal Institute of Technology in Zurich and became a Swiss citizen in 1917.

His famous studies on perfumes led to the isolation of two macrocyclic ketones (16- and 17-membered) muscone and civetone from the musk, the Himalayan deer (male musk deer), and civet cats (both male and female African wild civet cats), respectively. It was thought that such macrocyclic ketones would be unstable, but Ruzicka settled their structures by syntheses. This opened up a new area of organic chemistry. He elaborated the structures of various monoterpenes and completed their total synthesis (e.g., linalool, fenchone, etc.) and extended the Wagner–Meerwein rearrangement.

In 1916 Ruzicka started his own program financially supported by a Geneva Perfume Company. During this period his position at ETH carried no salary. In this endeavor he synthesized many lower terpenoids (e.g., nerolidol, farnesol). The University of Utrecht (Netherlands) offered him a job of Professorship where he stayed for 3 years (1926–1929).

He then joined ETH in 1929, spent there his active years (1929–1957), and retired in 1960.

In his seminal work on terpenoids he suggested that a C_5 unit (isoprene) acts as the starter and undergoes enzymatic chain elongation by repetitive head-to-tail condensation terminated at the desired length depending on the enzyme specificity. Cyclizations in varied ways gave rise to several skeletal patterns. This work will remain as the most fundamental concept in the terpene chemistry. This idea prompted chemists, biochemists, and enzymologists equally to search for the biological isoprene. In fact, the isopentenyl pyrophosphate (IPP) the biological isoprene has been discovered by Lynen (NL 1964). Ruzicka also worked on steroids, mainly the male hormones and his synthesis of testosterone brought him a lot of money since hormone treatment was popular in sexual diseases. With the money he started buying Dutch and Flemish paintings and became a well-known collector of the same.

For his outstanding work on natural products of plant as well as animal origin, more especially for his hormone work, he was awarded many prestigious prizes, and the most noted one was the award of the Nobel Prize in Chemistry in 1939 which he shared with Adolf Friedrich Johann Butenandt. He held 8 honorary doctorates (4 in Science, 2 in Medicine, 1 in Natural Science, 1 in Law), 7 prizes and medals, 24 honorary memberships of chemical, biochemical, and other scientific societies, and 18 honorary, ordinary, and foreign memberships of scientific academies. He felt that the honors which he had won should be distributed among the whole team of his coworkers. To mention only one example, the laudation of his 1936 honorary Doctor diploma of Harvard (tercentenary celebration of the oldest USA university) should more realistically be read in the plural form "... to the team of chemists, daring in their attacks, brilliant in their methods, successful in their interpretations of the architecture of nature's baffling compounds"; every member of the team helped to transform the youthful dreams of its oldest member into reality. Ruzicka lectureship was instituted by his PhD students and is organized by the laboratory of Organic Chemistry, ETH Zurich.

During World War II he founded Swiss-Yugoslav Relief Society and worked to secure the escape of several Jewish scientists from Nazis. He was instrumental in providing refuge to Prelog who succeeded him at ETH and subsequently received Nobel Prize.

Ruzicka married Anna Housmann in 1912; they were divorced in 1950. In 1951 he married Gertrud Acklin. He had no child. Alpine plants gardening and collection of old Dutch and Flemish paintings of seventeenth century as well as materials for an art library on that period were his hobbies. He bequeathed the paintings to the Zurich Art Museum. Ruzicka died on September 26, 1976.

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A.25 Karl Barry Sharpless (1940–) (NL 2001)

An intrepid explorer, passionate lover of the rivers, the oceans, fishing, the rare creatures in the catch, the mountains, the Periodic Table, catalysis, and fascinated by chirality commented "….because I handled my umbilical cord in utero …." [1]. His reagents compete with enzymes in enantio- and regioselectivity during asymmetric epoxidation (AE) asymmetric dihydroxylation (AD) and asymmetric aminohydroxylation.

K. Barry Sharpless was born in 1940 and raised in Philadelphia though he often claims to be from "*The Jersey Shore*" where his mother's family established a fishery on emigrating from Norway. Barry used to spend vacations and holidays there along with the family. His father, a busy surgeon, joined them whenever he got time. Barry was in love with the river and fishing since his childhood, and when

he was only 10, he started distributing crabs and other edible creatures from his catches to his known neighbors. He accompanied his uncle Dink in his boat as a helper. The boat was not in a good shape and they had innumerable memorable adventurous rides in the river. He repeatedly presented these adventures to his colleagues at MIT on their begging to repeat. He was more interested in rarity of the catch and not the quantity.

He studied at Dartmouth College (BA, 1963) with Professor T.A. Spencer, who sent him to Professor E. E. van Tamelen (mentor of T.A. Spencer) at Stanford from where he received his PhD in 1968. He did postdoctoral research with Professor J. P. Collman at Stanford (1968) and with Professor K. E. Bloch (NL 1964) at Harvard (1969). His passion for fishing was so intense that he spent much time there, he could not clear some exams to get entry for lab work and became a fish out of water. Van Tamelen asked him to do some library work to find out some inorganic species which might be capable of interesting transformations of organic molecules and left for a long tour in Europe as a Visiting Professor. Sharpless plunged into the Periodic Table and fished out selenium, titanium, and osmium as absolutely thrilling elements. In both the fields of fishing and chemistry he has been an intrepid explorer. His first laboratory was New Jersey's *Manasquan River*, whose astonishing rich variety addicted him to discovery. Later his laboratory became the Atlantic Ocean. "He now does the chemistry the way he used to fish" [2]. Van Tamelen was "one of the most creative people of his generation" [2]. He advised the students, "Try to do something that you can't do" [2].

Barry spent all the time in the library and had nothing to report on experimental side. His notebook was full with literature references and ideas. From van Tamelen, Sharpless "inherited enthusiastic disdain for 'safe' problems, deep admiration for traditional multistep organic synthesis and awe before selective biological catalysis: studying squalene oxide, lanosterol cyclase . . ." [1]. He was inspired by Derek Barton's research for new reactivity and conformational analysis. Further, Jim Collman ignited his interest to develop metal complexes as catalysts; he had discussions with Bob Grubbs (NL 2005) and the freedom of doing work with independent ideas while working in the laboratory of Konrad Bloch. All these led him to discover asymmetric epoxidation [3, 4], dihydroxylation (Sect. 6.2), and aminohydroxylation. They all find industrial applications for chiral molecules/drugs synthesis. He loves the smell of geraniol (roselike smell) and used it as a successful substrate having the built-in structural requirement to study the AE.

Sharpless served in several Chemistry Faculties: MIT 1970–1977, 1980–1990; Arthur C Cope Professor, 1987–1990; Stanford University, 1977–1980; at La Jolla, CA, The Scripps Research Institute, W.M. Keck Professor from 1990; Skaggs Institute for Chemical Biology; and TSRI Visiting Professor since 2002.

He received almost all the prestigious Awards and Prizes open to an organic chemist in the USA and outside for his creative work in organic synthesis. He won the *Nobel Prize in Chemistry* (jointly with W.S. Knowles and R. Noyori) in 2001 and has been elected Fellow/Member of American Association for the Advances of Sciences, 1984; American Academy of Arts & Sciences, 1984; National Academy

of Sciences, 1985; RSC (Hon.); UK, 1998; and Kitasato Inst. (Hon.), Japan, 2002, and received Honorary Doctorate from several universities.

His current research interests are centered on modular click chemistry *in situ* click assembly in biological hosts, homogeneous Os, Ru, and Rh oxidation catalysts, and transition metal-catalyzed asymmetric processes.

The following paragraph expresses the emotion of Sharpless on the receipt of congratulatory e-mails after getting the Nobel Prize.

"I've received way over a thousand e-mail messages since the Nobel Prize was announced. It's absolutely wonderful what people have written. I'm humbled. I'm moved to tears. I'm very proud of what I've contributed. But what's really touched me most have been the letters from former members of my research group, about what they learned. A former postdoc from Germany, who works on a polymer semiconductor project, something that couldn't be further away from what he did in my lab., 'Believe it or not, much of the work I am doing now has been inspired by our time together in the KBS lab, and it just represents the way that I think, which I learned in the many discussions we had. Sometimes people ask me how I get such weird ideas'." [2]

One of the authors (BT) while visiting Prof. S. Masamune at MIT in 1983 had the opportunity of listening to a talk by Sharpless who appeared first with a feathered native American Indian head dress and a hollow drumlike waist wrapper inscribed with his titanium complex catalyst; there was a roar of laughter and a welcome chorus "here comes Barry, here comes Barry." He then came in briefly and then came back with normal dress, dismantling the external outfits and delivered the lecture. The other author (SKT) had also a different type of experience while listening to his talks in International Symposia in Tokyo and Toba in 1982. He is really an unusual speaker and stands apart from many intellectually equals.

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A.26 Gilbert Jose Stork (1921-)

A momentous contributor to Organic Chemistry and Natural Products Synthesis

Gilbert Stork, currently Professor of Chemistry Emeritus (since 1993) at Columbia University, was born in Brussels, Belgium, on December 31, 1921, to Jacques and Simone Weil Stork. Thus, being born on a New Year's Eve, his birthday is celebrated by people all over the world. He received his secondary education in Paris. Frances Hoffman of Columbia University wrote about young Gilbert [1], "Certain of Gilbert's well recognized characteristics were evident in his youth. For example, his rigorous testing of reality began at an early age. One day his nurse took him to the park and carefully explained that he should under no circumstances go near a pond which was completely covered with water lilies. Since he found it difficult to believe that there could be any danger with what appeared to be a solid flower garden, he ran over to test the nurse's story. When he was pulled out of the pool with his felt hat still firmly fastened under his chin, he believed her; but the poor nurse lost her job."

Gilbert moved to USA in 1939. He earned a BS degree from the University of Florida in 1942 (2.5 years after enrolling) and a PhD degree from the University of Wisconsin, Madison, in 1945, working under Professor S. M. McElvain.

After working for a year as a medicinal chemist at Lakeside Laboratories in Milwaukee, he accepted in 1946 an Instructorship at Harvard University. In 1953 he moved to Columbia University as an Associate Professor where he became Professor in 1955, and since 1967 he has been the Eugene Higgins Professor of Chemistry at Columbia.

He is very much respected among the organic chemists all over the globe for his creative research that has a great impact on modern organic chemistry and for profundity of his intellectual style which imparts a strong influence in the way of thinking among the young generation of synthetic organic chemists.

Stork's research contributions fall into three "naturally occurring" areas: (i) the total synthesis of complex natural products; (ii) the creation of new selective synthetic methods; and (iii) the investigation of reaction mechanism. To separate (i) from (ii) is totally arbitrary because of the strong interplay between the two areas [1].

(i) *Total synthesis:* Stork's major interest from the beginning of his career has been to design controlled stereospecific/stereoselective and regioselective synthesis involving formation of C–C bonds which had not been much considered or recognized before him. Following this principle, now universally appreciated and used, he synthesized cincholoipon (1946), a *cis*-3,4-disubstituted piperidine related to hydroquinine, and achieved the total stereospecific synthesis of cantharidin. During his outstanding distinguished career he achieved the total stereospecific syntheses of a number of complex natural products—the more important of which are as follows: monoterpene—cantharidin (1951); sesquiterpenes—cedrol and β-vetivone; diterpene—dehydroabietic acid; triterpenes—a-onocerin and lupeol; steroids—progesterone and corticosteroids; steroidal amine—conessine; indole alkaloids—aspidospermine, quebrachamine, alloyohimbane, and its 3-*epi* derivative (1954), yohimbine; other alkaloids of different basic skeletons—gelsemine, lycopodine, and camptothecin; other bioactive miscellaneous natural products—griseofulvin, byssochlamic acid (a C_{18} compound having 3-propyl-8-ethyl-1,5-cyclononadiene-1,2,5,6-tetracarboxylic acid dianhydride structure), (+)-15-(*S*)-prostaglandin A_2, anhydrodeoxytetracycline, and cytochalasin B (an incredibly complex 14-membered macrolide with 8 chiral centers and two *E*-double bonds).

His recent first stereoselective chiral synthesis of quinine (2001) and reserpine (2004) remains as the masterpieces in the domain of natural product synthesis.

(ii) *Selective Synthetic methods:* Stork created many important synthetic transformations which contributed greatly to the explosive development of organic synthesis. In fact, according to Stork these are also his most valuable contributions to organic chemistry.

One of his new synthetic methodologies that have found most extensive use throughout the globe is briefly mentioned.

Formation of C–C bonds by the regiospecific monoalkylation or monoacylation of carbonyl compounds at the α-position with an alkyl or aryl halide or with an electrophilic olefin (Michael addition) *via* an enamine synthesis—known as *Stork reaction* [2, 3, 4, 5]. His paper on this topic has been very well cited. Twenty more types of synthetic methods have been devised by Stork.

His *radical cyclization* has also been much used in the synthesis of natural products.

(iii) *Mechanistic and Stereochemical Studies* with the potential of leading to controlled synthetic procedures include (a) investigation of the stereochemistry of the S_N2' reaction (1956, 1977), (b) stereochemistry of the Favorskii

rearrangement of α-haloketones (1960), (c) intermediates and stereochemistry in the metal-ammonia reduction of enones (1960, 1961, 1964), (d) stereochemistry of acyclic polyene concerted cyclization known as *Stork–Eschemoscher hypothesis* that leads to a bicyclic cation.

Stork was showered with almost all the major awards of Chemistry. Of them a few are American Chemical Society Award for Creative Work in Synthetic Organic Chemistry (1967); D.H.R. Barton Medal, Royal Society of Chemistry (2002); and Ryoji Noyori Prize, Society of Synthetic Organic Chemistry, Japan (2004) for his lifetime contribution to asymmetric synthesis.

Moreover, the Houston-based Robert A. Welch Foundation presented him the 1993 Award ($225,000 and a gold medal) for his significant research contributions having a positive influence on mankind.

Stork holds honorary doctorates from Lawrence University, Columbia University, and the Universities of Wisconsin-Madison, Paris, and Rochester. He was awarded *Honorary Fellowships* by the Pharmaceutical Society of Japan (1973), the Chemists Club of New York (1974), the Royal Society of Chemists (UK, 1983), and the Chemical Society of Japan (2002).

He was elected Chairman of the ACS Organic Division, 1966–1967; Foreign Member of: the French Academie des Sciences, 1989; US National Academy of Sciences, 1961; American Academy of Arts and Sciences, 1962; American Philosophical Society, 1995; and The Royal Society, UK, 1999.

Stork is an excellent teacher and a kind and charming man. He is loved and revered as a warm and supportive mentor by his former coworkers who have gone on to academic career. He met his wife Winifred Stewart in the English language classes in St. Petersburg. They have four children.

He likes to wear blue tie with an embroidered white stork on it, as described by one of his students. Stork's research has involved organic complex molecules. He says "This part of chemical science is more closely related to architecture than it is to physics or mathematics. What we deal with is really structure, often three dimensions."

In Cope Award address in 1980, "Stork presented his slide, a list of members of Stork Group who presently had positions in academia throughout the world. The slide listed over 110 names, an impressive number—indeed a possible world record for a single research professor. The names on the slide belong to distinguished chemists, and Gilbert must feel proud of this remarkable list—a superb testimony to him" [1].

References and Notes

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qualities and also contains a number of interesting photographs. Frances Hoffman first went to work with Gilbert at Harvard and moved to Columbia University with the Stork's group in 1953. After working with the Merck & Co. from 1954 to 1961 she returned to the Dept. of Chemistry, Columbia University, as the Director of Chemical Laboratories and was deeply involved in the development plans for a new chemistry building. At the time of publishing this article she had been a friend of Stork for over 30 years).

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A.27 Michel Tswett (Also Mikhail, Tsvet; Mikhail Semyonovich Tswett) (1872–1919)

—The Father of Chromatography

Chromatography is a technique of separating the components of a mixture, taking advantage of the differences in the rate of their movements on an adsorbing phase with the help of mobile phases. It is a very powerful technique of separation. It can separate components that have even close structures and properties. Tswett first devised this method and separated coloring pigments chlorophyll a and chlorophyll b from their mixture and from other plant pigments. During separation on the column containing the adsorbing material the components formed different rings of colors and the number of distinct ring stands for each component, as if the results of the separation are written on the column by color; hence the name chromato

(color) graphy (written) was given by Tswett. Some speculate that since the Russian meaning of "tsvet" (German spelling Tswett) is color or chroma, perhaps he named the technique after his name. However, Tswett emphasized at that time that this technique was not to be restricted to colored compounds separation, but might be equally applied to separation of colorless compounds from their mixture.

This technique has been rediscovered by Richard Willstatter (NL 1915) and Richard Kuhn (NL 1938). Kuhn used it to separate three isomers of dyes from their mixture in carrots in 1931. It reached such a level of sophistication and proliferation that it brought Nobel Prize to Richard Synge and Archer Martin (NL 1952) and entered into the work of many other Nobel Laureates. Sometimes chromatography columns are still known as Tswett columns.

Tswett, basically a botanist, was born in a small town of Asti, Italy, on May 14, 1872, while his parents (Russian Father, a civil servant in Russia, and Italian mother) were traveling to Switzerland. His mother died and his father left him to a nurse in Lausanne, finding no other alternative. Tswett grew up in Lausanne and Geneva, graduated in physical and natural sciences from Geneva University in 1893 from the Department of Physics and Mathematics, and obtained his doctorate in 1896. His thesis dealt with the investigations on the structures of plant cells, the movement of protoplasm, and the structure of chloroplasts. He delivered in 1903 a talk entitled "On a New Category of Absorption Phenomena and their Application to Biochemical Analysis" elaborating his discovery in a meeting of the Biological Section of the Warsaw Society of Natural Sciences. His first publication came out in 1894 on plant anatomy. He moved to Russia in 1897, but his foreign degrees were not acknowledged. He then did his MSc at Kazan University in 1901. He held different positions in Poland. In 1903 he separated the plant pigments using a column of powdered chalk as the adsorbing material and light petroleum as the mobile phase.

In 1908 he joined Warsaw Technical University from where he obtained a doctorate degree in Botany 2 years later. During World War I, the department was evacuated to Moscow, and under the constant threat of German invasion, he had to move again to Voronezh, South of Central Russia—where he died on June 26, 1919, at the age of 71.

The most sophisticated separation techniques that we are using in our research owe much to Tswett who sowed the seed of chromatographic separation. Though nowadays most of the compounds separated by this technique are colorless, the name chromatography remains and reminds us of Tswett's first separation of coloring pigments in 1903.

Tswett had his most famous statement on the name of the new technique "Like light rays in the spectrum, the different components of a pigment mixture, obeying a law, are separated on the calcium carbonate column and can thus be qualitatively and quantitatively determined. *I call such a preparation a chromatogram and the corresponding method the chromatographic method*."

At the International Congress, Pure and Applied Chemistry, London, 1947, Paul Karrer (NL 1937) said, "No other discovery has exerted as great influence and widened the field of investigation of the organic chemistry as much as Tswett's

chromatographic adsorption analysis. Research in the field of vitamins, hormones, carotenoids and numerous other natural compounds could never have progressed so rapidly and achieved such great results if it had not been for this new method, which also disclosed the enormous variety of closely related compounds in Nature."

Tswett started as a laboratory assistant with a meager salary. To supplement the latter, he sometimes taught in the schools. He finally became a privatdozent at the University and was allowed to lecture the students. Tswett was a lone man until he got married in 1907. In all his publications (~58) he was the sole author. He did not even have a definite home to stay. He practically spent his whole time in the university and slept on the laboratory table of the botany department. Every year since 1902 he used to travel to Europe and visited the universities and their libraries.

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A.28 Eugene Earle van Tamelen (1925–2009)

Do not go where the path may lead, go instead where there is no path and leave a trail
Ralph Waldo Emerson (1803–1882)

The above saying of Emerson is rightly applicable for E. E. van Tamelen, one of the most broadly creative chemists of his generation. To him "*The only rule is, 'there are no rules!' "* said his former PhD student K. Barry Sharpless, Professor at the Scripps Research Institute and a 2001 Nobel Laureate in Chemistry.

Eugene E. van Tamelen, "Gene" to his friends and "vT" to his students and chemists around the world, was born on July 20, 1925, in Zeeland, a small western Michigan town in Dutch America's heartland. He inherited, he thinks, his gift for spatial thinking, as well as lifelong love of the applied arts from his woodworker forebears. He enrolled at Hope College in Holland, Michigan, and initially wanted to become an automobile designer. But when he studied organic chemistry and experienced the three-dimensional space at molecular level, he found chemistry as the subject of his choice and future career. One of his proudest accomplishments was being the first Hope College student to publish original research in the world's most prestigious chemistry journal at that time, *The Journal of the American Chemical Society (JACS),* entitled "The Malonic Ester Reaction with 1-Halonitroparaffins." This was but the first of nearly a hundred *JACS* papers he authored.

Van Tamelen graduated from Hope College in 1947, earned his PhD degree from Harvard University in 1950, and joined the Chemistry Faculty of the Wisconsin University that year. He quickly rose to full professorship and became Homer Adkins Professor of Chemistry. In 1962 he moved to Stanford and chaired the Chemistry Department for several terms. He supervised the research of more than 200 doctoral and postdoctoral students. Many of them followed him into distinguished academic career.

Van Tamelen's skilled syntheses of complex natural products began at Harvard under the guidance of Professor Gilbert Stork. Van Tamelen was the major contributor to Stork's total synthesis (published in 1951), of a poisonous monoterpenoid cantharidin secreted by male blister beetles, *sometimes considered to be the first stereorational synthesis of a natural product*. Stork wrote about van Tamelen, "He was most unusual having already coauthored several papers as an undergraduate." The *Stanford Report* (See footnote at the end) wrote "His style was distinctive; he liked being different and being first, and the problems he worked on had to be big ones. He was an intuitive thinker . . ..who would 'graze' the chemistry library's disparate journals and come up with the novel connections that propelled his cross-disciplinary chemical investigations" [1]. He worked with Sir Alexander Todd (NL 1957) at the University of Cambridge in 1957.

He discovered squalene epoxide; the stereochemistry of the epoxide was deduced by Barton as 3-(*S*). This linear epoxy triterpene is the obligatory precursor of all forms of triterpenes and sterols including cholesterol. His seminal work on polyene cyclization is framed on biogenetic concept. He was the *first to introduce the concept of biomimetic synthesis in the field of synthetic chemistry*. Van Tamelen took the challenge of synthesizing Dewar benzene, which, chemists thought, is impossible to make because its bonds are in highly strained configuration. Sharpless said, "Sure enough, vT made it"—In his discoveries ideas and his personal style fuse. His academic career was marked by numerous signal achievements, ranging impressively from history making first total syntheses of the hallucinogenic alkaloid yohimbine and the antimitotic alkaloid colchicine. He was interested in *plant pigments especially of tulips,* inspired by his Dutch heritage. In 1967 on a royal appointment from the Queen of Netherlands he served as Professor Extraordinarius at the University of Groningen in the Netherlands.

Van Tamelen was an *avid reader*, an *avid traveler*, and a *passionate lover of architecture and cars*. He drove an Excalibur and then a Rolls and enjoyed the fellowship of Rolls Royce owners Club. He traveled extensively all over the globe and lectured.

His love for architecture especially of Frank Lloyd Wright is such that he owned a home designed and built by Wright and since then the house is known as Eugene van Tamelen House. He himself designed and built an open-air house in the tropical island of St. Lucia West Indies where his family and friends used to spend vacations.

He was honored with *many coveted awards*, of which special mention should be made of the Leo Hendrik Baekeleand Award (1975) and the ACS Awards in Pure Chemistry (1961) and Synthetic Organic Chemistry (1970). He received *Honoris Causa* from two universities, innumerable invited Lectureships within and outside the country, and the Membership of the National Academy of Sciences. Sharpless said, "...we were so proud to be his students, to be in a lab where five or six different, exciting problems were simultaneously under investigation." He used to give freedom to work and thinking to his students so that they could blossom to their fullest potential.

A life-size bronze figure of a woman seated on a bench with a book in her lap and engaged in reflection (*Contemplation*) was installed (2000) in van Tamelen Plaza (dedicated to him in1997) located between college's admission building and conference center by the artist and donor, Billie Houtman Clark whom van Tamelen met at a soda shop when young, and who recommended vT to meet her sister Mary. They met and got married. They have three children. The college offered two top scholarships—the Gene van Tamelen Prize for the creativity in the Sciences and the Mary van Tamelen Prize for the creativity in the Arts.

In retirement he kept traveling to exciting destinations, not just the world's reputed chemistry departments. One of his favorite hobbies was to sit and think—something he thought was missing from most people's lives. He was deeply interested in cosmology and "contemplating nature" was his enjoyable activity of all. On December 12, 2009, van Tamelen, an emeritus Stanford Professor, best known for the biologically inspired syntheses of complex natural substances and a resident of Los Altos Hills, CA, died of cancer at the age of 84 leaving behind his wife, Mary, his partner for 58 years and the former Mayor of Los Altos Hills, and their two daughters, one son and five grandchildren.

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A.29 Heinrich Otto Wieland (1877–1957) (NL 1927)

An outstanding natural products chemist, a pioneer in organic radical chemistry and a man with encyclopedqic knowledge in different branches of chemistry.

Heinrich Otto Wieland was born to Württernberger parents, Elise (neé Blom) and Dr. Theodor Wieland on June 4, 1877 in Pforzheim, Baden, Germany. His father, a pharmacist with a doctorate degree in chemistry, owned a gold and silver refinery.

Wieland studied at the Universities of Munich, Berlin, and Stuttgart and came back to Munich to join the Baeyer laboratory. He received the doctorate degree in 1901 from the University of Munich working under Johannes Thiele. In 1904 he completed his habilitation and continued to teach at the University of Munich. In 1913 he became a Senior Lecturer in the University Chemical Laboratory and then moved to the nearby Technical College as a full Professor (1917–1921). For a while during 1917–1918 he worked on defense at the Kaiser Wilhelm Institute in Berlin-Dahlem. In 1921 he joined the University of Freiburg where he started work on toad poisons and bile acids and stayed until 1925. In 1925 he returned to Munich and succeeded Professor Richard Willstätter (NL 1915) as the Chair Professor in the University of Munich and made Munich his home. Wieland was a cousin of Helene Boehringer, the wife of Albert Boehringer, who was the founder of a company named Boehringer-Ingelheim. From 1915 to 1920 he was the advisor at Boehringer-Ingelheim. He established the first scientific department of the company.

Wieland's scientific work, recorded in four hundred publications, covers a wide field in the realm of organic chemistry and biochemistry. His brilliant work on radical reactions, reactions of nitrogen oxides with olefins and aromatics, and production of stable organic nitrogen radicals of diphenyl nitrogen and its N-oxides has much impact on the modern development of organic radical chemistry. In later years he was entirely devoted to the chemistry of natural products. His

contribution to the clarification of the structure of complex alkaloids like morphine and strychnine (in association with Boehringer-Ingelheim), the constitution and synthesis of the *Lobelia* alkaloid, and his research into the *Curare* alkaloids were masterpieces. In 1941, he isolated the crystalline cyclopeptide toxins alpha-amanitine and phalloidine, the active principles of one of the world's most poisonous mushrooms, *Amanita* phalloides ("*death cap*" mushroom). His work on the pigment of butterflies led to the discovery of the biologically important class of compounds called pterins. He continued his work (1912–1932) on bile acids and the clarification of the steroid skeleton. His great school is evidence of his importance as an academic teacher.

While investigating the oxidation processes on living cells he *realized dehydrogenation as a universal oxidation process in Nature*. This work restores the unity of organic chemistry and biochemistry which had been lost since the time of Liebig.

He received a number of prestigious awards including Nobel Prize in Chemistry in 1927 for his work in isolating and deducing the structures of bile acids/steroids including cholic acid. He concluded his Nobel lecture with the statement that he had a "duty" to synthesize the bile acids even though the total task was insurmountable at that time. He was a Member of the great learned societies of the world. He received the Order of Merit and the Otto Hahn Prize. For 20 years he was the Editor of *Justus Liebigs Annalen der Chemie*.

Since 1964 Boehringer-Ingelheim instituted *Heinrich Wieland Prize*, awarded annually to promote research in chemistry, biochemistry, physiology, and clinical medicine of lipids and related substances. It is one of the most treasured international science awards.

In 1908 Wieland married Josephine Bartmann of Munich. They had three well-established sons, Wolfgang, a doctor of Pharmaceutical Chemistry, Theodore, Professor of Chemistry in the University of Frankfurt, and Otto, Professor of Medicine at the University of Munich. The only daughter, Eva, got married to Feodor Lynen (A.16) who shared 1964 Nobel Prize in Physiology/Medicine with Konrad Bloch (A.5).

Wieland was a kind man. His scholastic life was governed by hard work, but also by love and kindness both to his pupils and to his family. He successfully protected people, especially Jewish students, who were expelled because they were "racially burdened" after the Nuremberg Laws, by keeping them in his research group as chemists. He died on August 5, 1957, shortly after his 80th birthday in Starnberg, Bavaria, Germany.

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A.30 Richard Martin Willstätter (1872–1942) (NL 1915)

An outstanding organic chemist who did brilliant work on coloring matters of nature including the structure of chlorophyll, invented independently paper chromatography, and revived and developed the technique of chromatography in general.

Richard Martin Willstätter was born on August 13, 1872, to Jewish parents Maxwell Willstätter (a textile merchant) and Sophie Ulmann Willstätter at Karlsruhe, Germany. He went to school first in his home town and then to the Technical School (Gymnasium) in Nuremberg, when his parents moved there. He did brilliant performance in his high school. At eighteen he entered the Department of Chemistry, University of Munich, and studied under Adolf von Baeyer (NL 1905). He stayed there for the following 15 years, first as a student [BS Chemistry (1893) and PhD Chemistry (1894 under Alfred Einhor, and Bayer as the advisor)], then as lecturer in 1896, and worked independently. In early 1902 he became J. Thiele's successor as an Extraordinary Professor. For his dissertation he mainly worked on the structure elucidation of alkaloids and synthesized atropine and cocaine. Later, he worked on quinone and quinone-type compounds which are the basis of many dyestuffs. He then got involved in the work of plant pigments which required expanded facilities, and he moved to the University of Zürich as the Chair Professor in 1905. He spent there the most fruitful 7 years of his scientific life which resulted in his pioneering work on plant pigments—the anthocyanins and anthocyanidins from flowers and fruits and chlorophyll from green leaves. These investigations on plant pigments, especially the complicated structural work on chlorophyll, which he showed to be an intimate mixture of two, chlorophyll **a** and chlorophyll **b** (Chap. 3), both having magnesium at the core of the molecule, as we find iron in heme, brought him the 1915 Nobel Prize for Chemistry. But unfortunately, he was not allowed to accept the award until the end of World War I. The first synthesis of cyclooctatriene was achieved by him. Since 1912 he continued his work on pigments of flowers and fruits as Professor at the University of Berlin. In 1916 he

succeeded his old teacher Adolf von Baeyer as the Professor of the University of Munich.

In 1908, during his stay in Zürich he suffered a personal misfortune—the death (1908) of his wife Sophie Leser, a daughter of a Professor of the Heidelberg University, whom he married in 1903. They had one son Ludwig (b.1904) and a daughter Ida Margarete (b.1906).

In the years that followed in Munich Willstätter investigated the nature and activity of enzymes and made important findings on photosynthesis. In 1924 Willstatter resigned from his position at the University of Munich in protest against antisemitism (discrimination against Jews). His career came to a tragic end. Several offers and confidence of his colleagues and students in him could not convince him to undo his decision. He lived a retired life in Munich keeping contacts with some of his students and his nominated successor Heinrich Wieland.

In 1938, when he was targeted he fled from the Gestapo (German abbreviation of **Ge**heime **Sta**ats**po**lizei, secret state police), organized in 1933 and notorious for brutal methods and operations. The new Nazi regime was using Gestapo tactics. He was helped by his student Arthur Stoll (1887–1971), immigrated to Switzerland, and spent his last 3 years in Muroalto—near Locarno, writing his autobiography and died of heart attack in August 1942. His autobiography "*Aus meinem Leben*" was not published in German until 1949. It was translated in English as "From My Life" in 1965.

In an epilogue written by Arthur Stoll to Willstätter's biography the list of honors and distinctions accorded to this great scholar in different parts of the world occupies no less than three pages. Some of them are: *Fellow of the Royal Society, Iron Cross* 1932 and *Willard Gibbs Medal* 1933. In 1956 a memorial to Richard Willstätter was unveiled in Muroalto.

It is interesting to know through an example—how kind he was to his students. An American Chemist Michael Heidelberger came to work for a year with him. Willstätter helped his impecunious American student by sharing the cost of laboratory chemicals. When expensive chemicals like silver nitrate were to be purchased Willstätter paid the bill. On the other hand, the American student paid for chemicals like sulfuric acid. They remained friends for their whole life.

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A.31 Robert Burns Woodward (1917–1979) (NL 1965)

A consummate master of organic synthesis and the greatest synthetic organic chemist of all times

A child of extraordinary precocious abilities was born in Boston on April 10, 1917, to Arthur and Margaret Woodward. His mother being of Scottish origin (born in Glasgow) named their son after the poet Robert Burns. His father Arthur died the year (1918) after his birth in a great influenza epidemic and his mother Margaret remarried but was abandoned soon by her second husband, and she raised Robert single-handedly in straitened circumstances.

At 16 he was admitted as a chemistry major at the MIT. He was awarded the degree of MSc in June 1936 at 19 and the degree of PhD in June 1937 at 20 within a year. In 1938 he moved to Harvard University where he stayed until he died of a heart attack at only 62 on Sunday, July 8, 1979—the day when "a life that was characterized by an incredible sequence of brilliant and elegant achievements was concluded" [1]. The organic chemists throughout the world were saddened by his sudden untimely demise. At Harvard he became the Morris Loeb Professor in 1953 and the Donner Professor of Science from 1960 onward. He was the Todd Professor of Chemistry from 1973 to 1976 and a Fellow of the Christ College in Cambridge.

Woodward, a profoundly insighted chemist in whose heart resides an intensely imaginative child, said [2], "I love crystals, the beauty of their form—and their formation; liquids, dormant, distilling, sloshing!; the fumes; the odors—good and bad; the rainbow of colors; the gleaming vessels, of every size, shape and purpose. Much as I might think about chemistry, it would not exist for me without these physical, visual, tangible, sensuous things." He remarked in his lecture in organic synthesis (R. A. Welch Foundation Conference on Chemical Research, 1969) that "I fell in love with the field of organic synthesis when I was a small boy and the affair is still going on" [3]. He named one of his children "Crystal."

The highlights of his structural elucidations include the penicillins (1945), strychnine (1947), the tetracyclines (1952), ferrocene (1952), cevine (1954), the macrolides (1956, 1960), streptonigrin (1963), and tetrodotoxin (1964).

His unbelievable competence in structural elucidation of complex molecules will be evident by the comment made by W.D. Ollis [1], "The molecular complexity of strychnine prevented its effective structural investigation by organic chemists until the present century. During 1910–1947, mainly as a result of extensive degradative investigations by Leuchs (125 publications), Robinson (53 publications) and Wieland (30 publications), the structure of strychnine was eventually revealed. The contributions made by Woodward to this classical problem were incisive. In 1947 in a short communication, the correct formula for strychnine was proposed. Then a detailed full paper was published (1948) containing the final sentence, 'We conclude that the structure for strychnine is established.' The paper described only 7 new experiments, but provides, with an appealing logic, brilliant interpretations of the whole array of earlier degradative transformations of strychnine" [1]. A preliminary communication [2] on the total synthesis of strychnine was published by him in 1954, and the detailed full paper [3] was published in 1963. The breathtaking list of his total syntheses includes quinine (1944), cortisone (1951), cholesterol (1951), strychnine (1954), lysergic acid (1954), lanosterol (1954), reserpine (1956), chlorophyll (1960), tetracycline (1962), colchicine (1963), and cephalosporin C (1965). In collaboration with Eschenmoser's group at ETH, Zürich, he completed the total synthesis of vitamin B_{12} (1972) and erythromycin (1981, completed by his coworkers at Harvard). Elegance and ingenuity of his synthetic methods are incredible.

Because of not attending classes in chemistry and neglecting the other subjects he was advised to withdraw from the college. He then worked as an employee in the MIT Biology Department. However, Professor James F Norris, the then Director of Research Laboratory of Organic Chemistry at MIT, permitted Woodward to reenroll since Norris realized that this talented boy with an unusual mind would make a name for himself in the scientific world. Barton wrote [4] in his autobiography, "Woodward was in everyway an exceptional person. He had taught himself more chemistry at the age of 18 than the professors at Massachusetts Institute of Technology (MIT) had acquired in their life time. . . .. Fortunately, the professors at MIT eventually recognized Woodward's astuteness in chemistry and allowed him to take examinations without attending the classes." "His phenomenal memory was beyond anything I'd ever seen," said William S. Knowles (NL 2001) about Woodward in his Nobel lecture [5]. "Woodward was the man against whom the greatest chemists of his day measured themselves and their research," wrote J. F. Seeman [6]. Apart from his work on structural and synthetic organic chemistry, he offered almost everything of organic chemistry. His structure diagram drawings on blackboard were so immaculate that they almost got proverbial status. The basic concept of the *conservation of the molecular orbital symmetry* occurred to him in the course of the vitamin B_{12} synthesis where he came across a seemingly inexplicable example of a counter-thermodynamic stereochemistry of ring closure of a hexatriene derivative. This example was reported to him by one of his postdoctoral

associates, S. Ranganathan (ex-Professor, IIT, Kanpur, India). This baffling example of cyclization could be well explained as a disrotatory electrocyclic ring closure of a hexatriene in terms of the conservation of orbital symmetry. The latter concept also provided the explanations of many other perplexing observations of cyclization reactions. Woodward collaborated with a brilliant young physical chemist at Harvard, Roald Hoffmann, and propounded a series of rules for predicting the stereochemistry and feasibility of such reactions, classified as pericyclic reactions[5]. The rules are known as Woodward–Hoffmann rules.

Woodward was overwhelmingly decorated in his own country and outside, by awards, medals, Honoris causa, Fellowships, Lectureships, and Professorships. In 1965 he was awarded the Nobel Prize for his "outstanding achievements in the art of organic synthesis".

He was very fond of blue color and used to wear a blue tie, sometimes with embroidered molecules he synthesized. In fact, after the completion of the total synthesis of strychnine [7, 8], he presented each of his collaborators a tie embroidered with the structure of strychnine. One such collaborator had been Professor Michael P. Cava with whom one of the authors (SKT) worked as a postdoctoral research associate at the Ohio State University. Both the authors (SKT and BT) saw that memorable tie Cava wore when he came to attend a dinner party at their residence (Columbus, Ohio) in 1964. We heard from Professor Cava that he used to present such ties after the completion of a synthesis to the authors of the paper.

Woodward has been extremely courteous and possessed a delicate sense of humor. While introducing Professor Woodward to the audience before his plenary talk on the total synthesis of vitamin B_{12} at the 8th IUPAC symposium on the Chemistry of Natural Products held at New Delhi in 1972, Professor Morris Shamma (Penn State University) said that after receiving the Nobel Prize Woodward faced a string of interviewers including a lady journalist. She was so overwhelmed by the personality and handsomeness of Woodward that she reported next day a lot of data with a heading—"Professor reads three books a day." Woodward was very much amused to find such a comment about him. He utilized it in a Woodwardian way. When he did not want to attend a party, he used to say, "I have to finish three books" and when he was willing to go he would say, "I just finished three books." In that IUPAC meeting both the authors (SKT/BT) were present.

In the memoir [9] of Prelog, an interestingly unusual photograph taken by Koshland Jr. was printed in which Woodward, dressed in a suit and a tie, was standing on the Baltic beach, with one leg on the back of V. Prelog and the other one almost on the back of C. Djerassi—both wearing swimming trunks. The caption of the photograph reads—"to Prelog this photograph showed that we are the solid fundaments of His glory." According to Djerassi the chemists were "staging the supposed superiority of Harvard over ETH and Stanford." Apparently, in every

[5] Roald Hoffman received the Nobel Prize in 1981, jointly with Kenichi Fukui for this theory predicting the products of different types of concerted reactions product and the necessary conditions.

man's heart, independent of achievements, there resides a child who sometimes peeps out of the shield. The photograph is also available in reference [10].

At the IUPAC meeting held in Australia, after reporting his total synthesis of chlorophyll involving 55 steps, Woodward made his final comment "it remains to thank with all the warmth at my command those who fought and enjoyed the battle with me." The concluding remark by Robinson was "I believe for the first time, the outstanding achievement of the total synthesis of chlorophyll. Professor Woodward is not only the most brilliant synthetic organic chemist, who gives us metaphorically left hooks and right jabs in bewildering quick succession, but also an expositor able to convey a sense of the drama of the development to his audience."

Woodward was the first scientist to show that spectroscopic analysis could be used for structural elucidation. From the study of the UV spectra of a number of natural products he arrived at the empirical rules for calculating the λ_{max} values for conjugated dienes and dienones, which agreed well with the corresponding observed values (Sect. 4.2). Thus, these rules were widely applied for structure determination of unknown natural or synthetic molecules. In 1958 he discovered *octant rule* (see Sect. 2.19.5) jointly with other stalwarts (see ref. [134] of Chap. 2). A picture showing the playful mood of Woodward and William Moffitt in one afternoon of 1958, after the discovery of the octant rule, appeared in reference [11]. The synthetic designs and methodologies he developed for the total synthesis of a great number of biologically active complex natural products have made Woodward the greatest synthetic organic chemists of all times.

"His interests in chemistry is wide, but the main arena of his first-hand engagement has been the investigation of natural products—a domain he regards as endlessly fascinating in itself, and one which presents unlimited and unparalleled opportunities for the discovery, testing, development and refinement of general principles" [12].

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Appendix B

A Chronology of Landmark Inventions/Discoveries Leading Directly or Indirectly to the Development of Natural Products Chemistry

Introduction

The development and advancement of any branch of science depend on the cumulative effects of observations (major or minor), reflections, findings (designed or serendipitous), ideas, discoveries, inventions, noticeable events, identification of the unexpected etc., and their subsequent uninterrupted expansion, diffusion, and application. Different branches of science sometimes derive benefits from a single invention as well as discovery, e.g., chromatography, spectroscopy, X-ray crystallography, etc.

In this Appendix information regarding some landmark inventions/discoveries as well as a few simple but useful inventions which appear to be pertinent to the development of organic chemistry in general and natural products chemistry in particular have been narrated very briefly and chronologically. These brief historical events may stimulate the readers to study the subject matters farther.

1812: Discovery of Optical Rotation

Jean Baptiste Biot (French Physicist and Astronomer, 1774–1862) in 1812 discovered [1, 2] that a quartz plate cut at right angle to its crystal axis rotates the plane of linearly polarized light by an angle proportional to the thickness of the plate. In **1815** he showed that organic compounds, liquids like turpentine, and solids like camphor, tartaric acid, or sugar in solutions also showed such properties. This phenomenon is known as optical rotation (Sect. 2.2.8). He gave the modern definition of *Specific rotation* [α] which he thought to be a characteristic constant

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for each individual optically active compound (Sect. 2.2.9). Biot is regarded as the pioneer of polarimetry.

Impact: The phenomenon of optical rotation of chiral compounds enormously proliferated later and more precisely the specific rotation led to

(i) the identification of the optically active enantiomer,
(ii) the bioactivity, if any, and its correlation with the enantiomer,
(iii) the measurement of optical purity (*ee*) of the product derived by asymmetric synthesis (see Sect. 2.11.2.1), and
(iv) the use as chiral synthons since late twentieth century.

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1821/1822: Opposite Optical Rotation of Nonsuperposable Mirror Image Crystals

Sir John Frederick William Herschel (British Astronomer 1792–1871) observed [1] that the quartz crystals capable of rotating the plane-polarized light in opposite directions possess hemihedral faces of two types, which are nonsuperposable mirror images to each other, called enantiomorphous (from the Greek *enantios*' meaning opposite and *morphe* meaning form).

Impact: This concept of enantiomerism of nonsuperposable mirror images of optically active quartz crystals had great potential of its subsequent application in organic molecules (*vide infra* **1948, 1950**)

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1825: Discovery of Benzene

Michael Faraday (English Chemist and Physicist 1791–1867) [1, 2, 3] discovered benzene by distilling the sticky condensate that was plugging London's the then newly fixed gas pipes. Initially, benzene was named "bicarburete of hydrogen" by Faraday and was renamed as benzene by Liebig [3]. Faraday determined its m.p., b.p., and density with great accuracy. Its correct formula C_6H_6 was given by the Italian Chemist, Stanislao Cannizzaro (1826–1910).

The structure of benzene remains a riddle for a long time because of its carbon: hydrogen ratio. Though the German Chemist Friedrich August Kekulé (1829–1896) is well known for the ring structure in its present form for benzene, the ring structure was also suggested prior to Kekule by an obscure contributor, an Austrian Chemist-Physicist, Johann Josef Loschmidt (1821–1895) [4].

Impact: Benzene ring is the core structural moiety of most of the aromatic compounds. Its study by organic chemists, theoretical organic chemists, and physical chemists is responsible for the development of aromatic chemistry, especially the aromaticity—one of the finest tenets of organic chemistry. It serves as the major example of Hückel's (Erich Armand Arthur Joseph Hückel, German Chemist (1896–1980)] rule and resonance stabilization theory. Because of its ring current effect in NMR spectroscopy, the aromatic protons are deshielded (Sect. 4.2.6) and could easily be identified. This helps a lot in structural elucidation of aromatic compounds containing benzene or substituted benzene ring/s and in many cases reveals the stereochemical features (cf., shielding of protons above the aromatic plane).

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1826: Empirical Formula of Rubber

Faraday arrived at the correct empirical formula [1] $(C_5H_8)n$ for the natural macromolecular rubber.

Impact: This empirical formulation gave an idea about the basic structure of natural rubber as the polyunsaturated polymer of pentadiene. Perhaps this finding is partially responsible for the formulation of *"isoprene* rule" of terpenoid and steroids biosynthesis, leading subsequently to the discovery of isopentenyl pyrophosphate (IPP) as the C_5-precursor in the terpenoids, steroids, and rubber biosynthesis [2].

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1828: First Synthesis of an Organic Compound, Urea, from an Inorganic Molecule: Genesis of Synthetic Organic Chemistry and of the Concept of Structural Isomerism

The year 1828 witnessed the first synthesis of an organic compound, urea, from an inorganic salt, ammonium isocyanate by Friedlich Wöhler (German Chemist, 1800–1882) (Fig. B.1) [1, 2]. This is an accidental synthesis and serves as the milestone in Organic synthesis.

Impact: Prior to this synthesis an idea prevailed that organic compounds cannot be synthesized outside an organism and a vital force is needed to effect that. In 1837, Liebig wrote "the extraordinary and to some extent inexplicable production of urea without the assistance of vital functions, for which we are indebted to Wöhler, must be considered one of the discoveries with which a new era of science has commenced" [3]. This synthesis of urea gave a great blow to this long-held belief and commenced a new field—the synthetic organic chemistry. "The following year, Wöhler and Liebig in a joint paper on uric acid drew the conclusion that all organic compounds would be capable of preparation" [3]. To quote Nicolaou, "The first synthesis of urea was a profound moment in the history of science, making both death of all-pervasive theory of vitalism, and the birth of synthetic organic chemistry as its own distinct, creative and valuable discipline" [4]. The concept of *structural isomerism of molecules having the same molecular formula also emerged*.

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1843: Liebig Condenser [1]

Distillation is a process of converting a liquid into its vapor and condensing back the vapor into the same liquid. For this purpose, a countercurrent cooling devise was first made by Christian Ehrenfried Weigel (1748–1831) in 1771 and later independently by French Chemist P.J. Poisonnier in 1779 and a Finnish chemist Johan Gadolin (1760–1852) in 1791. They used two concentric metal tubes and inside the inner metal tube, a glass tube is suspended. Through the annular space between the two metal tubes cooling water is passed and the distillate comes though

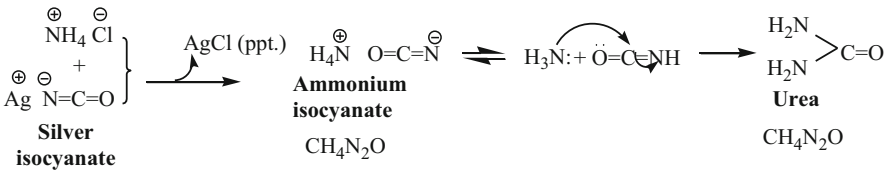

Fig. B.1 Wöhler's Synthesis of Urea from an inorganic compound

the inner tube which is not in direct contact with the cooling water. Later this device was further modified by Johann Göttling (German Pharmacist, 1755–1809) and by Justus (pronounced as yoos-toos) Baron von Liebig (German chemist, 1803–1873) for easy and convenient use. He used two concentric tubes, the inner one being made up of glass and the outer one of metal. The inner glass tube is directly connected to the distilling flask and sealed with the outer metal jacket through cork at both ends. The annular space is continuously supplied by water by means of two rubber tubings—one at the bottom for inlet and the other at the top for outlet. The glass tube comes directly in contact with the cooling water and thus the cooling is efficient. The condenser we use today is of Liebig type in which the metal jacket is replaced by a glass concentric tube fused with the inner one at two ends.

Impact: Refluxing and distillation, the two important everyday operations in the chemical , could be done easily and safely using Liebig condenser. The inner tube may be modified as glass hollow spiral tubes, wavy tubes, etc., to make the cooling most efficient by increasing the inner surface. In almost all chemical reactions, Soxhlet operations, winning of materials from an extract, etc., involving low-pressure distillation, Liebig condenser is used in various modified forms. In industry where water is used as cooling agent the principle of Liebig condenser is also applied.

Reference

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1844/1845: Synthesis of Acetic Acid from Elemental Carbon: First Synthesis of an Organic Compound with C–C Bond

Kolbe (Adolf Wilheim Hermann Kolbe, German Chemist, 1818–1884) synthesized acetic acid from elemental carbon. Unlike single carbon containing urea, acetic acid contains C–C bond, the lifeline of organic molecules and it was a planned synthesis (Fig. B.2).

Impact: This synthesis strongly supported Wöhler's work on the synthesis of an organic molecule in the absence of "*vital force*" and thus helped in the gradual demolition of the "*vital force*" theory of organic synthesis. This is the second

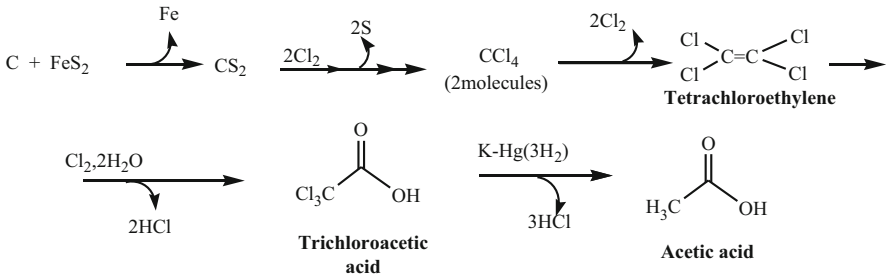

Fig. B.2 Kolbe's synthesis of acetic acid starting from carbon

organic molecule (CH_3COOH) synthesized. The organic synthetic chemistry, which commenced with Wöhler's synthesis of urea, strode a major step forward with the synthesis of acetic acid by Kolbe [1–3].

References

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1848, 1850: Concept of Symmetric and Dissymmetric Carbon Compounds and Structures

Louis Pasteur in 1848 extended Herschel's concept of dissymmetric quartz crystals to the realm of molecules. He divided the molecules into two categories—(i) symmetric molecules with superposable mirror images devoid of optical rotation and (ii) dissymmetric molecules with nonsuperposable mirror images exhibiting optical rotation (Sect. 2.2.5).

Impact: In 1850 Louis Pasteur cited the irregular tetrahedron as well as the helix as examples of dissymmetric structures and thus sowed the seeds of stereochemistry. He first introduced many pioneering concepts of stereochemistry and hence is regarded as the "Father of Stereochemistry."

1856: Mauve, The First Synthetic Dyestuff—A Serendipitous Product—Beginning of Chemical Industry [1–5]

Quinine, the magical drug of malaria, has attracted the attention of synthetic chemists. At the insistence of August Wilhelm Hofmann (1818–1892), William Henry Perkin (1838–1907), then a young student of 18 years, attempted the synthesis of quinine in his home laboratory from N-allyltoluidine molecule in the presence of oxygen. Though his endeavor failed to synthesize quinine, instead he got a black solid. Being a man of intuition, keen inquisitiveness, patience, and outstanding laboratory skill, he did not throw away the black solid, as the usual practice of many, and instead tried to extract material from it using different solvents. Finally, he could extract a material by alcohol in which it formed a brilliant purple solution. On concentration beautiful purple crystals were thrown out and they stained the fiber, and he named it Mauve (Chap. 25).

Impact: It is the first synthetic dye and at that time was in great demand all over the globe. This demand needed its commercialization and the first chemical industry began—an event of great importance to science and society.

References

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1858, 1874: Tetrahedral Carbon (Friedrich August Kekulé, 1858, 1867) and Asymmetric Carbon (Jacobus Henricus van't Hoff, 1874; Joseph Achille Le Bel, 1874)—Three-Dimensional Asymmetry [1–4]

All the above related concepts are clubbed together. Kekulé in 1858 suggested that carbon is tetravalent. In 1867 he envisaged the possible tetrahedral arrangement of the four valences of a carbon atom. Van't Hoff and Le Bel independently in 1874 suggested that carbon atom possesses a regular tetrahedron configuration and that its valence bonds are directed toward the four corners of a regular tetrahedron. They further suggested that a carbon atom with four different groups or atoms is asymmetric in nature and their mirror images are nonsuperposable. They are optically active and the two isomers will rotate the plane of polarized light in opposite directions with equal magnitude (Chap. 2). Kekule′ also suggested carbon–carbon

bond formation in a chain. However, Archibald Scott Couper proposed this concept prior to Kekule' in 1858. Unfortunately his teacher delayed in presenting Couper's paper, and Couper lost his priority. Depressed Couper left his work and since then spent an isolated life.

Impact: The above concepts with those of Louis Pasteur who suggested rotation arises out of the dissymmetry in the molecule, opened a new horizon in Chemistry—the Stereochemistry. The chemical properties as well as biochemical activities, if any, of optically active molecules depend on the stereochemical features of the molecules. It has an immeasurable impact on organic chemistry (e.g., asymmetric synthesis), medicinal chemistry, and natural products chemistry.

Incidentally, it may be mentioned that van't Hoff received the first Nobel Prize (Chemistry) in 1901 for the laws of chemical dynamics and osmotic pressure and not for his original concept of asymmetry.

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1879: Invention of Soxhlet Apparatus

Franz Ritter von Soxhlet (German Agricultural Chemist, 1848–1926) devised an apparatus with an arrangement capable of using a constant level siphon for returning the solvent from the container containing plant materials (using Soxhlet apparatus), after the completion of each extraction cycle, to the extraction flask which is kept boiling for constant supply of the solvent to the plant-containing container [1]. As a consequence, almost a constant volume of solvent can be repeatedly used for the extraction of plant materials (Sect. 4.1.4). The apparatus has been named Soxhlet after the inventor.

Impact: Extraction of the plant materials is the basic need for the isolation work and for that matter—work on natural products chemistry. Judicial and economical use of solvents is possible in this device. Further, volatile solvents such as ether could also be used unlike the cold extraction which requires less volatile solvent like alcohol. Small-scale extraction is also possible by this method. The apparatus though initially designed for the extraction of the plant materials has been used for other extraction purposes. The apparatus and the procedure have been modified to meet the need of the work on other branches too, e.g., pharmaceutical chemistry, biochemistry, botany, physiology, etc.

Reference

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1891: Fischer Projection Formula

Emil Fischer published a paper in 1891 [1, 2] on the configuration of glucose and its isomers using van't Hoff–Le Bel's concept of asymmetry. He further suggested in a follow-up publication [3, 4] that stereostructures could be written in a plane following some conventions (see Sect. 2.2.10) which is known as Fischer projection formula.

Impact: Fischer projection formula is the easiest way of writing the relative (more than one chiral centers) and absolute configuration (at least one chiral center) of chiral molecules, although Fischer projection formula represents the high-energy unstable eclipsed conformation [5]. R/S configurations can be depicted very convincingly using priority sequence of four different ligands (groups/atoms) around the chiral center in Fischer Projection formula. It helps in our understanding the configurations of chiral molecules (Sect. 2.2.10).

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1894: The Concept of Stereoselectivity of Enzymes and the Lock and Key Relationship between Enzymes and Substrates in the Enzyme-Catalyzed Reactions

In 1894 Emil Fischer published his famous paper [1] on the stereoselectivity of enzyme-catalyzed reactions supported by experimental evidence and put forward the concept of lock and key relationship between the participating enzyme and the substrate. An enzyme called "invertin" by Fischer was found to hydrolyze α-D-glucosides and β-D-glucoside remained untouched while the latter was hydrolyzed by enzyme emulsion and α-D-glucoside remains unaffected. According to Fischer

the enzymes are choosy. It picks up the substrate that fits in configuration with it like lock and key. "The main subject to which Fischer addressed his powerful experimental skills and penetrating intellect was stereoselectivity in enzyme catalysis, a field still of current significance; the ability of enzymes to select between stereoisomers has proved one of their most alluring properties" [2].

Impact: "Fischer's paper (1894) is remarkable for its discoveries themselves and also for the insight of a man of genius into future development" [2]. The lock and key theory remains a central tenet in enzyme catalysis and asymmetric organic synthesis with enzyme or chiral auxiliaries. Stereoselective synthesis is important especially in cases of chiral molecules during synthesis or their intermediates.

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1895–1945: Instrumental Analysis: X-ray. Mass Spectrometer. NMR

X-ray 1895: William Konrad (Conrad) Roentgen (Röntgen) (1845–1923) discovered X-ray in 1895 and was the first recipient of Nobel Prize (1901) in Physics. The application of X-ray has been expanded and applied in various fields. Through years of research and sophistication of this technique, it has been successfully employed in the structural elucidation of complicated as well as challenging natural and synthetic molecules.

Impact: Dorothy Crowfoot Hodgkin (1910–1994) received the Nobel Prize (1964) for the advancement of this technique and its application in the structural elucidation. She showed the β-lactam structure for penicillin. She arrived at the correct structures for quinine, morphine, strychnine, etc. This technique as well as its proliferated branches is extensively and easily used for the structural elucidation of natural products with new skeleton patterns and complicated structures. It is also applied in guest–host chemistry to find out the binding configuration.

Mass Spectrometer: 1922 Nobel Prize went to Francis William Aston (1877–1945) who invented and developed mass spectrometer and discovered most of the isotopes. The mass spectrometer attained modifications and sophistications through years of research (Sect. 4.2).

Impact: The enormous potential of mass spectral analysis was realized by Klaus Biemann (MIT), Carl Djerassi (Stanford), McLafferty (Cornell), and others. The first compound analyzed was a hydrocarbon. The exact molecular weight of the compound could be obtained, and in many cases generalized mass fragmentation

patterns (e.g., McLafferty fragmentation, retro Diels–Alder fragmentations due to buttressing effect., α-cleavage, etc.) provide definitive clue for the structural detail of the molecules. Moreover, high-resolution mass spectrometry developed later gives the exact molecular formula from the accurate *m*/*z* value of the molecular ion peak.

NMR 1945: Nuclear Magnetic Resonance phenomenon was first observed by Purcell (Harvard) and Bloch (Stanford) independently in 1945. Though it has been discovered in the physics laboratory, its influence as an essential tool for structural elucidation of organic molecules is immeasurable. In course of decades, the instrument has been modified, updated, and designed to handle organic molecules of complex structure. Its regular use in structural analysis started from late fifties.

Impact: Of all the techniques of instrumental analysis for structural elucidation of organic molecules (Sect. 4.2) NMR tops the list. Its proliferation is such that it may replace X-ray and it is more advantageous since the composition in equilibrium can be detected. It can be studied in solution.

1897: First Cell-free Enzyme Extract Prepared and Cell-free Fermentation: A Serendipitous Finding

It was believed that the enzyme-catalyzed fermentation of sugar needed the presence of enzyme in yeast cells i.e., in the presence of living organisms and this theory was supported by Louis Pasteur.

Eduard Büchner (German Chemist 1860–1917) prepared cell-free extracts from yeast cell with the help of his bacteriologist elder brother Hans for therapeutic uses and when preserved that extract in sugar solution a startling result was obtained. Sugar fermented to alcohol and carbon dioxide. Büchner thus demonstrated that fermentation is caused by the action of enzyme itself taken out of the yeast cells and the yeast cells are not needed to be present [1].

Impact: It is indeed a great blow to the belief of vital force theory since fermentation takes place outside the living organism. It is a key step in biochemistry and in enzyme-catalyzed synthesis. Various metabolic paths could be studied. The impact of the cell-free extracts of enzyme (cell-free fermentation) is such that Büchner received 1907 Nobel Prize in chemistry.

Reference

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1897–1930: Discovery of Electron. Concept of Bond Formation and Bond Breaking

Sir Joseph John Thomson (British Physicist, 1856–1940, NL 1906, father of Sir George Paget Thomson, NL 1937 [shared]), discovered "electron" in 1897. "It was an epochal moment in the history of science for the electron was the first "sub-atomic" particle and its appearance in the scientific scene dealt a death blow to the 2,000-year-old idea that atom is the indestructible building block of all matter. Atoms, to everyone's shock and amazement, were made of much smaller things [1]. Thomson was not alone with this idea; some more people like Walter Kaufman, a Physicist at the University of Berlin, and Emil Wiechert, another German Physicist at the University of Königsberg, were there also. Wiechert, for the first time in a talk delivered in January 1897, had proposed that cathode rays consisted of very light particles with a mass 2,000–4,000 times smaller than the lightest atom. But Thomson alone measured its charge, and charge-to-mass ratio in 1889, and he is universally remembered as the father of the electron [1]. The benefit of this discovery has been shared by both physicists and chemists. In an attempt to associate electrons with bonds, Gilbert Newton Lewis (1875–1946) and others suggested electron transfer and sharing between component atoms during bond formation (by S_N1, S_N2, S_N2', E1, E2 etc.). Later Sir Robert Robinson and Sir Christopher Kelk Ingold suggested the use and direction of arrow for the electron pair transfer and their role in bond formation and bond breaking during reactions (S_N1, S_N2, S_N2', E1, E2 etc.). Later, quantum theory has been applied to chemical bonding.

Impact: The concept of bond formation and bond breaking is the primary recipe in chemistry and has immense impact towards our understanding—why does a reaction occur and how—thus, the entire organic chemistry's life line is bonding. Even enzymatic reactions may be explained mechanistically in terms of S_N1, S_N2, S_N2' substitutions, E1, E2 eliminations, antiperiplanar migration with electron pair, etc. Thus, organic chemists might be able to explain many of the enzymatic reactions without adequate knowledge of enzyme chemistry. Moreover, chemical properties of elements depend on the behavior of atomic electrons in the outermost shell. The spectral properties (electromagnetic radiations) of the molecules and chemical reactions are the results of transition of electrons between different energy levels. Important structural information on the molecule could be obtained from these studies. Mass spectrometric studies are based on electron removal from the molecule and its subsequent fragmentation to yield structural information and the exact molecular weight leading to the correct chemical formula of the molecule.

Reference

1. Marcus Chown, Just Who did Discover the Electron? *New Scientist*, **1997** (Forum) (29 March), page 49.

1928: Discovery of Penicillin

Penicillin has been isolated from the mold *Penicillium notatum* by Alexander Fleming in 1928 and subsequently developed into a wonder drug against a variety of pathogens.

Impact: It is the first antibiotic drug delivered to the users which serves as the remedial agent to great sufferings and *since then microbes have joined the plants as the source of natural drugs*, and subsequently many other antibiotics have been isolated from microbes. The antibiotics are our first line of defense against well-known and emerging pathogenic bacteria.

1948: Concept of Prochirality

While interpreting the experimental observations of some metabolic processes using isotopic tracer elements, Ogston [1] came out with an idea of nonequivalence of two identical groups in a symmetrical molecule of the general formula ($Cabd_2$) toward asymmetric reagents like enzymes. This special ability of distinguishing two identical groups in a molecule ($Cabd_2$) by enzyme has been demonstrated by regiospecific decarboxylation of an α-aminomalonic acid [1]. In a three-point combination between the substrate and enzyme to form the enzyme–substrate complex, the catalytic activities of the points, at which two identical groups are attached, behave catalytically differently. This nonequivalence is reflected in decarboxylation. Hence one of the carboxylic groups gets decarboxylated, while the other does not. Since in a molecule ($Cabd_2$) when one of the identical groups undergoes virtual replacement by another unlike ligand, the carbon atom becomes chiral; hence, the previous situation prior to attending chirality is called prochirality. The carbon of $Cabd_2$ is thus called *prochiral carbon* and each d of d_2 is called *prochiral d* (vide Sect. 2.9). This is a case of central chirality. The term prochiral was introduced by Hanson [2].

Impact: The significance of this prochirality was fully realized by Cornforth who mapped the biosynthetic path of terpenoids by labeling the key prochiral hydrogens (Chap. 5) in collaboration with G. Popjak. This concept has also been employed in explaining some of the steps of other biosynthetic processes (Chap. 13) involving prochiral atoms and has been extended in cases of axial and planar chirality (vide Sects. 2.16 and 2.17).

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1948–1950: Conformational Analysis—An Outstanding Concept and the "Holy Grail" of Organic Chemistry

Conformational analysis adds a new conceptual dimension to our understanding of chemical reactivity of molecules. Different rotational organizations of bonds carrying atoms and or groups within a molecule in space give arise to different nonsuperposable arrangements having different shapes. These different shapes of a molecule keeping unaltered bond connectivity and with different angle strains are called conformers (see Sect. 2.3). Theoretically, barring the diatomic molecules, they could have innumerable number of conformers. However, energetically preferred conformers exist in rapid equilibrium [1, 2]. The conformers may have same or different internal energies, but the energy barrier between them is much less than 16 kcal/mole (see Fig. 2.24), and all attempts to isolate the conformers are frustrating. The reactivity of each conformer varies toward a particular reagent. The population of the conformer having less nonbonded interactions is generally more in the equilibrium.

Impact: The impact of conformational analysis is immeasurable in our understanding of chemical reactivity of organic molecules. The immeasurability of the impact may now be expressed in the words and comments of Barton himself who shared Noble Prize for conformational analysis with Hassel (Odd Hassel, Norwegian Chemist, 1897–1981) in 1969. He gave the title "*The Holy Grail has been found"* for his talk in "Frontiers in Chemistry, Lecture Series, on February 6, 1998, a seminar [3] arranged to commemorate the 80th Birthday of Sir Derek H.R. Barton at La Jolla, California. During Seminars at Harvard, around 1948 some reactions of Steroids could not be explained [4] and the Holy Grail of the Chemical world when found could explain all [1]. We now quote "The Light came on with conformation analysis"; the title was given by W. S. Johnson for the Advanced Topics in Organic Chemistry, Stanford University fall quarter, 1963 [5] and K. Barry Sharpless (NL 2001) commented "Sir Derek Barton received a Nobel Prize for blasting Organic Chemistry out of the dark age. . ." [5].

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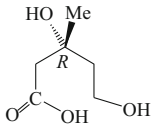
1956: The Discovery of Mevalonic Acid as a Bacterial Growth Factor

This is one of the most striking examples of serendipity (1956, by Merck group) (by Karl Folkers and his associates).

Impact: It deciphered the biosynthesis of two major classes of natural products viz terpenoids (Chap. 5) and steroids (Chap. 11). The name mevalonic acid (MVA) was assigned to this physiologically active compound and the name is derived from its systematic name (*R*-β,δ-dihydroxy-β-methylvaleric acid).



***R*-(+)-Mevalonic Acid**

1966: The Use of ^{13}C-labeled Precursors in the Biosynthetic Study of the Natural Products: A Major Breakthrough

The natural abundance of ^{13}C is much less (1.1 % only). Thus, the ^{13}C-labeled anticipated precursor of the natural product under investigation, when added to an appropriate culture, may lead to the ^{13}C-rich product, if incorporated. Prior to the feeding biosynthetic experiment, all the carbon atoms of the externally non-labeled natural product should be structurally identified. The product obtained from the experimental culture will show, in case of incorporation, the significant appearance (excess that of natural abundance) of the incorporated label carbons and the label distribution and location of labels can be determined from a comparison with the spectrum (^{13}C) of the unlabeled compound and the ratio of the ^{13}C satellites in the proton NMR spectra of the labeled and unlabeled compounds. From these data the metabolic path may be delineated.

Impact: The ^{13}C isotope is nonradioactive and NMR active. The biosynthetic studies with ^{13}C-rich precursor/s are considered to be a major breakthrough since it is a nondestructive process and involves the analysis of ^{13}CNMR and proton NMR spectral data for the identification of the location of incorporation. The method avoids time-consuming and sometimes hazardous degradation methods, isolation, and identification of the label-containing fragmented molecule/s. This type of

degradation sometimes incorporates inadvertent errors in the result and hence in the conclusion. Thus, the use of ^{13}C label precursor for the study of biosynthetic work is a breakthrough in the field of natural products chemistry and it is superior to radioactive labeling. Tanabe and Detre first employed this method for the biosynthetic study of a fungal metabolite griseofulvin [1]. Subsequently the method has been used by many others and is now used extensively [2–5].

Some reaction mechanisms on biosynthetic studies have been done with ^{13}C-labeled compounds.

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1982–1990: Combinatorial Chemistry: Drug Design and Discovery

Combinatorial Chemistry, a very important tool for drug design and discovery, was invented during 1982–1990, and since 1990 has been taken to industry. Its root was embedded in the work of Robert Bruce Merrifield (NL 1984) (1921–2006) who introduced solid-phase synthesis of peptides in and around 1960.

Conventionally for drug design and discovery the individual compound is tested at various stages necessary to get the drug/prodrug status. The main source for such molecules has been the nature as well as laboratories. The natural products are mostly complicated in structures and their isolations have been labor-intensive and a slow process, and their synthesis in most cases involve multisteps with low yields. Further, tests with these molecules, though in some cases (Chap. 33) ended up in miraculous results, many others have not shown effective/promising biological properties, and thus the efforts are not always economic in terms of both time and cost. In cases of unnatural compounds, the individual compound is synthesized, purified, and tested. Such synthesis is also time-consuming. On the whole, the procurement of the anticipated drug/prodrug molecules from such sources is thus a long-drawn slow process and economically not cost-effective.

In view of the vast demand of new drug molecules (mainly caused by drug resistance and revelation of various unwanted side effects which call off the drug from the market and call for new drug molecules) and to avoid the bottleneck of drug research, a method has been developed and modified through years to synthesize a large number of different compounds within a short period of time. The term

combinatorial synthesis is self-explanatory since in this process an array of compounds are synthesized involving the combination of a privileged motif/template/scaffold (designed synthetically or synthetically copied from bioactive natural products) with different reagents with the hope of emulating the biological properties of the bioactive natural products as such or in an improved state. There are various combinatorial approaches. In one such approach the reactions are carried out in a number of small vessels (V), and in each the same starting compound (C) anchored to a solid resin bead through an appropriate linker is taken. To each vessel different reagents (R) are added and allowed to react in a well-defined identical condition for a short time when a number of different compounds are formed. The statistical probability of combination is increased by mixing up the products of the individual vessel (pool system) and then again the resulting mixture is distributed (split system) in a series of vessels and different reagents are added to each vessel and allowed to react as before. The process is repeated forming a large number of compounds with the desired motif. The motif is generally selected when it is present as a subunit in a number of biologically active natural compounds and can accommodate chemical diversification on its frame. The large number of compounds thus formed constitutes the library of compounds carrying that motif/template. Each compound should carry a tag containing its chemical identity and other relevant data for future biological evaluation. Each library thus provides several compounds and may be thousands with a particular template chemically diversified in all possible desired ways. The technique heading for more combination is thus based on split and pool strategy. A simplified version of combinatorial synthesis is shown in Fig. B.3. The content (mixture) of each vessel at each step may be biologically evaluated prior to the isolation of individual compound and inactive mixtures may be kept aside. The compounds are screened by high-throughput screens (HTS), a very rapid and sensitive *in vitro* screen developed during 1989–1991. Now thousands or more compounds may be robotically screened per day on submicro scales. This leads to the probability of getting more lead compounds from large number of screenings.

Impact: A large number of compounds with a desired template could be prepared and thus could be used extensively for drug research.

Advantages: Bead-tagged individual compound could easily be separated and purified by washing the impurities including the unused reagents, which are generally used in excess to push the reaction in the forward direction. The products will remain anchored to the beads and could be delinked from the anchor easily.

The possibility of discovering drug molecules is more since a library of compounds with privileged motif could be tested for drug evaluation. So, the bigger is the library, higher is the probability of discovery of the drug. However, on several occasions, the reactions do not take place as desired or expected. The possibility of obtaining novel-structured compounds compared to natural products is less. In this regard Roald Hoffmann (NL 1981) commented that nature took millions of years to optimize the condition to biosynthesize her designed novel molecules.

Nicolaou and his collaborators have utilized solid-phase combinatorial synthesis to construct focused libraries of natural products analogues (small molecules). He reported combinatorial libraries with α,α-dimethyl benzopyran scaffold [1–3].

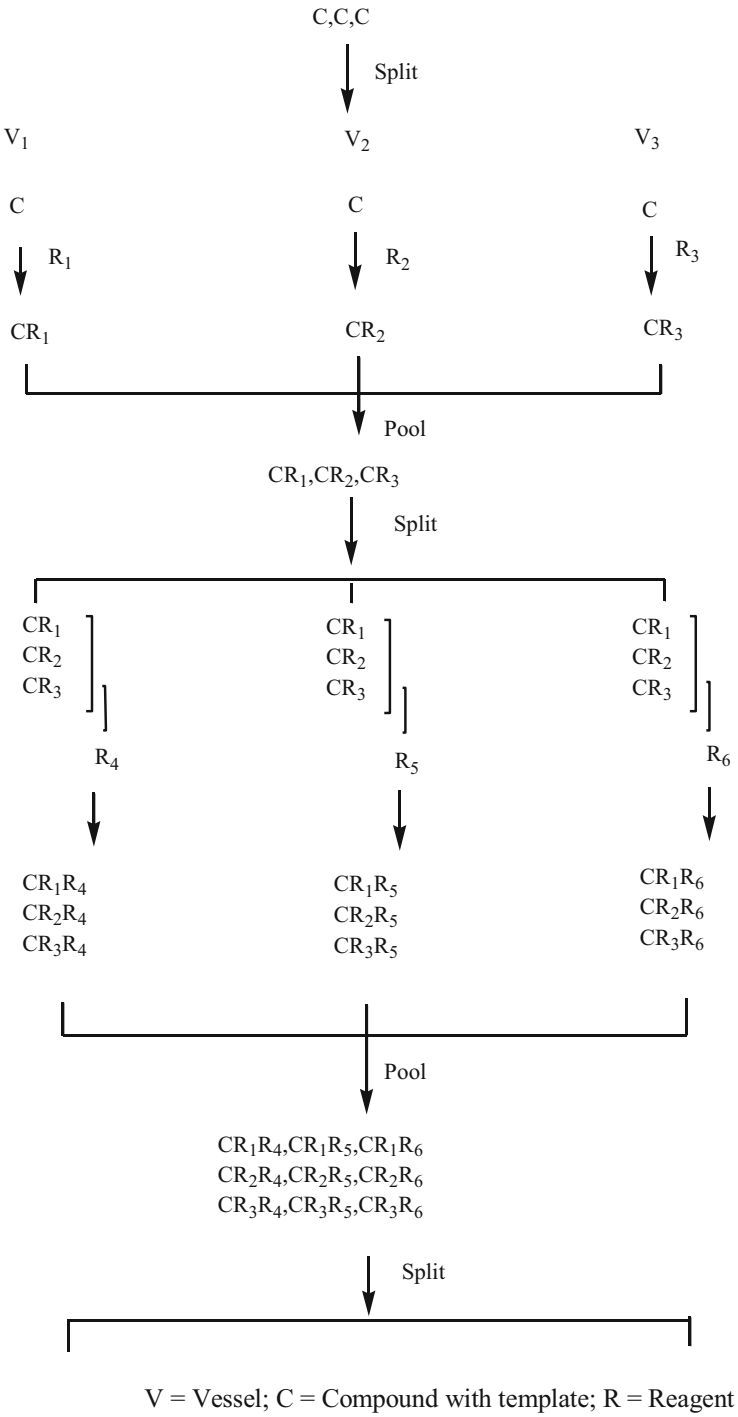

Fig. B.3 A simplified version of combinatorial chemistry

1903: Chromatography—An Indispensable Separation Technique

See Sect. 4.1 and the life sketch of Tswett (A.27).

1917: Concept of Biomimetic Synthesis and Multicomponent Reaction

Tropinone is a common structural moiety of several alkaloids of which atropine, cocaine, etc., are familial names. Tropine has been synthesized by Richard Willstälter (NL 1915, Appendix A, A-30, German Chemist) in a multistep process (Sect. 19.3.1, Fig. 19.10). But Robinson from the symmetry of the tropinone molecule could understand that it could be synthesized in good yield in a very simple way close to that of Nature. In fact he successfully synthesized tropinone from salicyldehyde, methylamine, and acetone [1] (Sect. 19.3.2, Fig. 19.11).

Impact: Robinson's tropinone synthesis serves as the first example of biomimetic synthesis involving the Nature's synthetic strategy—atom economy, step economy, and mild conditions. These points are in the primary focus of the synthetic organic chemists of the present day. Thus biomimetic synthesis of tropinone opened a new avenue to the synthetic organic chemists [2]. Further, the concept of multicomponent reactions (MCRs) in the synthesis has been demonstrated by tropinone synthesis of Robinson. MCRs are broadly defined, regardless of their mechanistic nature, as "one-pot" processes that combine three or more substrates either simultaneously (so-called "tandem," "domino," or "cascade" reactions) or through a sequential addition procedure that does not involve any change of solvent. By minimizing the number of synthetic operations while maximizing the buildup of structural and functional complexity, these highly step economical reactions are particularly appealing in the context of target-oriented synthesis [3]. Robinson's tropinone synthesis, nearly a century ago (1917), demonstrated a 3CR process (i.e., 3-component reaction in one pot). This MCR concept though overlooked for decades has now become an important criterion in the strategy of the synthesis of target molecules. However, the Strecker reaction for the synthesis of α-aminonitriles reported in 1850 (*vide* Fig. 31.24) may be considered as the first example of a multicomponent synthesis.

References

1. Robert Robinson, A Synthesis of Tropinone, *J. Chem. Soc*., **1917**, 762-768.
2. E. E.van Tamelen, Biogenetic-type Synthesis of Natural Products, *Fortschritte d.chem. org. Naturst*. **1961**, *19*, 242-290.
3. Barry B. Touré and Dennis G. Hall, Natural Product Synthesis Using Multicomponent Reaction Strategies, *Chem. Rev.,* **2009**, *109*, 4439-4486.

Arpad Furka is considered to be one of the pioneers of combinatorial syntheses (1982) and H. Mario Geyson developed in 1984 a technique for synthesizing peptides on pin-shaped solid support. In 1985, Richard Houghten introduced the "tea bag" method for rapid multiple peptide synthesis, and Ellman *et al* started in 1992, solid-phase synthesis of small molecules [4].

Combinatorial synthesis has been proliferated and sophisticated, and libraries of molecules with complex structures could be generated. Dynamic combinatorial chemistry [5] has been a very useful tool which provides diversity and complexity as an efficient means to discover the drugs.

References

1. K. C. Nicolaou, J. A. Pfefferkorn, A. J. Roecker, G.-Q. Cao, S. Barluenga and, H. J. Mitchell, Natural Product-like Combinatorial Libraries Based on Privileged Structures. 1. General Principles and Solid-Phase Synthesis of Benzopyrans, *J. Am. Chem. Soc*., **2000**, *122*, 9939-9953.
2. K. C. Nicolaou, J. A. Pfefferkon, H. J. Mitchell, A. J. Rpecker, S. Barluenga, G.-Q. Cao, R. L. Affleck, and J. E. Lillig, Natural Product-like Combinatorial Libraries Based on Priviledged Structures. 2. Construction of a 10000-Membered Benzopyran Library by Direct Split-and-Pool Chemistry Using Nanokans and Optical Encoding, *J. Am. Chem. Soc.,* **2000**, *122*, 9954-9967.
3. K. C. Nicolaou, J. A. Pfefferkorn, S. Barluenga, H. J. Mitchell, A. J. Roecker and G.-Q. Cao, Natural Product-like Combinatorial Libraries Based on Privileged Structures. 3. The "Libraries from Libraries". Principle for Diversity Enhancement of Benzopyran Libraries, *J. Am. Chem. Soc*., **2000**, *122*, 9968-9976.
4. Richard B. Silverman, *The Organic Chemistry of Drug Design and Drug Action*, Elsevier, Second Edition, Amsterdam, Boston, **2004**, pp. 34-43.
5. Sophie R. Beeren and Jeremy K. M. Sanders, History and Principles of Dynamic Combinatorial Chemistry in *Dynamic Combinatorial Chemistry*, Ed. N. H. Reck and Sifbren Otto, Wiley-VCH, **2010**, pp. 1-22.

Further Reading

- Graham L. Patrick, *An Introduction to Medicinal Chemistry*, Oxford University Press, Fourth Ed., 2009 (Indian Ed., pp. 307-331)
- K. C. Nicolaou and T. Montagnon, *Molecules that Changed the World*, Wiley VCH, **2008,** p. 256.

Appendix C

Miscellaneous Helpful Information for Students

(1) **Spellings Generally Confused. Some examples**

(a) **Hoffmann:** Hoffmann-La-Roche, Total Synthesis of Quinine; Woodward-Hoffmann Rule; Jules A Hoffmann (NL 2011, in Physiology/Medicine); Bayer chemist Felix Hoffmann synthesized aspirin.

Hofmann: Hofmann Degradation (Elimination)

Hoffman: Frances Hoffman, Director of Chemical Laboratories, Columbia University; (see [1] of Appendix A.26)

Limonene (a monoterpene) C_{10}, **Limonin** (a tetranortriterpenoid) C_{26}

Cinchona alkaloids: Countess of **Chinchon** was treated with *Cinchona* bark extract for her fever (See **25.1**).

Chrysin (a ring B unsubstituted flavone, Fig. 14.7), **Chrysene** (a tetracyclic aromatic hydrocarbon, **10.29**)

Sclarene (a C_{20} bicyclic diterpene), **Sclarin** (a C_{25} sesterterpene), **Sclerin** (an aromatic dicarboxylic acid anhydride, a plant growth hormone), **Scleroin** (2,5-Dihydroxy-3,4-dimethoxybenzophenone)

(b) (**R** means **R**ight, **W** means **W**rong spelling for the following words): equ**a**torial (**R**), equ**i**torial (**W**); des**icc**ator (**R**), des**i**cator (**W**), des**s**icator (**W**); station**a**ry phase (**R**), station**e**ry phase (**W**); tartrate (**R**) as in diethyl tartrate (DET), tart**a**rate (**W**); **ph**t**h**alic Acid (**R**), phtalic Acid (**W**), thalic acid (**W**); sep***ara***te (**R**), sep**e**rate (**W**), separ**e**te (**W**).

(2) **Chemical Bonds**

—— Bond in the plane of the paper

► Bond projecting forward in front of the paper

Bond projecting backward behind the paper

▬ Bond in front of other bonds

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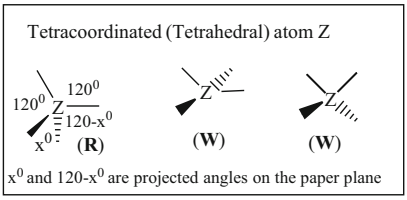
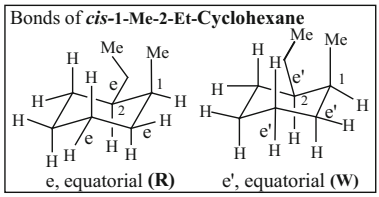
DOI 10.1007/978-3-642-45410-3, © Springer-Verlag Berlin Heidelberg 2015

----- Partly formed bond (for example, transition state, H-bond)
〰 Stereochemistry unidentified/not specified/random, also used in part structure

(3) **Some Incorrect Representations**
(**R**) Means **R**ight representation, (**W**) means **W**rong representation)

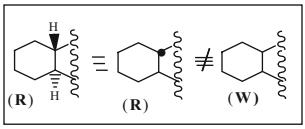


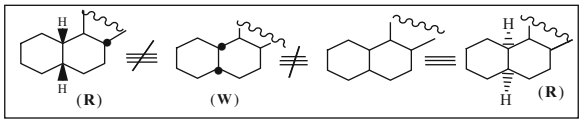
The β-, and α- bonds are in a plane perpendicular to the plane of the paper containing the other two bonds. The two planar bonds are usually directed opposite to the two nonplanar bonds, as shown in **(R).**

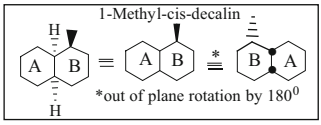


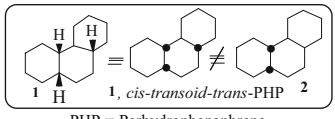
Directions of the equatorial bonds are to be noted; equatorial bond is parallel to the second ring bond on either side ***All axial bonds mut be parallel***

S_N2 (**R**), SN^2(**W**); S_N1(**R**), SN^1(**W**); Mass spectrometry (**R**), Mass spectroscopy (**W**)

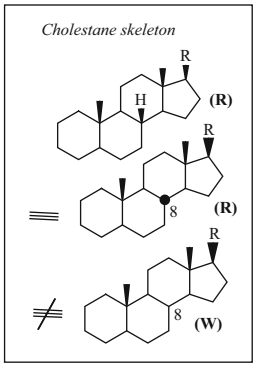


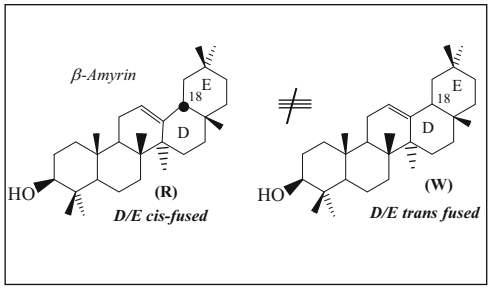






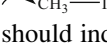
PHP = Perhydrophenanhrene
2, *cis-transoid-cis*-PHP



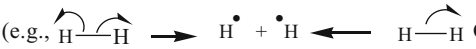


Note: *Whenever there is a β-H at the ring juncture of any structure, it should be shown with a β-bond or with just a dark solid circle at the juncture. In the absence of such presentation, conventionally, the hydrogen at the ring juncture will be considered as α-H. By oversight the β-H or a solid circle at the ring juncture is commonly not shown in the literature, especially in cases of steroid skeleton at C8 and in cases of triterpenes with cis fused D/E rings at C18.*

(4) **Significance of Arrows**

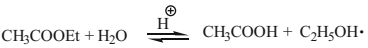
(a) ↷ movement of a pair of electrons in the direction of the arrow (e.g., ). The arrow should avoid touching any bond or atom, but should indicate the bond being formed or broken with electron pair.

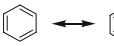
(b) ↶ fishhook arrow indicates the movement of one electron in the direction of the arrow.



(c) ⇵ indicates pair of electrons with *opposite spin in an orbital;* sometimes expressed vertically written reversible reactions (see, Fig. 3.9).

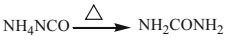
(d) ↑ ↑ indicates two electrons with *same spin in two different orbitals*.

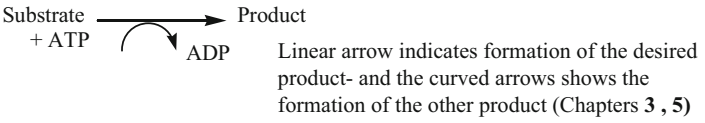
(e) ⇌ sign of equilibrium in a chemical reaction, e.g., 

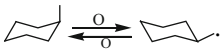
(f) ⟷ sign of resonance between canonical forms (e.g., ).

(g) ⇉ represents a multistep process.

(h) ⟶ indicates irreversible path of a reaction or, sometimes to show advancement in the forward direction, e.g.,

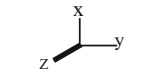




(i) ⇄ indicates ring inversion or flipping, e.g.,  .

(j) ↶ indicates addition followed by elimination.

(5) **Symbols of Various Types of Rotations About Specified Axes**



(left-handed Cartesian co-ordinates)

Rotate clockwise (*cw*) in the plane of the paper by ~ x^0 (about z-axis)

Rotate anticlockwise (*acw*) in the plane of the paper by ~ x^0 (about z-axis)

Rotate *acw* about the vertical axis (x-axis) by ~ x^0.

Rotate *cw* about the vertical axis (x-axis) by x^0

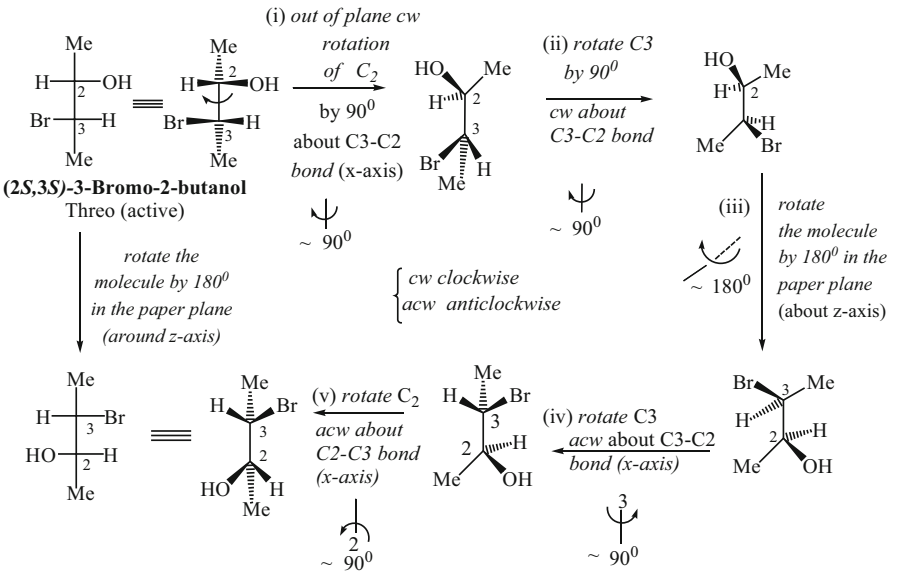
Rotate *acw* about the horizontal axis (y-axis) by x^0.

Rotate *cw* about the horizotal axis (y-axis) by x^0.

Rotate *acw* about the given axis by x^0.

Rotate *cw* about the given axis by x^0.

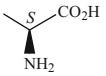
(6) **Rotations about C2-C3 Bond of (2S,3S)-3-Bromo-2-butanol**

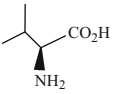


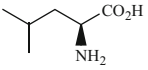
(7) **Protein Forming 20 Amino Acids Encoded by DNA** (Structures, Names, Three-Letter and One-Letter Abbreviations)

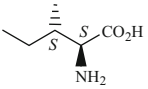
Aliphatic Amino Acids :


Glycine Gly G

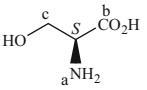

Alanine Ala A

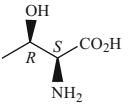

Valine* Val V

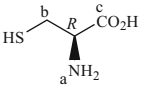

Leucine * Leu L

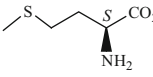

Isoleucine * Ile I

Aliphatic Amino Acids with Hydroxyl or Sulfur Containing Side chain

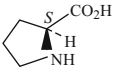

Serine Ser S


Threonine * Thr T

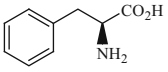

Cysteine Cys C

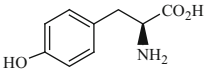

Methionine* Met M

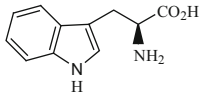
Cyclic Amino Acid (α-NH_2 group as a part of a ring)


Proline Pro P

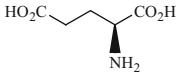
Aromatic Amino Acid

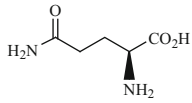

Phenylalanine * Phe F

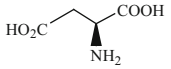

Tyrosine Tyr Y

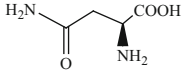

Tryptophan* Trp W

Acidic Amino Acids and Their Amides

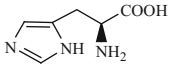

Glutamic Acid Glu E

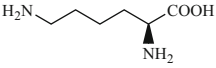

Glutamine Gln Q

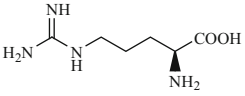

Aspartic Acid Asp D


Asparagine Asn N

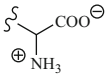
Basic Amino Acids


Histidine * His H


Lysine* Lys K


Arginine Arg R

Zwitterion Form of Amino Acids



Notes: i) *The α -carbon of each of these amino acids excepting the achiral glycine possesses* **L-** *configuration. The α-carbon of each of these chiral amino acids excepting cysteine is specified (S); the α-carbon of cysteine is assigned (R) due to the reversal of the CIP priority sequence, as shown.*

ii) *The essential amino acids marked with asterisk can not be synthesized in human body, and must be acquired in the diet.*

(8) **Full Description of an Optically Active Compound** should be represented by rotational sign as well as absolute configuration (*R*-*S*), if known, of the chiral center/s, e.g., *R*-(+)-mevalonic acid, *S*-*(*−)-mevalonic acid. Quite often the former appears either as *R*-mevalonic acid or (+)-mevalonic acid. Likewise, biologically active form of glyceraldehyde appear in the literature as **D**-glyceraldehyde or less frequently as (+)-glyceraldehyde. Simple (*R*)-mevalonic acid or (*S*)-mevalonic acid will not suggest the absolute configuration for (+) or (−) variety. However, (*R*)-mevalonic acid and **D**-glyceraldehyde are often used well-known compounds; their rotational senses are also well-known, and hence may not always appear in their representations. Many examples of full descriptions of optically active compounds, displaying both their sign of rotation and absolute configuration, may be found in the Chaps. 6–29.

D/L notations represent Absolute configuration as applied to amino acids and sugars (not related to rotation); d/l represents dextro/levo rotation (not related to absolute configuration).

(9) **Some Commonly Used Terms and Symbols**
(L) Latin, (Gk) Greek

de novo (L)	A new; again; from the beginning
et al. (L)	And others
ibid (L)	In the same book, journal, as in the preceding reference
idem (L)	The same authors as mentioned in the preceding reference
in situ (L)	Existing place
quasi (L)	As if
in vivo (L)	Made to occur within the living organism
in vitro (L)	Controlled experimental condition outside the living organism
per se (L)	of, on, in itself
domino process/ effect (L)	The cumulative process/effect that results when one event precipitates a series of like events
tandem (L)	In conjunction with; one following the other
modus operandi (L)	Mode of working; way a thing operates
enantios (Gk)	Opposite
syn/anti (Gk)	Together/against
supra (L)	Top; above; especially used to refer to an earlier part of the text
infra (L)	Under; below; especially used to refer to a later part of a text
e.g. (L) (exempli gratia)	As for example
i.e. (L) (id est)	That is
viz (L) (videlicet)	Namely
rectus (L)	Right
sinister (L)	Left
homos (Gk)	Same
morphe (Gk)	Form
topos (Gk)	Place, physical surrounding
cheir (Gk)	Pertaining to hand
etymology	Derivation of word (Gk/L etymologia)
tincture	An alcoholic solution of medicine or similar things
marc	Residue left after the solvent extraction of the plant materials or some vegetable drugs

(10) **NMR Solvent Data** (taken on a Varian Gemini 200 Spectrometer at 22 °C)

Solvent (Boiling point, °C)	Chemical shift (ppm from TMS), (**Multiplicity**, Coupling constant J_{HD}/J_{CD})	
	^{1}H	^{13}C
Acetic acid-d_4 (118)	11.65 (**1**); 2.04 (**5**, 2.2 Hz)	178.99 (**1**); 20.0(**7**, 20Hz)
Acetone-d_6 (57)	2.05 (**5**, 2.2 Hz)	206.68 (**13**, 0.9 Hz); 29.92 (**7**, 19.4 Hz)
Acetonitrile-d_3 (82)	1.94 (**5**, 2.5 Hz)	118.69 (**1**), 1.39 (**7**, 21 Hz)
Benzene-d_6 (80)	7.16 (**1**)	128.39 (**3**, 24.3 Hz)
Chloroform-d (62)	7.27 (**1**)	77.23 (**3**, 32.0 Hz)
Dimethylsulfoxide-d_6 (189)	2.50 (**5**, 1.9 Hz)	39.51 (**7**, 21.0 Hz)
Ethanol-d_6 (79)	5.29 (**1**), 3.56 (**1**)	56.96 (**5**, 22 Hz)
	1.11 (m)	17.31 (**7**, 19 Hz)
Methanol-d_4 (65)	4.87 (**1**)	49.15 (**7**, 21.4 Hz)
	3.31 (**5**, 1.7 Hz)	
Methylene Chloride-d_2 (40)	5.32 (**3,** 1.1 Hz)	54.00 (**5,** 27.2 Hz)
Pyridine-d_5 (116)	8.74 (**1**)	150.35 (**3**, 27.5 Hz)
	7.58 (**1**)	135.91 (**3**, 24.5 Hz)
	7.22 (**1**)	123.87 (**5**, 25 Hz)
Trifluoroacetic Acid-d (72)	11.50 (**1**)	164.2 (**4**)
		116.6 (**4**)

Notes:
(i) *The samples for the ^{1}H and ^{13}C NMR spectra contain a maximum of 0.05 % and 1.0 % TMS v/v respectively*
(ii) *The spin of deuterium being 1, the $CDCl_3$ peak in ^{13}C spectra appears as a triplet caused by coupling of carbon with deuterium with the intensity ratio 1:1:1*
(iii) *The multiplet "m" at 1.11 ppm (of ethanol-d_6) denotes a broad peak with some fine structures*
(iv) *The chemical shifts, in particular, can depend on solvent, solute concentration and temperature*
(v) *"Solution: Water (as H_2O, HDO, or D_2O) can be minimized by adding molecular sieves to the solvent, agitating the mixture, and allowing it to stand for a few hours. The water content may be reduced to about 10–20 ppm in this manner. If exchange still causes a problem, it is recommended to use less hygroscopic solvent, such as chloroform, methylene chloride, or acetonitrile"*
The above data are taken from the on-line link "NMR_Solvent_Data_Chart.pdf", Cambridge Isotope Laboratories

(11) **Isotope Distribution of Elements**

Element	Mass	Relative abundance	Element	Mass	Relative abundance
Hydrogen (H)	1	99.99	Phosphorus P	31	100
(D)	2	0.01	Chlorine Cl	35	75.77
Nitrogen (N)	14	99.60	^{37}Cl	37	24.23
^{15}N	15	0.40	Carbon C	12	98.9
Oxygen O	16	99.76	^{13}C	13	1.1
^{17}O	17	0.04	Bromine Br	79	50.5
^{18}O	18	0.20	^{81}Br	81	49.5
Fluorine F	19	100.00	Iodine I	127	100

If an organic compound contains one bromine atom per molecule, a twin peak [e.g., M^+ and $(M+2)^+$ (1:1)] and, in case of one chlorine, a twin peak (M^+ and $(M+2)^+$ (1:0.3] will appear in their mass spectra.

(12) Numerical Prefixes in Chemical Words

1/2	semi-, hemi-, demi-	11	undeca-, hendeca-	24	tetracosa-
1	uni-, mono-, holo-	12	dodeca-	25	pentacosa-
1½	sesqui-	13	trideca-, triskaideca-	26	hexacosa-
2	bi-, di-, diplo-	14	tetradeca-	27	heptacosa-
2½	hemipenta-, sester-	15	pentadeca-	28	octacosa-
3	ter-, tri-	16	hexadeca-	29	nonacosa-
4	tetra-, quadri-	17	heptadeca-	30	triaconta-
5	penta-, quinto-	18	octadeca-	40	tetraconta-
6	Hexa-, sexa-	19	nonadeca-	50	pentaconta-
7	hepta-, septa-	20	eicosa-	60	hexaconta-
8	octa-, okta-	21	heneicosa-	70	heptaconta-
9	nona-, ennea-	22	docosa-	80	octaconta-
10	deca-	23	tricosa-	90	nonaconta-
100	hecta-	101	henhecta-	102	dohecta-
110	decahecta-	120	eicosahecta-	200	dicta-

(13) SI Prefixes (Système International d'Unités)

Prefix	Symbol	Fraction	Prefix	Symbol	Multiple
Deci	d	10^{-1}			
Centi	c	10^{-2}	Deca	da	10
Milli	m	10^{-3}	Hecto	h	10^2
Micro	μ	10^{-6}	Kilo	k	10^3
Nano	n	10^{-9}	Mega	M	10^6
Pico	p	10^{-12}	Giga	G	10^9
Femto	f	10^{-15}	Tera	T	10^{12}

(14) Formation of Plurals

Latin

aquari**um**, bacteri**um**, corrigend**um**, dat**um**, maxim**um**, medi**um**, memorand**um**, minim**um**, quant**um**, sept**um**, spectr**um**, strat**um**, (for plural **um** → **a**)
alumn**us,** calcul**us**, nucle**us**, radi**us** (**us** → **i**)
alg**a**, lacun**a**, lamell**a**, lamin**a** (**a** → **ae**)
apex, apices; calx, calces; helix, helices; latex, lattices; matrix, matrices; vertex, vertices.

Greek

criteri**on,** dodecahedr**on**, icosahedr**on**, octahedr**on**, phenomen**on**, polyhedr**on**, tetrahedr**on** (**on** → **a)**
analys**is**, diagnos**is**, dialys**is**, electrolys**is**, hydrolys**is**, hypothes**is**, prosthes**is**, pyrolys**is**, synthes**is**, thes**is**, prognos**is** (**is** → **es**)

(15) Brief Etymology (Origin of Words) of Some Traditional Chemical Names

Consult (i) Alex Nickon and Ernest F. Silversmith, *Organic Chemistry: The Name Game,* Pergamon Press, New York, Oxford, **1987,** pp 304-317.

(ii) Stanley C. Bevan, S. John Gregg, and Angela Rosseinsky, *Concise Etymological Dictionary of Chemistry*, Applied Science Publishers, London, **1976**, pp 140.

The present book is concluded with a quotation from Göethe[6]

Nature! Out of the simplest matter it creates most diverse things, without the slightest effort, with the greatest perfection, and on every thing it casts a sort of fine veil. Each of its creations has its own essence, each phenomenon has separate concept, but everything is a single whole.

[6] Taken from the *Amazingly Symmetrical World* by L. Tarasov, Mir Publishers, 1986 (English Publication).

Plant Index[1]

[1] Note: Page numbers followed by f denote figures and page numbers followed by t denote tables.

[2] The family of each genus is written in parenthesis against a species of that genus, appearing first when there are more than one species of that genus in this list.

[3] The families Leguninosae = Fabaceae; Umbelliferae = Apiaceae; Compositae = Asteraceae; Liliaceae = Alliaceae; Brassicaceae = Cruciferae; Cornaceae = Nyssaceae

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Subject Index[1]

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