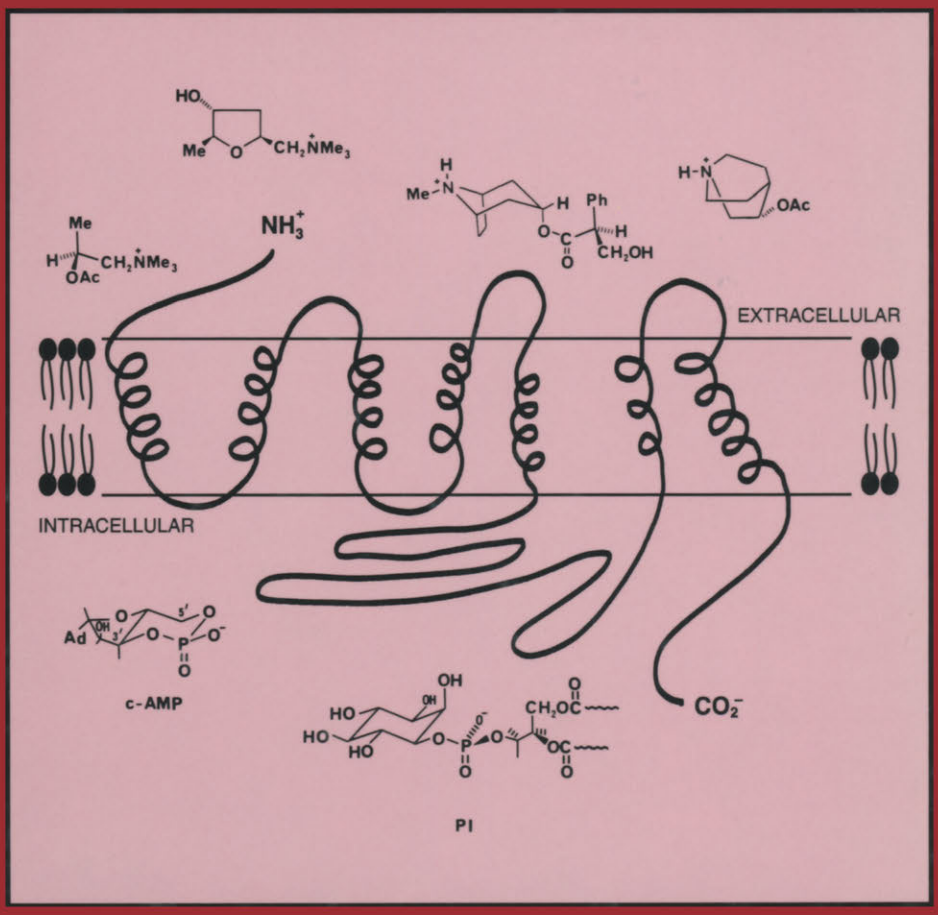
THE STERIC FACTOR IN MEDICINAL CHEMISTRY

Dissymmetric Probes of Pharmacological Receptors



ALAN F. CASY

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With love to my wife, Mary

Foreword

Stereochemistry was born in the second half of the 19th century with the publications of Pasteur in 1860, van't Hoff in 1874, and Le Bel in 1874, a remarkable period that followed rather closely the progressive appearance of chemistry as we understand it now. But chemical compounds are three-dimensional entities, implying that, in some sense, the whole of chemistry *is* stereochemistry. The two-dimensional *description* of chemical structures necessarily results in a loss of information, and this is why stereochemistry is of particular relevance when it comes to designing drugs and other bioactive compounds and investigating their structure–activity relationships.

The study of stereoselective phenomena in chemistry, biochemistry, and pharmacology has proven remarkably fruitful in deepening our understanding of chemical and biological processes. In such investigations, stereoisomeric compounds are probes of particular efficiency, and to neglect or ignore their avail can only lead to scientific impoverishment and limited vision (see Fig. F1 below). There is thus a

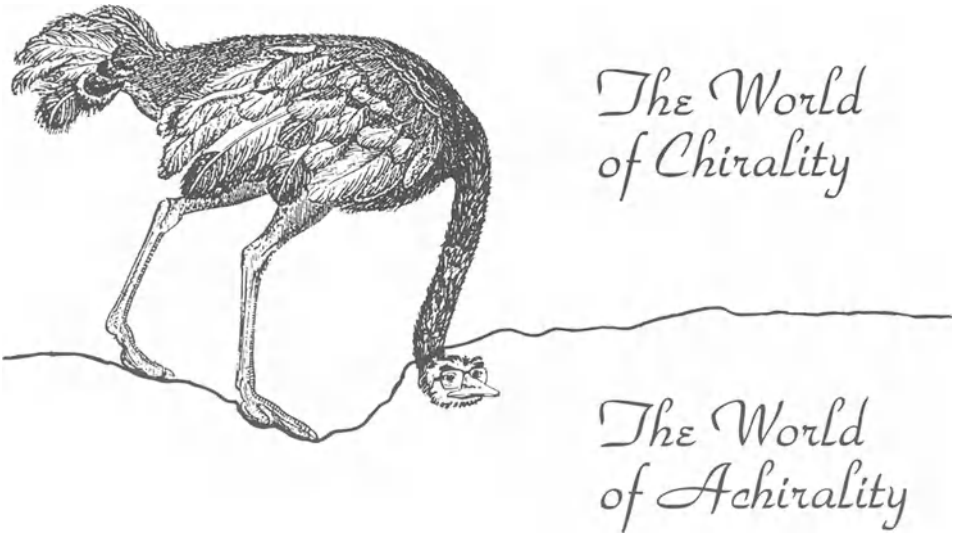

FIGURE F1. Ignoring chirality? (Cartoon by Patrick Bertholet. Copyright B. Testa.)

need to confront such ignorance and encourage the study of stereoselective phenomena and the use of stereoisomeric probes, but this must be done without obsession and exclusiveness. Such a reasonable, balanced, and lucid frame of mind inhabits every chapter of the book you now read. It is indeed the merit of Dr. Alan F. Casy to bring to these pages a clear and comprehensive view of medicinal stereochemistry, a discipline in which he has been active and successful for many years both as a teacher and a researcher.

Written for graduate students and research workers in medicinal chemistry and pharmacology, this book will contribute significantly toward a better *education* of scientists by removing the fear of stereochemistry caused by ignorance, moderating the overconfidence of possible zealots, and outlining a broader context. This is what education is about.

Bernard Testa

Lausanne, Switzerland

Preface

This book sets out to provide source material on stereochemical influences in medicinal chemistry and pharmacology. While there is much review literature on individual areas of these disciplines, the absence of a coordinated account which presents both the biological data and details (together with evidence) of the spatial characterization of stereoisomeric sets is the prime reason for the present undertaking. A secondary motivation, which I advance as some jusitification of my role as author of such a work, is my early introduction to the field and the underlying stereochemical theme of much of my research career. I was fortunate when still in my mid-20s to have Arnold H. Beckett as my mentor, a man who was one of the first to recognize the significance of molecular shape in medicinal chemistry and the fact that "nature undoubtedly carries out her reactions on a three-dimensional basis." In the early 1950s, when I joined AHB at Chelsea, stereochemical studies of this kind were relatively rare and restricted to a few areas, but they were rapidly to burgeon and encompass the entire spectrum of biological activity. Today one has only to scan a current issue of the *Journal of Medicinal Chemistry* to appreciate how many articles carry some aspect of a stereochemical role. Advances in the methodology of both pharmacology and organic stereochemistry have been profound over the past 40 years and have enabled the realization of such a growth of interest.

Clearly, no one book (let alone one with a single author) can present the entire field of stereochemical medicinal chemistry. Because of my own special interest, I have chosen to select material that relates to well-investigated neurotransmitter–receptor systems, namely, ligands of adrenergic, dopaminergic, cholinergic, histaminergic, and serotoninergic receptors, and also to opioid receptors. The stereochemical aspects of each type are treated in depth with a literature coverage extending up to the end of 1990 (with some later additions). The book opens with an introductory chapter that covers bibliographical sources, the objects of stereochemical investigations, important concepts, and the pattern of presentation. The second chapter deals briefly with matters of nomenclature and methodology with emphasis on modern developments, while the third is devoted to pharmacokinetics. Specific topics are presented in Chapters 4 through 14. The Appendix and Postscript provides some concluding remarks and guides the reader to stereochemical studies of areas not covered in the main text.

While the book is directed at medicinal chemists and pharmacologists actively engaged in research, whether in academia or the pharmaceutical industry, it is hoped that this volume will prove valuable and enlightening to postgraduate and senior undergraduate students of chemistry, medicinal chemistry, pharmacology, pharmacy, and biochemistry (and to students of the life sciences in general).

Colleagues who have responded so generously to my many letters of enquiry and requests are too numerous to be listed here in entirety. I should, however, like to express special appreciation to Bernard Testa who has kindly provided the Foreword, and George Dewar for his contribution to Chapter 10 on neuromuscular blocking agents. Thanks are also due to Richard Barlow, Robin Ganellin, Arthur Jacobson and Kenner Rice (of NIH, Bethesda), Håkan Wikström, Uli Hacksell, and Anette Johansson (of the Uppsala group), Günther Lambrecht, John Neumeyer, Popat Patil, Robert Ruffolo, Walter Schunack, Jan Tollenaere, and Bill Trager. Finally I thank my typist, Helen Thame, for her careful and patient work, and Robert Parfitt for his encouragement and support.

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Alan F. Casy

Bath

Abbreviations

ACE	angiotensin converting enzyme
ACh	acetylcholine
AChE	acetylcholinesterase
ACTM	1-acetoxy-2-trimethylammoniumcyclopropane (ACh agonist)
ADTM	aminodihydroxytetralin (DA agonist)
AGP	alpha (α_1)-glycoprotein
Ar	aryl
ATP	adenosine triphosphate
AUC	area under curve (pharmacokinetic usage)
ax	axial
BOC	*t*-butyloxycarbonyl
c	*cis*
c-AMP	cyclic adenosine monophosphate
CBM	cyclobutylmethyl
CD	circular dichroism
CNA	chlornaltrexamine
CNS	central nervous system
COMT	catechol-*O*-methyl transferase
CPM	cyclopropylmethyl
CP-MAS	cross-polarization magic-angle spinning (solid-state NMR)
CSP	chiral stationary phase
CSR	chiral shift reagent
DA	dopamine
4-DAMP	benzilate of 1-methyl-4-piperidinol methiodide
DCI	dichloroisopropenaline
DMF	dimethylformamide
DOPA	dihydroxyphenylalanine
DPAT	2-dipropylaminotetralin
E	entgegen
EAQ	eudismic affinity quotient
ED_{50}	effective dose in 50% of a population
EHT	extended Hückel treatment (MO methods)

EI	eudismic index
EKC	ethylketazocine
EM	extensive metabolizers
EPMR	equipotent molar ratio
eq	equatorial
ER	eudismic ratio
FNA	funaltrexamine
GC	gas chromatography
GC-MS	gas chromatography — mass spectrometry (in combination)
GI	gastrointestinal (tract)
GITC	2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate
GP	guinea pig
GPI	guinea pig ileum
GTP	guanosine triphosphate
HPLC	high performance liquid chromatography
HSA	human serum albumin
5-HTP	5-hydroxytryptophan
HYP	hydroxypindolol
IC_{50}	concentration of drug that displaces 50% of a radioactive ligand (in binding assays)
ICYP	iodocyanopindolol
INPEA	4-nitro analogue of isoprenaline
IR	infrared (spectroscopy)
iv	intravenous
J	coupling constant in Hz, ^2J over two, ^3J over three bonds etc. (in NMR spectroscopy)
LAH	lithium aluminum hydride
*m*AChR	muscarinic cholinergic receptor
MAO	monoamine oxidase
MHP	mouse hot-plate (opioid assay)
MO	molecular orbital
MTPA	2-methoxy-2-trifluoromethylphenylacetic acid
NA	noradrenaline (NE norepinephrine)
*n*AChR	nicotinic cholinergic receptor
NIH	National Institutes of Health (Bethesda, MD)
NMR	nuclear magnetic resonance (spectroscopy)
NMS	*N*-methylscopolamine
NOE	nuclear Overhauser enhancement (in NMR)
NPA	*n*-propylapomorphine
NSAID	nonsteroidal antiinflammatory drug
ORD	optical rotatory dispersion
OXO	oxotremorine
OXO-M	methiodide of oxotremorine
pA_2	negative logarithm of dose of antagonist that converts response to a double dose of agonist to that of a single dose
PBZ	phenoxybenzamine
PCP	phencyclidine
PI	phosphoinositol

PJ	Pharmaceutical Journal
PM	poor metabolizers
PNMT	phenylethanolamine *N*-methyltransferase
3-PPP	3-(*m*-hydroxyphenyl)-1-*n*-propylpiperidine
QNB	benzilate ester of 3-quinuclidinol
R (*S*)	Rectus (Sinister) configurational symbols of the Cahn–Ingold–Prelog protocol
r	reference (configurational usage)
RIA	radioimmunoassay
RVD	rat vas deferens
SSB	stereospecific binding
t	*trans*
TiPS	*Trends in Pharmacological Sciences*
TLC	thin layer chromatography
TMQ	trimetoquinol
Ts	tosyl (*p*-tolylsulfonyl)
Tz	telenzepine
UV	ultraviolet (spectroscopy)
Z	zusammen

Contents

1

Introduction

I am addicted to medicinal chemistry. I get high on it.
— SIR JAMES BLACK, Nobel Laureate.
(*Observer Profile*, London, October 23, 1988)

Examples of stereoisomeric pairs in which the members show pronounced differences in their biological effects have long been known. It is fitting that what is probably the first recorded case should be due to an observation of Louis Pasteur from whom the study of stereoisomerism itself has arisen. In 1858 Pasteur reported that the *dextro* rotatory form of ammonium tartrate was more rapidly destroyed by the mold *Penicillium glaucum* than the *levo* isomer.[1] Over the following century and beyond numerous similar examples from diverse biological fields have accumulated, particularly noteworthy ones of early vintage being those of the differing effects of (−)- and (+)-adrenaline (epinephrine) on blood pressure,[2] and the superior anticholinergic potency of hyoscyamine over its racemic counterpart atropine.[3] Between 1920 and 1940 pharmacologists such as Cushny[4] and Easson and Stedman[5] began to speculate on the causes of activity variations among stereoisomers, and by 1959 the subject had attracted sufficient attention to warrant a number of reviews.[5–12] The immediate post-World War 2 period saw a burgeoning of interest in the chiral aspects of pharmacology and medicinal chemistry prompted, in particular, by individuals such as Arnold Beckett at Chelsea and Everardus Ariëns at Nijmegen. Today such investigations are regarded as routine and accepted as an essential element and extension of classical structure–activity analyses.

More recent reviews have been published by Casy (1970, a chapter of Burger's *Medicinal Chemistry* monograph),[13] Stenlake (1979, a chapter of a monograph),[14] Ariëns,[15,16] Simonyi,[17] and Taylor and Insel (a chapter of a monograph).[18] Ariëns and co-authors also edited a book presenting the proceedings of an international meeting held at Noordwijkerhout on the stereochemistry and biological activity of drugs.[19] Details of meeting held at Tübingen (1988)[20] and Cambridge (1990)[20] on the same topic are also available in book form. Two handbooks of CRC Press edited by Donald Smith provide valuable source material in regard to a wide range of pharmacological areas[22,23] and many references to these publications will be found in the present monograph. The 1988 publication of Wainer and Drayer, *Drug Stereochemistry*, contains both analytical and pharmacological infor-

mation,[24] as does a 1990 volume edited by Simonyi.[25] Chirality has featured in a series of reviews appearing in the 1986 issues of *Trends in Pharmacological Science* (TiPS). These include articles by Mason (origin of chirality in nature),[26] Ariëns (chirality in bioactive agents and its pitfalls),[27] Lehmann F (stereoisomerism and drug action),[28] Testa (chiral aspects of drug metabolism),[29] Hoyer (implications of stereoselectivity in radioligand binding studies),[30] Walle and Walle (pharmacokinetic parameters obtained with racemates),[31] and Simonyi and others (chirality of bioactive agents in protein binding, storage and transport processes),[32]

Biological data on stereoisomers may be found in a vast range of scientific periodicals but the single most valuable source—certainly in regard to pharmacologically active compounds—must be *Journal of Medicinal Chemistry* as has been the case since its inception in 1957. Review series in medicinal chemistry (*Advances in Drug Research*, *Progress in Drug Research*, and *Progress in Medicinal Chemistry*) often include articles which emphasize stereochemical aspects as does the ACS publication *Annual Reports in Medicinal Chemistry*, now in its 25th year.

In response to the ever-increasing interest in molecular geometry, the last few years have seen the publication of two specialist journals: *Chirality* (devoted to the pharmacological, biological, and chemical consequences of molecular asymmetry) and *Tetrahedron: Asymmetry*, an offshoot of the well-known Pergamon publications which included biological data as well as synthetic and other chemical aspects.

1.1. Purpose and Value of Stereochemical Investigations in Medicinal Chemistry

Primarily stereochemical studies are directed at the search for evidence of the characterization of bioactive macromolecules at the molecular level. In medicinal chemistry knowledge of such target molecules relates to receptors which mediate the pharmacological/physiological effects of endogenous ligands and their exogenous (xenobiotic) analogues, and to enzymes responsible for the biosynthesis of endogenous ligands and the biotransformation of xenobiotics. Enzymes which are implicated in the generation of second messengers, such as adenylate cyclase, should be included in this target category along with G-regulatory proteins.

The very concept of a receptor arises only as a result of the discovery of a molecule which produces a specific biological response, so it is natural that examination of ligand molecules be a prelude to study of the receptor itself. Such work, generally referred to as structure–activity relationship (SAR) studies, i.e., analyses of the relationships between structure and activity, has been in progress for most of this century. Stereochemical investigations may be regarded as a finely-tuned extension of this traditional approach to medicinal chemistry in which a complementary relationship between the pharmacophoric features of the ligand and the active sites of its receptor is assumed.

Post-World War 2 advances in the isolation and purification of enzymes followed more recently by those of many pharmacological receptors themselves[33,34] mean that many of the target macromolecules are already available for direct study, a trend which will continue to develop as we enter the 21st century.

As such macromolecules become available in a viable state, direct study of their interactions with ligands will be possible and it is at this stage that stereo-

chemical data accumulated on the affinity and efficacy of such small molecules for their receptors will achieve its full potential in regard to the identification of active sites and the consequences of ligand–macromolecule association. Information of this kind will greatly facilitate drug design in general, in addition to advancing knowledge of the processes of life.

Apart from the overall aims outlined above, evidence of activity differences between stereoisomers provides evidence on (1) whether a compound has a general or specific mode of action, and (2) whether a series of ligands, which may include both agonists and antagonists, have the same type of association mode with the receptor. Thus agents with specific pharmacological effects generally display a large activity difference between their separate stereoisomers, while agents with more general actions—commonly depressant effects—show isomeric potency difference of low order which probably relate to pharmacokinetic rather than pharmacodynamic factors (see Chapter 3); a pertinent example is the lack of stereospecificity of antipodes of the general anesthetic halothane ($F_3C^*CHBrCl$) in two model systems,[35] When the more active (eutomeric) forms of a series of chiral ligands of related pharmacology are shown to have the same absolute configuration, it may be assumed that all associate with the receptor in a similar manner—when such is not the case, evidence of differing modes of ligand–receptor binding is provided, e.g., eutomers of methadone and diampromide (page 517), muscarine and muscarone (page 240). The mode of association of formally achiral ligands with their receptors may also be probed by study of chiral analogues, e.g., studies of the reversed ester of pethidine (page 484) and acetylcholine (page 231).

It is important from a clinical point of view to study the properties of the separate antipodes of a drug administered in racemic mixture form. If the therapeutic efficacy of the two forms differs significantly, then the question of administration of the eutomeric rather than the racemic mixture form arises. This matter is currently of great interest to the pharmaceutical industry in regard to the licensing of novel agents of chiral structure and is discussed in detail in Chapter 3.

Such investigations also bring to light the influence of one antipode on the other at pharmacokinetic and/or receptor levels—for example, one form may be an agonist and the other a competitive antagonist.

1.2. Concepts

A number of proposals to account for differing activity between stereoisomers were advanced early in the investigations of such phenomena (the terms *eutomer* and *distomer*, derived from the Greek for good and bad, for the more and less active members of an antipodal pair, respectively, are now in general use, Chapter 2, page 17, and employed throughout this book). The most fundamental, perhaps, is that of the three-point fit concept applicable to antipodal pairs. If three functions or structural features attached to a chiral center of a bioactive molecule are involved in its binding to a receptor of high stereoselectivity, a specific molecular orientation or configuration of the three groups will be necessary for ligand–receptor association. Hence, in such cases of three-point fit, a clear-cut difference in affinity between an object–mirror image pair is to be anticipated because only one member will be able to present the required stereochemical sequence to the receptor surface. Figure 1.1 represents an isomer of high affinity (a) which has a

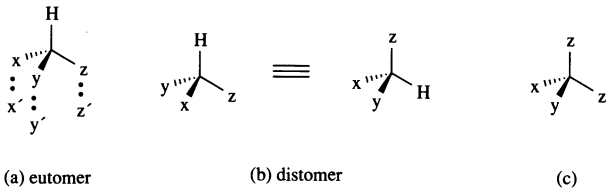


FIGURE 1.1. Representation of (a) high- and (b) low-affinity members of an antipodal pair, and (c) an achiral analogue.

configuration of the binding groups X, Y, and Z that is complementary to that of the corresponding active sites X′, Y′ and Z′ of the receptor; in the form of lower affinity (b) only two of the binding features may be aligned correctly with the receptor. Isomeric affinity differences are also possible when only two functions of the ligand contribute to receptor binding (two-point fit), since the third group, while adding little or nothing to the affinity of the more active isomer, may seriously impair the close association of the two binding functions to the receptor in the less active form. One means of assessing the role of a third group in this context is to compare the activity of the less potent member of an antipodal pair with that of the analogue in which the group in question is replaced by hydrogen (c). If the group has binding significance in the eutomer and is without influence in the distomer, the latter should have an activity similar to that of the more symmetrical analogue lacking this function. On the other hand, if the same group impedes binding when orientated as in the distomer, the potency of this antipode should be lower than that of the analogue which lacks the third group. This approach was first applied by Easson and Stedman[5] in regard to the secondary hydroxyl linked to the chiral center of sympathomimetic amines, as fully discussed in Chapter 4.

Another approach is to examine analogues in which one of the substituents linked to the chiral center is duplicated giving an achiral product. Structure (c) of Fig. 1.1 represents such an analogue of the eutomer (a), Z being the duplicated group. This analogue possesses the same configurational arrangement as the active isomer (a)—its inactivity would indicate that the additional group Z, positioned as in the distomer (b), prevents effective ligand–receptor association. The case of antipodes of methadone and the achiral derivative 6-methylmethadone provides evidence that such is the case for the methyl group attached to the C-6 chiral center of the distomeric form of methadone (Chapter 14).

This discussion has so far been limited to consideration of the *affinity* of a ligand for its receptor. Strictly, such a treatment is valid only when one is dealing with an agent which, once bound, fails to trigger a pharmacological response. Such agents effectively block the receptor and act as *antagonists*. In the case of ligands which induce a response or cascade of responses, i.e., *agonists*, aspects of *affinity* and *intrinsic activity* (also known as *efficacy*) need to be assessed, since both may be subject to stereochemical control. Pure antagonists are defined as ligands of zero efficacy (*cf* enzyme–substrate investigations in which the Michaelis constant K_m represents affinity while catalytic efficiency is expressed by V_{max} or the turnover number k_{cat}).

Apart from binding studies, which provide measures of affinity alone, most other pharmacological data in regard to agonists give a measure of the net influence of the two factors (pharmacokinetic considerations are disregarded at this point). Experiments aimed at quantitating both affinity and efficacy are, however, becoming more common in spite of the greater sophistication of the necessary procedures. One method of characterizing efficacy, for example, involves determination of the degree of receptor occupancy needed to elicit a standard response—the greater the efficacy of a ligand the lower the degree of occupancy required. The procedure rests on the assumption of spare receptors, i.e., existence of a surplus over those needed to be occupied at the maximum response level, and employs irreversible ligands to reduce (inactivate) their number prior to agonist evalution.[35]

In a update of the three-point attachment model of Easson and Stedman, Testa[37] has emphasized the three conceptual steps of penetration, binding, and activation which bear upon the interaction of a ligand with its receptor. He proposes that the original concept be extended to encompass points of repulsion as well as attraction, intrinsic activity, and trigger/catalytic (rather than binding) sites.

Further insight into ligand–receptor associations may be gained by determining the effect of temperature on affinity constants. Free-energy changes, so derived, lead to evidence of entropy (ΔS°) and/or enthalpy (ΔH°) contributions to the ligand–receptor interaction.[38] Different types of interactions contribute to ΔH° and ΔS°. The formation of the ligand–receptor complex increases the order of the system and hence leads to an unfavorable entropy change ($-\Delta S^\circ$). Hydrophobic interactions, which are highly enthalpic ($-\Delta H^\circ$), are accompanied by dehydration which increases the disorder, producing a favorable entropic contribution ($+\Delta S^\circ$). Insight into the relative importance of these thermodynamic parameters aids the characterization of the molar determinants of recognition at a receptor site. Examples are included in Chapter 4, 9, and 11 which relate to adrenoceptors, cholinergic and histaminergic receptors, respectively.

1.3. Conformational Effects

The influence of substituents (chiefly methyl and small alkyl groups) on conformational equilibration is a further factor in analyses of stereochemical SAR, particularly when insertion of such a group into an achiral bioactive molecule leads to enhancement of potency. In such cases the activity-raising action of the additional substituent may be due, not to a secondary binding effect, but to its leading to an increase in the population of a conformer favorable for receptor binding but unfavored thermodynamically in the achiral parent. This aspect is of special relevance to bioactive molecules based on small alicyclic rings, as exemplified by opioid ligands based on 4-phenylpiperidines (Chapter 13, page 465).

1.4. Pfeiffer's Rule

The magnitude of the isomeric potency or affinity ratio (eudismic ratio) clearly reflects the degree of stereoselectivity of the receptor for its ligands. Attention to this aspect of stereochemical SAR was first drawn by Pfeiffer in 1956.[39] He plotted the average human dose of 14 chirals drugs used in racemic mixture form

against the corresponding antipodal activity ratios from tests on animals and animal tissues and obtained a positive correlation between the two parameters. The conclusion reached was that the greater the difference between the pharmacological activity of isomers of an antipodal pair, the greater the specificity of the active isomer for the response of the tissue under test. Agents of high potency as judged from the dosage of their racemic mixtures should thus display correspondingly high eudismic ratios.

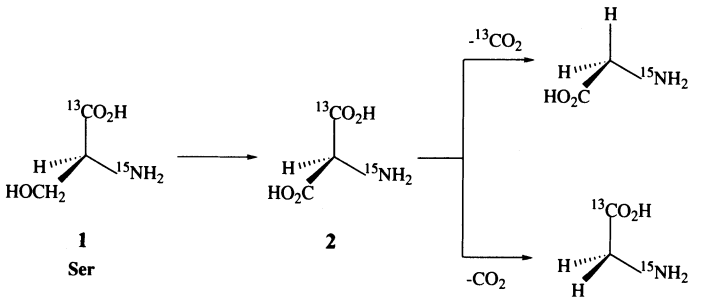
Pfeiffer's observations have been extended by Ariëns and his colleagues,[15,40] who showed that stereoselectivity does not occur randomly but is a function of the potency or affinity of the more potent isomer. Barlow[41] has pointed out that the data used by Pfeiffer are comparatively crude—no allowances for aspects of stereoselective transport to the active site were made, nor was evidence of optical purity available at that time; Pfeiffer's observation is thus all the more remarkable for these limitations. Pfeiffer's rule certainly appears to make sense in the simplest situation where activity depends only on binding, as with competitive antagonists; the higher the affinity of a compound, the more it matters how groups are arranged about a chiral center. Barlow has warned, however, of the danger of accepting Pfeiffer's rule as part of established teaching before its implications have been fully explored. Examples of distomers which, although less effective than their eutomeric partners, are nevertheless of high potency are not uncommon, notably in field of muscarinic antagonists (Chapter 9). Thus although the conclusion, drawn from Pfeiffer's rule, that the eudismic index (a log term, see below) is approximately linearly related to the logarithm of the affinity constant (log K) is acceptable, the corollary that the same parameter determines log K for the weaker enantiomer seems improbable.

De Miranda and others[15b] have attempted to trace the general principle underlying correlations between the enantiomeric potency ratio and potency. In methods of so-called eudismic analysis which involve plots of *eudismic index* (EI) (the logarithm of the ratio of eutomer and distomer affinities, the eudismic ratio ER) against the negative logarithm of the molar dissociation constant of the eutomer (pEu), the slope of the line (*eudismic-affinity quotient*, EAQ) is a quantitative measure of the stereoselectivity of a binding site toward a given series of related antipodal pairs. The positive EAQs usually found imply that the EIs displayed by a given receptor increase as a function of the affinities with which the eutomers of the antipodal pairs bind to it.[42] EAQ values close to unity imply the situation in which the affinity contribution of a given substituent is present in the eutomer and absent in the distomer, as in the Easson–Stedman model. When the stereoisomers being compared are *epimers*, i.e., compounds with two or more chiral centers which differ in configuration at only one of these, a plot of the epimeric eudismic index (EEI) against pEu gives evidence of how critically a given center of chirality is to the receptor–ligand interaction (the case of some 1,3-oxathiolane muscarinic ligands is discussed in Chapter 9).

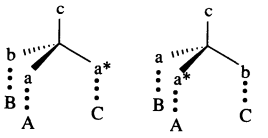
1.5. Ogston's Ideas and Prochirality

Proposals that certain biochemical transformations proceeded via symmetrical intermediates drew criticism on the basis of the results of isotopic labeling studies.

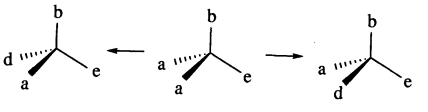
If, for example, the conversion of ^{15}N, ^{13}C, labeled serine **1** to glycine involved decarboxylation of aminomalonate **2**, the isotopic ^{15}N: ^{13}C ratio should change in the Gly product because of the equal possibility of CO_2 loss from either of the two equivalent carboxylate functions. The fact that the ratio was unchanged was evidence against a symmetrical intermediate. Similar evidence and arguments were directed against citrate being intermediary between oxalacetate and α-ketoglutamate metabolism (fixation of $^{11}CO_2$ or $^{13}CO_2$ led to labeling of CO_2H adjacent to carbonyl of α-ketoglutamate only).



This line of reasoning implied the inability of metabolizing enzymes to distinguish identical groups attached to the same carbon atom. Ogston pointed out the fallacy of such arguments in a much cited 1948 paper[43] by proposing his three-point attachment concept. If the enzyme–substrate interaction concerned solely the function to be transformed, then non selective changes of a symmetrical ligand such as aminomalonate (**2**) would be reasonably anticipated. However, if association were of the three-point kind—more likely in view of the now-recognized dissymetric character of enzyme macromolecules—then only one arrangement (**3**) positions one of the pair of identical groups above the catalytically active site of the receptor (arrangement **4** likewise aligns function "a" with the catalytic site but



binding of the substrate receives no contributions from interaction of the additional substituent a and/or B). Such a concept leads to the expectation of the specific transformation of one member of a pair of chemically identical functions, as observed experimentally by means of isotopic probes. Hanson,[44] in addressing the need of identifying each of two identical groups of this kind, introduced the term *prochirality*. Thus the central carbon of an assembly such as **5** becomes chiral when



one of the pair of "a" substituents is replaced by a fourth group "d" (different for a, b, or c) and is hence described as a prochiral center. The absolute chirality (*R* or *S*) will depend on which of the "a" substituents is replaced by "d."

Prochirality theory is now accepted as a cornerstone of biochemistry[45,46] and is equally relevant to study of the interactions of pharmacological receptors with their ligands. Prochirality considerations help elucidate, for example, the manner in which formally symmetrical ligands approach and bind to their receptors. Larson and Portoghese[47] were the first to apply such concepts to medicinal chemistry in work on the reversed esters of pethidine as described in Chapter 13. See also Chapter 2, page 15 and review by Young.[48]

1.6. Pattern of Presentation

This book is intented to serve as a source book of information on stereochemical influences in medicinal chemistry with a coverage that includes the more extensively investigated classes of pharmacological receptors and their ligands. In all topics included, details of the pharmacological difference between isomeric sets are provided together with essential evidence (chemical and/or physical) of stereochemistry. Chapter 2 provides a review of stereochemical nomenclature and methodology—the latter includes some of the biological procedures—with special regard to the appreciation of material included in subsequent chapters. Chapter 3 is devoted to pharmacokinetics and to the influence of stereochemistry on the various processes that govern the transport of the ligand to its receptor. Considerations of the clinical use of racemic mixtures as opposed to the corresponding eutomeric (homochiral) isomers also form part of this chapter. The remaining chapters present stereochemical data relevant to adrenergic, dopaminergic, cholinergic, histaminergic, serotoninergic, and opioid receptors. In many cases separate discussions of agonist and antagonist ligands are given. In general each section includes an account of:

1. the endogenous ligands and aspects of their biosynthesis, storage and release, and metabolism;
2. exogenous (chiefly synthetic) ligands, including pharmacokinetic investigations as available;
3. aspects of receptor subtype classification, including the design of selective ligands;
4. conformational analysis of endogenous and exogenous ligands and investigations of conformationally restrained analogues;
5. data interpretations and stereochemical correlations in terms of proposals of receptor molecular features and ligand–receptor interaction modes.

Emphasis is placed throughout on ligand structure and deductions of receptor events at the molecular level that follow from such knowledge. For an interesting review of structure–activity relationships from the receptors' point of view, the reader is referred to a paper by Hollenberg.[49]

Some discussion of literature sources has already been given earlier in this chapter. Novel data are continually being reported and the need for updating sections of this book during the several years of its preparation has been a common

experience of the author. It has been possible, however, to include material published up to (and beyond) the end of 1990.

A guide to stereochemical investigations of areas of pharmacological import not discussed in the text, such as benzodiazepine, excitatory amino acid, and GABA receptor systems, is provided as a conclusion to this book.

1.7. Notes on References

References are provided at the end of each chapter in the usual Plenum Press house style except for papers where co-authors exceed six in number when the style A. F. Brown *et al.* is employed. In the case of frequently mentioned reference books, notably *Martindale: The Extra Pharmacopoeia*, pagination relating to the most recent edition (29th, 1989) is included in the text, e.g., (*Martindale* **29**, page 562), for easy reference.

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2

Nomenclature and Methodology

2.1. Introduction

It is assumed that most readers of this book will already be familiar with stereochemical principles and will have knowledge of pharmacological concepts and methods. Nevertheless, a summary of basic principles of these kinds is held to form an appropriate and useful starting point for the main topic of the book. The purpose of this summary is to serve as an "aide-mémoire," to update prior knowledge, and to direct attention to stereochemical aspects which recur throughout the main text. Material presented relates, on the one hand, to the molecular geometry of organic structures and, on the other, to pharmacological methodology.

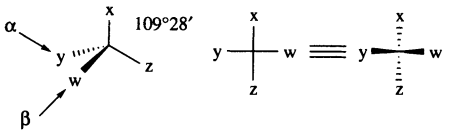
2.2. Stereochemical Nomenclature and Methodology

Many texts on the basic concepts of stereochemistry are available. To mention one of a few familiar to the author, that of Eliel[1] remains a comprehensive and lucid guide in spite of its having being published almost 30 years ago. Eliel's latest text, *Stereochemistry of Organic Compounds*, with co-author S. H. Wilen, will soon be published by Wiley and will contain a comprehensive glossary of stereochemical terms. More recent presentations include those of Kagan,[2] Testa,[3] and Nasipuri.[3a] The Wiley-Interscience series *Topics in Stereochemistry* (19 volumes up to 1989) includes valuable reviews of relevance to this book.

At the most fundamental level, the three-dimensional shape of an organic compound hinges on the hybridization state of its constituent carbon atoms. Hybridization of the sp^3 type provides the familiar tetrahedral geometry **1** in which the carbon nucleus is visualized to be at the center of a regular tetrahedron to the corners of which are directed its four bonding orbitals separated by angles of 109°28′ in the ideal state. The representation **1** shown with two bonds in the plane, one above (β) and one below (α) the plane of the paper, will be employed throughout

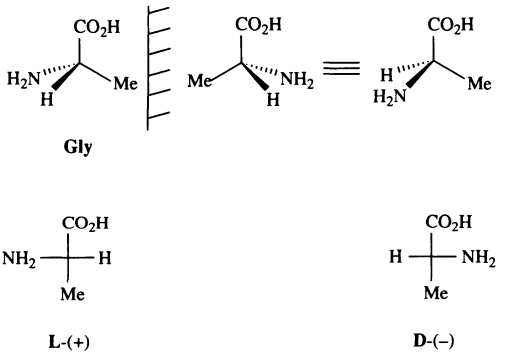
* A stimulating account of the development of Eliel's interest in stereochemistry is presented in his recent book, *Profiles, Pathways and Dreams: From Cologne to Chapel Hill*, ACS, Washington (1990).

this book together with the Fischer projection **2** in which vertical bonds project below and horizontal bonds above the plane. The facility to mentally correlate the two representations (**1** and **2**) is a useful acquisition. The criterion of molecular



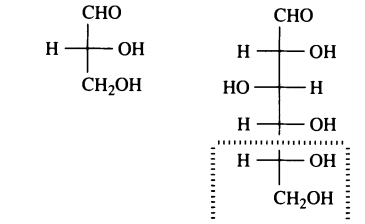
1 **2**

symmetry is the possibility of superimposition of a molecule upon its mirror image. When this operation may not be carried out the molecule is revealed as dissymmetric or *chiral* (from the Greek "cheir" meaning hand). Dissymmetry arises as a result of the presence of one or more chiral centers in the molecule, the commonest of which is the *asymmetric carbon atom* where the nucleus is linked to four different substituents. In such cases the substituents may be arranged in two possible manners around the central carbon giving rise to a pair of molecules which are related as object to mirror image, termed *enantiomorphs*, *enantiomers*, *antimers*, or *optical antipodes*. As an example, antipodes of alanine **3** are shown depicted in tetrahedral and Fischer projection manners.



3

The small capital letters "L" and "D" are used to designate *absolute configuration* following the convention introduced by Fischer.[1] To use it the main chain of the molecule is drawn vertically with the most oxygenated carbon at the top. Substituents linked to the central asymmetric carbon form the horizontal arm of the cross—these are usually hydrogen and larger grouping. When the larger substituent falls to the left the designation L is used, and when to the right, D. When the convention was proposed in the 1890s, no means was available of establishing absolute configuration and Fischer arbitrarily chose L to depict the dextro rotatory antipode of alanine. Fischer made a choice (actually in regard to dextro glyceraldehyde) which leads to the symbolism shown for the dextrorotatory antipode of alanine. Fortunately, modern methods of establishing absolute analysis (page 21) required no revision of his assignment. The D/L system continues in use for amino acids and also for glyceraldehyde **4** and related carbohydrates **5** where the chiral center adjacent to terminal CH_2OH is employed as the label of absolute configuration.



D-(+)-glyceraldehyde D-(+)-glucose

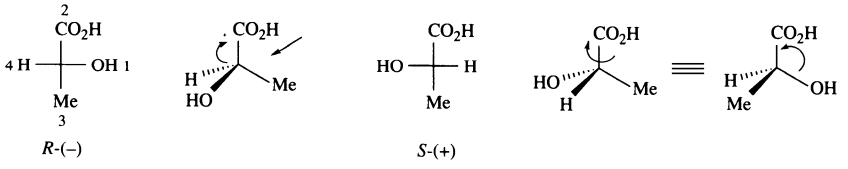
4 **5**

Designation of the sign of optical rotation of plane polarized light is by positive and negative symbols enclosed in parentheses: (+) dextrorotatory, (−) levo (laevo) rotatory. The lower case symbols, d and l, should not be used because of possible confusion with capitals which relate to absolute configuration. It is worth stressing that the sign of rotation (a parameter sensitive to many factors including wavelength, temperature, solvent, and concentration) bears no relationship to the absolute geometry of a chiral molecule.

Fischer's D/L system adequately defines the absolute configuration of many small biochemical molecules but lacks universal application. For this reason the *R*/*S* system, devised by Cahn, Ingold, and Prelog,[4] was introduced during the 1950s.

To apply this convention three operations are necessary:

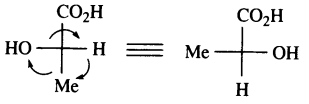
1. rank the substituents attached to a chiral center according to a set of sequence rules of which atomic number is the first criterion;
2. view the chiral center from a direction remote from the substituent of lowest rank (often hydrogen);
3. trace the sequence of substituents in order of decreasing rank (1→3)—if this is clockwise (left to right) the configurational is *R* (rectus), if anticlockwise (right to left), it is *S* (sinister). The example of antipodal lactic acids **6** is shown:



R-(−) *S*-(+)

6

Most applications of the *R*/*S* system are trivial but require care when molecules with many chiral centers are in question—especially for polycyclic systems. Access to simple molecular models of the Dreiding type is useful in such cases, or a molecular graphics VDU. A Fischer projection may be used to make an *RS* assignment provided the group of lowest rank occupies the *lower* arm of the cross (see **7**).

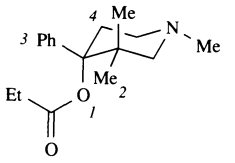


S-(+)

7

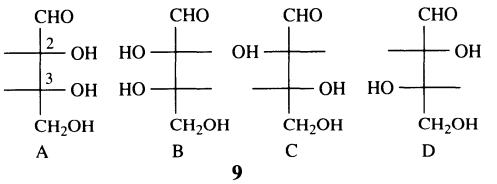
Misapplications do occur in the literature and the reader should be alert to such errors and be prepared to confirm assignments made.

An antipode of 3-methylprodine **8** (page 487) provides a case where care is needed in its *R*/*S* assignment.[5] The chiral C-4 center leads to oxygen (rank 1) and CH_2 (rank 4), and to two quaternary carbons (ArCq and C-3)—these trace back first to three carbons (or three carbon equivalents in the case of Ar C-q); further extension of the C-3 center leads to nitrogen (and two hydrogens) while that of ArCq leads to carbons only, hence the C-3 carbon has the higher rank.



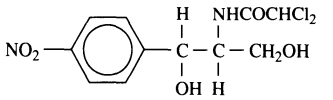
8 *S* (–)

When two or more chiral centers are present in a dissymmetric molecule, the number of optical isomeric forms (*N*) increases according to the formula $N = 2^n$ where *n* is the number of chiral sites. Thus when $n = 2$, four isomers are possible comprising two sets of antipodal pairs. Isomers among the group which are not related as object to mirror image are described as *diastereoisomers*. The tetrose sugars **9** provide an example:



9

A and B (erythrose) and C and D (threose) are the two antipodal pairs while A and C, A and D, B and C, B and D are diastereoisomers. The terms *erythro* and *threo* are often employed to designate Fischer projections of *vicinal* (*vic*, 1,2) diols or *vic* amino/hydroxyl derivatives; e.g., the derivative **10** is described as a D-*threo* form (chloramphenicol).

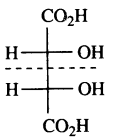


10

Diastereoisomers, unlike antipodal pairs, differ in their physical interactions with media of a symmetric (achiral) nature, and may be separated on the basis, for example, of differences in their solubilities in common solvents and retentions in various chromatographic systems. Methods of chiral analysis depend, in fact, upon the prior conversion of antipodes to diastereoisomers by reaction with an optically pure chiral reagent (see page 33). Likewise weak (noncovalent) diastereoisomeric

interactions form the basis of most classical resolution procedures and, indeed, are involved in all biological phenomena where small chiral molecules (endogenous or exogenous) interact with dissymmetric macromolecules such as enzymes and receptors.

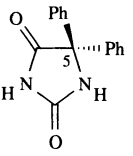
Elements of symmetry (planes, centers) are absent in optically active stereoisomers. When present the stereoisomer has no influence on plane polarized light and is described as a *meso* form, such as *meso* tartaric acid **11**.



11

Many medicinal agents in clinical use are chiral and the majority are marketed in *racemic mixture* form—this term is a general one. The term *racemate* should be reserved (but is often not!) for cases where antipodes interact to form a racemic compound as revealed by plots of antipodal composition versus melting point. In this book the abbreviation *rac* is employed to denote a racemic mixture; other designations used in literature are (±), *RS*, and DL.

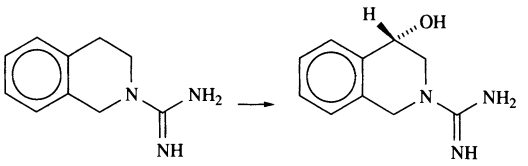
Another term often met in biochemistry and medicinal chemistry is *prochiral*. This term denotes the center of an achiral substrate which has the potential of becoming chiral once the molecule is structurally modified. Thus C-5 of phenytoin **12** is a prochiral site since it becomes chiral following metabolic *para* hydroxylation



12

of one of the phenyl substituents. The *p*-hydroxylated metabolite proved to contain more of the *S* than the *R* antipode (5.5–16.3% *R*), as judged from analysis of urine samples from epileptic patients after treatment with various types of glucuronidase (antipodes were resolved on a Cyclobond I column).[6] The term prochiral harks back to Ogston[7] who first drew attention to how enzymes differentiate chemically alike paired groups of substrates of the Caabc type such as citrate, as discussed earlier (page 6).

Another example is the antihypertensive agent debrisoquine **13** which is converted to the 4-hydroxy derivative **14** with high enantioselectivity (98% and above)

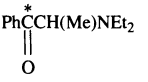


13 **14**

by extensive metabolizers (EM). In poor metabolizers (PM), not only is total 4-hydroxylation reduced, but a loss in product enantioselectivity is also seen (5–36% of *R*-**14** was isolated).[8]

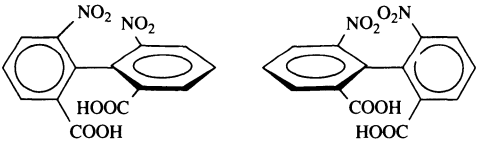
When an optically pure (homochiral)* form of an antipodal pair suffers a change of configuration, it is said to be *inverted* (unidirectional invertase enzymes are found in the walls of the GI tract). This process is usually energetically unfavorable because it requires bond cleavage. However, it may be facilitated by the presence of a carbonyl function adjacent to the chiral center since formulation of a symmetrical intermediate is then possible. The intermediate may lead to the inverted product (invertomer) or revert to the original antipode, and a racemic mixture results in a process termed *racemization* if both routes are equally likely.

Pertinent examples are the racemization of the stimulant drug diethylpropion **15** (Ref. 10) and the anticholinergic alkaloid hyoscyamine (atropine) (page 287).



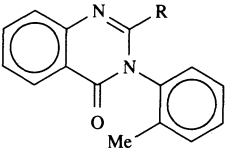
15

Optical isomerism sometimes arises as a result of restricted rotation (*atropisomerism*). The classic examples are biphenyls of the type **16** the antipodes of which cannot interconvert at room temperature because of the large barrier to rotation about the central bond. Racemization takes place at elevated temperatures. A few clinical agents owe their chirality to this phenomenon, as will be described.



16

The example of hypnotic agent methaqualone **17**a is of relevance here. The 100-MHz ^{1}H-NMR spectrum of its 2-benzyl analogue **17**b displayed a CH_2Ph signal of the AB type (2 doublets, ^2J 14.5 Hz), proof of the presence of a chiral



(a) R = Me; (b) R = CH_2Ph

17

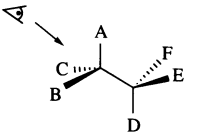
* Some authorities maintain that the term homochiral should be reserved for molecules of the same handedness which are not identical apart from their configurational relationship.[9] They would use the term "enantiomerically pure" to describe a sample made of identical chiral molecules, such as a sample of (+)-tartaric acid. The term homochiral is, however, frequently employed to describe such materials.

center adjacent to the benzyl substituent; the signal collapsed to a singlet on raising the temperature.[11] The NMR spectrum of methaqualone itself run in the presence of a chiral shift reagent showed a pair of aryl methyl signals—again evidence of the analyte behaving as a racemic mixture.[12] Antipodes judged to be about 70% optically pure were obtained by chromatography of methaqualone on triacetylcellulose. These had ED_{50} values of 35.7 and 26.5 mg/kg for dextro and levo products, respectively, in the mouse electroshock test for anticonvulsant activity. Products were optically stable at room temperature.

Before leaving this section, terms relating to differences in the biological actions of members of an antipodal pair are presented. The situation where one isomer is active while the other is totally inactive is rare and may even be nonexistent. The description more active and less active antipode (enantiomer, etc.) may be used, but in this book terms advocated by Ariëns and his colleagues[13] will be generally employed. These are *eutomer* for the more active and *distomer* for the less active isomer. The *eudismic ratio*, also known as the stereospecific index,[14] refers to the numerical value obtained by dividing the potency or affinity of the eutomer by that of the distomer—the greater the value, the higher the degree of stereoselectivity exhibited by the macromolecule with which the antipodes interact. The *eudismic index* is a log term (log affinity of eutomer − log affinity of distomer).

Aspects of *conformation* must now be introduced. This term refers to the actual positional relationships in space of the constituent atoms of a molecule at any given instant in time, and has biological importance in regard to both chiral and achiral molecules. The study of stereochemical relationships of this kind is known as *conformational analysis*.[15] The different conformations (*conformers*) of a molecule are often legion and these freely interconvert by rotations about single bonds. At any one instant, however, the populations of certain arrangements may exceed those of others—such conformers are *preferred* or *favored* because of their minimal energy content or special stabilizing intramolecular interactions.

The conformations of small acyclic units are best represented by *Newman diagrams*. These are drawn from the aspect of an observer viewing a molecule along one of its carbon–carbon bonds, e.g., **18** giving the diagram **19** in which the front carbon obscures (eclipses) the rear carbon. The conformation shown is one in which substituent groups are at their maximum distance apart—rotation about the linking C−C bond converts **19** into the form **20** in which A obscures F, etc. The



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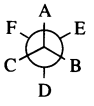


19
staggered

20
near-eclipsed

two conformers are described as *staggered* and *eclipsed*, respectively (**20** actually depicts a near-eclipsed conformer). The *torsion* or *dihedral angle* (τ) most conveniently describes the relationship between a pair of vicinal substituents in the system X−C−C−Y. It is defined as the angle between two planes, one containing the C−C and C−X bonds and the other the C−C and C−Y bonds, and is best depicted by means of a Newman projection (Fig. 2.1). The torsion angle is con-

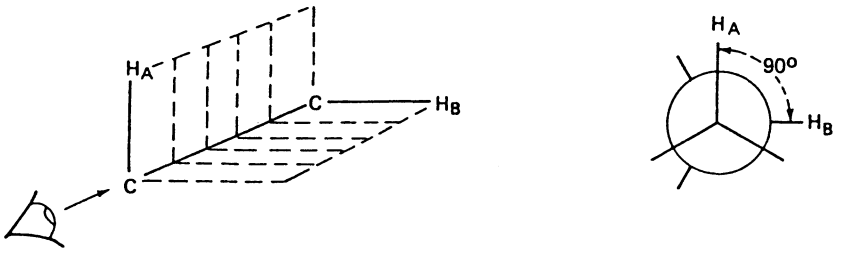
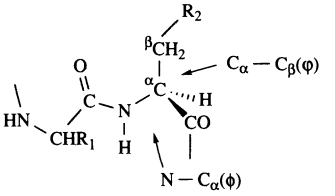


FIGURE 2.1. Representation of a dihedral (torsion) angle of 90° in a H_ACCH_B fragment: one plane is defined by the C — C and C — H_A bonds and the other by the C — C and C — H_B bonds (after Casy).[139]

sidered positive or negative according to whether the bond to the front atom X requires rotation to be right or left, respectively, in order that its direction may coincide with that of the bond to the rear atom Y. Descriptions of various conformations are shown in Fig. 2.2 with positive dihedral angles depicted (X → Y, L → R) (see also IUPAC rules).[17]

Conformational studies of small peptides in solution rely heavily on estimates of N-Cα (φ) and Cα-Cβ (ψ) torsion angles (see **21**) based on ^{1}H-NMR spectral analysis,[18] as further discussed in the opioid chapter of this book (IUPAC rules).[19]



21

2.2.1. Cyclic Systems

When the ends of an acyclic chain composed of sp^3-hybridized atoms are linked, conformational restraint is imposed on the system and conformational options are reduced. In a six-membered cyclohexane ring the tetrahedral angle of 109° 28′ may be maintained, provided the molecule adopts a nonplanar (puckered)

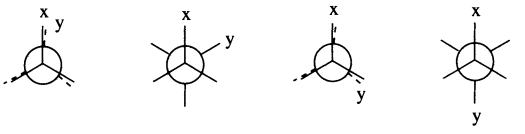
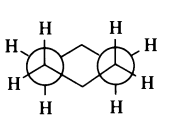
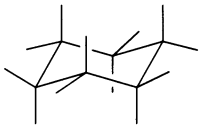


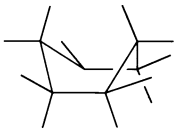
FIGURE 2.2. Conformational descriptions of vicinally related substituents (after Casy).[16]

conformation of either the *chair* **22** or *boat* **23** kind. Only the chair form has a completely staggered conformation—in contrast four pairs of eclipsed hydrogens plus a pair at the top of the molecule in specially close proximity exist in the boat, and the two forms differ in energy content by 5–6 kcal/mole. This barrier is not high



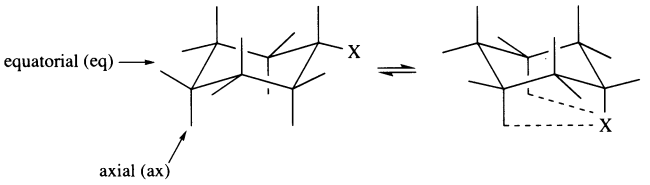
chair **22**





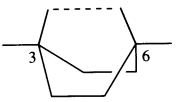
boat **23**

enough to prevent rapid chair interconversions (*flipping*) which proceed via boat forms. The bonds in a cyclohexane chair **24** fall into two distinct groups. Those at right angles to the mean plane of the ring are called *axial* (*ax*) bonds; those pointing sideways close to the mean ring plane are called equatorial (*eq*). The equatorial conformation is favored for a substituent X since it suffers energy-raising nonbonding interactions with its two 1,3-diaxial hydrogen partners when placed axially.



24

In half-chair and boat conformation (**25**) of six-membered saturated rings the terms pseudoaxial and pseudoequatorial are preferred for substituents at positions 3 and 6.

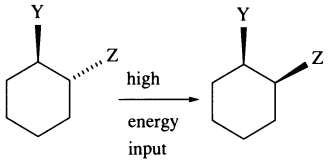


25

Anet[20] has proposed the general use of the terms axial and equatorial for substituent positions at a tetracoordinated atom in a ring of any size based on the local environment of the atom alone.

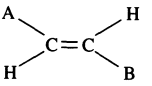
2.2.2. Geometrical Isomers

Isomers which exist as a result of restricted rotation are common among cyclic molecules since their interconversion requires a bond-breaking and a bond-making step, and hence is energetically unfavorable (see **26**).



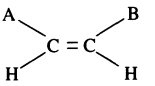
26

Terminology derives historically from geometrical isomers which owe their existence to restricted rotation about a carbon–carbon double bond. Thus the configuration of a 1,2-disubstituted ethene **27** is described as *trans* and that of its isomer **28** as *cis*. To avoid the ambiguity of these terms that occurs in more extensively substituted alkenes, the *E* (German: *entgegen*, apart) and *Z* (German: *zusammen*, together) system is employed. Selection of the subsitituents to be employed in the designation of configuration is based on the sequence rule as usual.



trans (E)

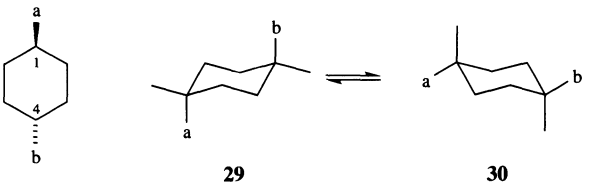
27



cis (Z)

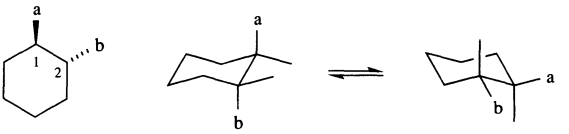
28

The *trans* description of a 1,4-disubstituted cyclohexane is evident from both diaxial **29** and diequatorial **30** chair conformations. In the case of the 1,2-isomers



29 **30** (preferred)

31 it is formally appropriate only to the diaxial conformer—nevertheless the diequatorial form, with X and Y close together (*gauche*), is likewise described as *trans*. *Cis* conformers have an axial/equatorial or *eq*/*ax* relationship, whether substituents are 1,4 or 1,2.



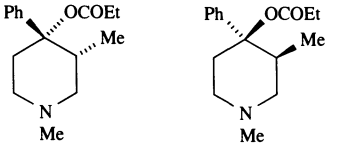
31

In 1,3-disubstituted cyclohexanes *cis* isomers **32** carry *eq*/*eq* or *ax*/*ax* substituents, while *trans* are *eq*/*ax* or *ax*/*eq* forms.

32

The *E*/*Z* convention is generally restricted to geometrical isomers of the alkenic type, while *R*/*S* designations are employed in the case of most geometrical isomers of saturated cyclic systems since these, in addition, usually carry the feature

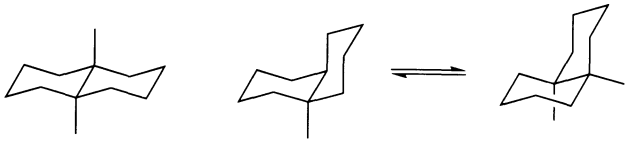
of *chirality*. When the need is only to indicate *relative* configuration, the terms *cis* (*c*) and *trans* (*t*) are employed following the sequence rule with the substituent of highest rank being designated the *reference* (*r*) group, such as **33**.



c 3-Me, *r* 4-=COEt (for each antipode)

33

In bicyclic systems, such as decalin **34**, the possibility of *trans* and *cis* ring junctions obtains: only *cis*-joined bicyclic rings may interconvert.



trans **34** *cis*

Stereochemical principles established for saturated cyclic molecules in which all ring members are carbon atoms effectively apply when one or more ring member is replaced by nitrogen or oxygen as, for example, in the case of piperidine and tetrahydropyran derivatives.

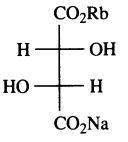
Chirality is not confined to carbon—chiral agents of biological interest are occasionally encountered that owe their dissymmetry to nitrogen, phosphorus, or sulfur. Because nitrogen bases freely invert, chiral nitrogen molecules are only met in the case of quaternary ammonium derivatives **35**, as described in the opioid ligand chapter of this book. In contrast, inversion of trigonal sulfur derivatives, e.g., sulfoxides **36**, requires a substantial input of energy, and antipodes of this kind are stable at room temperature. Dissymmetric sulfoxides from part of the cholinergic and opioid ligand chapters.

35 **36**

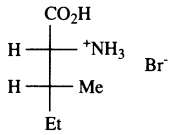
2.3. Methods of Establishing Relative and Absolute Stereochemistry[1]

Originally the only method of elucidating the relative stereochemistry of a pair of compounds of like dissymmetry was by a sequence of *chemical interconversions*. Thus if an optically active molecule X could be converted to an optically active molecule Y after a series of chemical changes, the two molecules were assumed to share the same relative configuration. The same conclusion could be made if each could be converted to the same antipodal form of a third compound Z(X→Z←Y).

Firm evidence of a stereochemical relationship is only available, however, when the steps of the sequences can be shown to be without influence on the original chiral centers. If chiral centers are directly involved in the chemical transformations, then evidence of steric consequence (retention or inversion of configuration) must be available. Many sequences of this kind are described in this book, and chemical procedures continue to be employed. The methods provide evidence of *absolute configuration* only when molecules of established absolute geometry form part of the reaction scheme. Such absolute standards have only been available since the development of absolute methods in X-ray crystallographic analysis by Bijvoet and his colleagues in 1951.[21] The methods require the incorporation of a heavy atom in the crystal and the use of the technique of anomalous dispersion. It was first applied to the sodium rubidium salt of (+)-tartaric acid (**37**) and to (−)-isoleucine hydrobromide (**38**).



37



38

Following recent advances in the technology of X-ray crystallography, reports of absolute configuration determinations are now common, and most problems of absolute geometry are now solved in this manner on a routine basis. Inclusion of a heavy atom in the crystal analyte is now no longer necessary. Increasingly X-ray analyses are reported of complexes (generally salts) which include a component of known configuration that serves as an internal reference, such as the salt of a chiral base with L-(+)-tartaric acid. As an exponent of the chemical sequencing method, the present author appreciates the simplicity and unambiguity of X-ray methods but feels that much of the fun and challenge of stereochemical elucidation has been removed as a result of these advances.

Physical methods based on the optical rotatory properties of chiral molecules also play a role in configurational investigations. Rotational data are now routinely available over a range of wavelengths, rather than restricted to that of sodium light (λ = 589 nm) and may thus be recorded with greater accuracy since extents of rotation often increase with decrease in the wavelength of the polarized light. Earlier methods of relating the configurations of sets of chemically similar antipodes by comparing the signs of their specific rotations and the influence of solvent change upon rotational directions[1] have been superceded by the advent of the technique of *optical rotatory dispersion* (ORD) and *circular dichroism* (CD).[22,23] In these refinements the signs and appearance of Cotton effect phenomena (anomalous dispersion curves or CD bands) are compared rather than simple rotations, and offer the means of correlating chiral molecules without resort to more time-consuming methods.

X-ray methods also provide accurate descriptions of *solid-state conformations* and a vast body of data of this kind is available (Cambridge Data collection, see later). The prime tool for investigating the conformation of molecules in solution (solute state) is *NMR spectroscopy*.[24] Polar molecules can usually be examined in deuterium oxide (D_2O), a solvent which closely mimics the physiological condition.

Today, techniques are available that suppress the water signal (which otherwise restricts the dynamic range of the spectrum and prevents the resolution of solute signals) enabling studies to be carried out in water or H_2O–D_2O mixtures.[18] The polar solvent deuterated dimethylsulfoxide (DMSO-d_6) has been employed for NMR studies of small peptides but is a less appropriate solvent. While an X-ray analysis gives esentially the complete conformation of a molecule, information from NMR spectroscopy is usually limited to a few features. The two techniques do not necessarily provide the same stereochemical answer, e.g., crystalline diprotonated histamine (as the diphosphate) is exclusively antiplanar with respect to the amino and imidazole features[25] while histamine as solute in 0.1 M D_2SO_4 is populated by about 50% of the antiplanar and 50% of the two synclinal conformations.[26]

It is often difficult to divorce relative stereochemistry from conformation in such NMR studies and information on both often accrues. In noninterconverting isomers of the alkenic type, configurational problems may be solved from the comparative ^3J values of vicinal protons of disubstituted alkenes ($^3J_t < {}^3J_c$) or by measurement of nuclear Overhauser enchancement (NOE) in higher substituted cases. The latter technique, which enables protons closely placed in space to be identified, may now be carried out efficiently by recording difference spectra under normal 1D or 2D (NOESY, ROESY) conditions.[27] Evidence on the conformational preferences of saturated acyclic and cyclic molecules rests heavily upon application of the relationship between the magnitude of coupling between vicinal protons and their dihedral angle relationship as developed by Karplus[28] (Fig. 2.3). Many examples are presented in this book. Solution of the stereochemistry of the diastereoisomeric *sec*-alcohol precursors of α- and β-eucaine (local anaesthetics) by ^{1}H NMR is shown in Fig. 2.4.

Second-order treatment of spectral data is sometimes required for the conformational analysis of small molecules such as acetylcholine and histamine but the

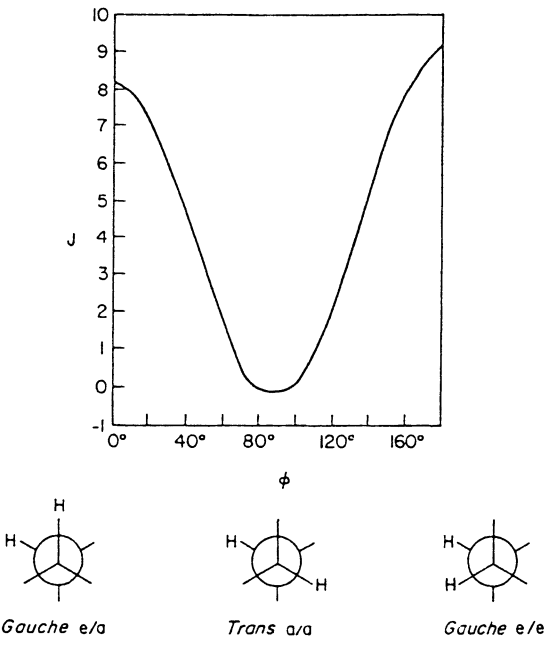


FIGURE 2.3. The Karplus function describing the magnitude of vicinal proton–proton J coupling as a function of the dihedral angle ϕ in the H—C—C—H bond system. The Newman projections denote *gauche* e/a, *trans* a/a, and *gauche* e/e coupling (after Casy).[24]

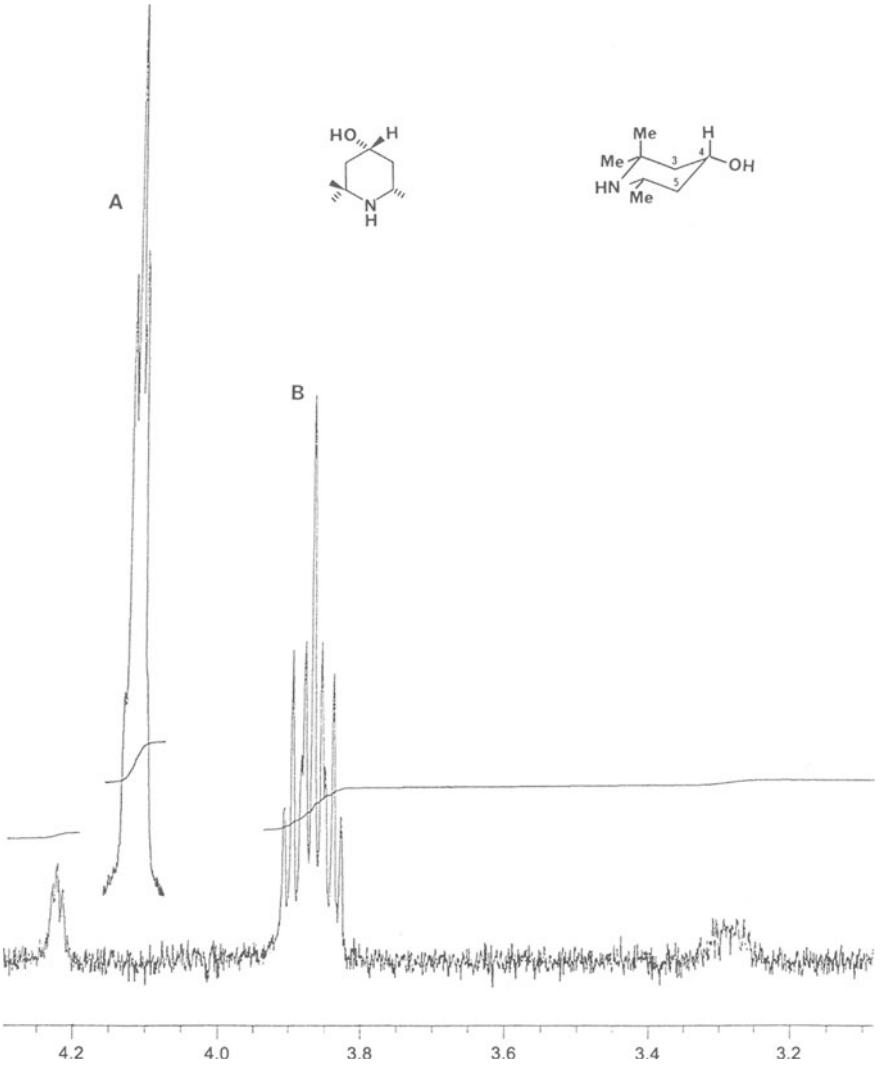


FIGURE 2.4. Part of the 400-MHz ^{1}H-NMR spectrum of α-2,6,6-trimethyl-4-piperidinol in $CDCl_3$. The sample contains the β-isomer as an impurity as evident from the low-intensity signals near 3.3 and 4.25 ppm. Insert A is an expansion of the β-4-H signal taken from the spectrum of a pure sample of the β-isomer. The width at half-height ($W_{1/2}$) of the α-4-H signal near 3.85 ppm (B: 22.5 Hz) is three times as great as that of the β-4-H signal near 4.25 ppm (A: 7.5 Hz), evidence that 4-OH is equatorial in the α-isomer (whence ax 4-H is coupled to axial protons at C-3 and C-5) and axial in the β-isomer (eq 4-H suffers no large couplings in consequence).

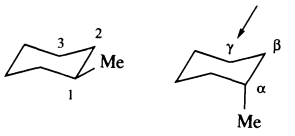
need for this is now less as a result of the introduction of high-frequency spectrometers which operate at 270 MHz and above.

Chemical shift considerations also play a role in solving problems of conformation—especially allowance for the shielding effects of aromatic rings (protons above the aromatic ring plane are shielded while those in the plane are deshielded). Differential influences of shift reagents upon ^{1}H chemical shifts are sometimes

of stereochemical value, such as configurations of psychotropic thioxanthenes (page 209).

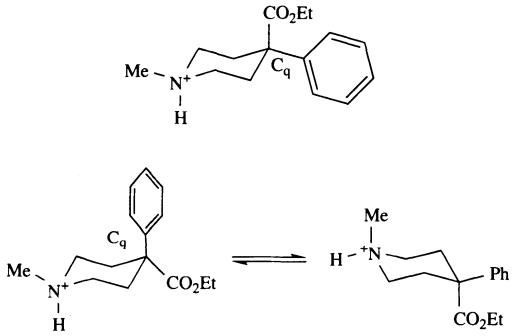
Unpublished high-resolution ^{1}H-NMR spectral data recorded at Bath are included throughout the text of this book.

Although most stereochemical data based on NMR spectroscopy are derived from proton parameters, the potential of carbon-13 (^{13}C) magnetic nuclei should not be overlooked in this regard.[29] A much applied principle is that of the γ-shielding influence of methyl substituents. When methyl is equatorial in a cyclohexane **39** it has little influence on the C-3 chemical shift; however, when axial it moves the C-3 shift upfield by several ppm as a result of steric polarization.[30]



39

The NMR technique is also valuable for the study of slowly interconverting (NMR-slow) conformers as occurs in the case of certain N-protonated piperidines, such as pethidine **40**, where both epimers are significantly populated as demonstrated by observation of duplicate NMR signals (page 465).[31]



40

2.4. Computational Methods

By the methods of quantum mechanics it is possible, in principle, to establish the preferred conformation(s) of any molecule. The techniques involve computer aided calculations in which various molecular parameters such as bond angles and bond lengths, and the Coulomb integrals of electrons in specific atomic orbitals are employed. By these means the energies of a range of molecular conformations may be derived and conformers of lowest energy identified.

Advances in computer technology have now made it possible to carry out molecular energy calculations on a routine basis. Most of the work is directed at

predicting the more probable conformations (of low energy content) of ligand molecules. Provided sufficient computer resources are available, quantum chemical calculations yield the whole conformational domain of a molecule in the isolated state, the relative stabilities of the various conformers, the energy levels, the charge distributions, and other quantities that can be derived from the wave function.[32, 33] The difficulty with a systematic examination is one of cost, and the approach is "governed by compromise between the limitations on computational power available."[34] The assumptions of fixed bond angles and bond lengths are usually made, leaving only torsional variables to specify a conformation.

Calculations are usually guided by X-ray crystallographic information relating to the ligand itself or closely related molecules, drawn for the extensive data base presently available, such as that of the Cambridge Crystallographic Data Centre which began operations in 1965. A serious shortcoming to the application of quantum mechanical methods to pharmacological ligands is that calculations are nearly always performed as though the molecules existed *in vacuo*. Methods for the simulation of the solvent environment are now, however, being developed.[35] Tollenaere *et al.*[32] have emphasized that as conformational data based on computer technology accumulates, a strong need is felt to visualize the results of analyses, and to be able to manipulate structures in a more sophisticated way than is possible by use of conventional molecular models. These problems have led to the development of computer graphics by means of which three-dimensional structures may be visualized and manipulated on a visual display unit.[36] A bibliography of quantum pharmacological studies is presented in the text of W. G. Richards[37] and accounts of several of its applications are included elsewhere in this book.

Höltje *et al* have recently reviewed the use of quantum chemical methods to study molecular mechanism of drug action.[38]

2.5. Sources of Stereoisomers[39]

Means of acquiring homochiral forms of dissymmetric molecules fall roughly into four divisons;

1. Resolution of the racemic mixtures that result from conventional synthetic procedures.
2. Use of optically active natural products as precursor molecules in synthesis (chiral building blocks).
3. Exploitation of the stereoselectivity of enzymes.
4. Use of methods of asymmetric synthesis based on catalysts which control the introduction of one or more asymmetric center.

Most methods ultimately depend upon the existence of a chiral pool produced by natural metabolic processes, that is continually being regenerated.

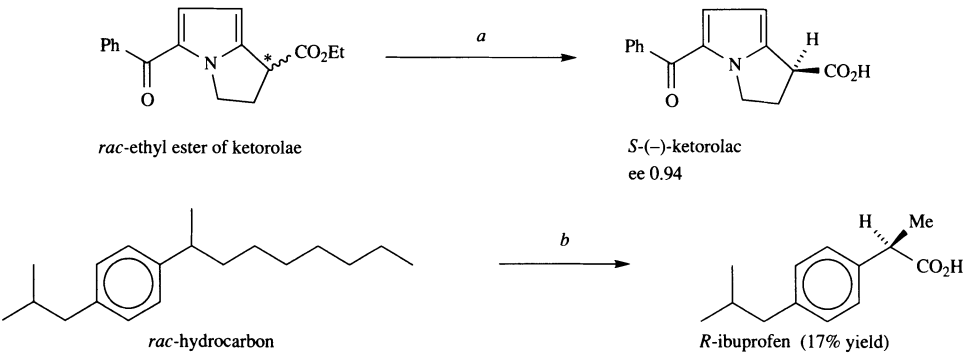
Methods (3) and (4) are being applied increasingly to the production of homochiral products on both economic grounds, and the demands of regulatory authorities that novel eutomers rather than racemic mixtures be introduced into clinical practice.

2.5.1. Resolution[40–43]

Classical methods involving the recrystallization of diastereoisomers formed from a racemic mixture by combination with an optically pure molecule are still in wide use. Tartaric acid [now available in its unnatural D-(−)-form] and its derivatives (*O,O*-dibenzoyl, etc.) form the mainstay of the resolution of *rac*-bases, while a variety of alkaloids are employed to resolve *rac*-acids. The isolation of each member of an enantiomorphic pair of bases is now often achieved by first crystallizing diastereoisomeric salts formed with L-(+)-tartaric acid (or derivative), yielding one pure salt, then recovering base enriched in the second isomer from mother liquors and recrystallizing salts formed with D-(−)-tartaric acid. Neutral racemic mixtures may often be linked to ionizing functions (usually acids) by derivatizing processes, the products also being separable via diastereoisomeric salts. The progress of resolution monitored by polarimetry is more readily accomplished if rotational readings are taken over a range of wavelengths—a facility offered by modern photoelectric polarimeters (sensitivities of the order of a few hundredths of a degree are typical of such instruments—these may be greatly enhanced if a laser light source is employed; see below). Today, however, knowledge of optical purity may be achieved with great accuracy as a result of the development of methods of chiral analysis based on the *separate quantifications* of *each* antipode (see page 33). Certain chiral compounds are found to resolve spontaneously upon crystallization and the enantiomers of these conglomerate species may be separated by direct crystallization when seeded with the pure enantiomers.[136] Conglomerates are mechanical mixtures of antipodes equivalent to an ordinary mixture of two compounds whose phase diagram shows a single eutectic point. Their occurrence is rarer than those of racemic compounds.[44] This technique has been used to resolve *rac*-α-methyl DOPA (Chapter 4, page 79), and has been applied succesfully to obtaining both enantiomorphs of methadone in 50% total yield by Zaugg.[45]

2.5.1.1. Enzymatic Resolution

The use of enzymes to accomplish resolutions dates back to the late 1940s when it was discovered that when *N*-acetyl-*rac*-alanine is treated with hog-kidney



[a] protease enzyme from *Streptomyces griseus* (Ref. 48) at pH 9.7
[b] *Rhodococcus* sp BPM 1613 (Ref. 49).

Scheme 2.1. Enzymatic resolutions.

acylase until about half the acyl groups are hydrolyzed, the residual acyl amino acid is a derivative of D-Ala, while the free amino acid is the L-antipode (the two products are readily separated).[46] There are now many examples of such processes (see the review of Sih and Wu [47]). Thus the NSAIDs ketorolac and ibuprofen in racemic mixture form may be obtained in a homochiral state by processes of stereospecific enzymatic hydrolysis and oxidation, respectively (Scheme 2.1.).[48,49]

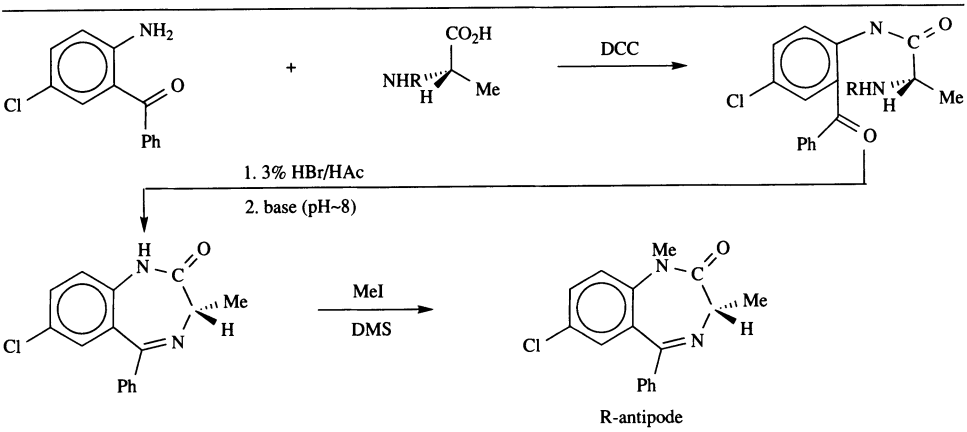
2.5.2. Use of Chiral Building Blocks[50–52]

Natural products provide a rich source of chiral precursors for use in organic synthesis, and include alkaloids (e.g. ephedrine and quinine), L-α-aminoacids, carbohydrates and related products, terpenes (e.g. camphor and borneol), and α-hydroxy acids (e.g., lactic, mandelic, and tartaric acids). Perhaps exploitation of sugars and their relatives has been greatest in regard to the use of natural chiral synthons in the field of medicinal chemistry. The discovery that D-mannitol could be converted to D-(+)-glyceraldehyde has proved of particular value to the synthesis of chiral β_1 beta-blocking agents of the propranolol type, fully described in Chapter 5. Several asymmetric syntheses based on sugars are described in the cholinergic chapters of this book. The preparation of chiral benzodiazepines from *S*-(+)-alanine provides an example of use of a natural amino acid (Scheme 2.2).[53]

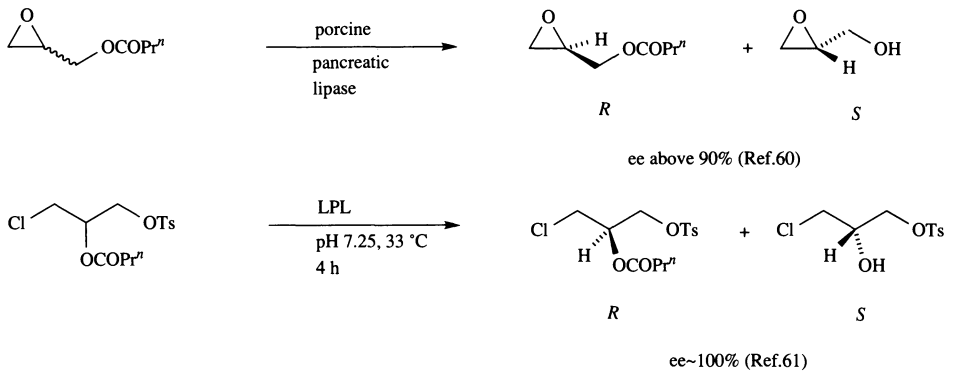
2.5.3. Use of Enzymes in Chiral Synthesis[54–59]

Apart from the use of enzymes in achieving the resolution of racemic mixtures (see above), their application to stereoselective synthesis is a vigorously developing technology in its own right as judged by the numerous reviews devoted to this topic.[54–59] A few examples are included here. The first relates (yet again) to chiral precursors of beta-blocking drugs. Both *R* and *S* chiral epoxides or epichlorhydrins may be obtained by the stereoselective hydrolysis of *rac*-esters (Scheme 2.3).

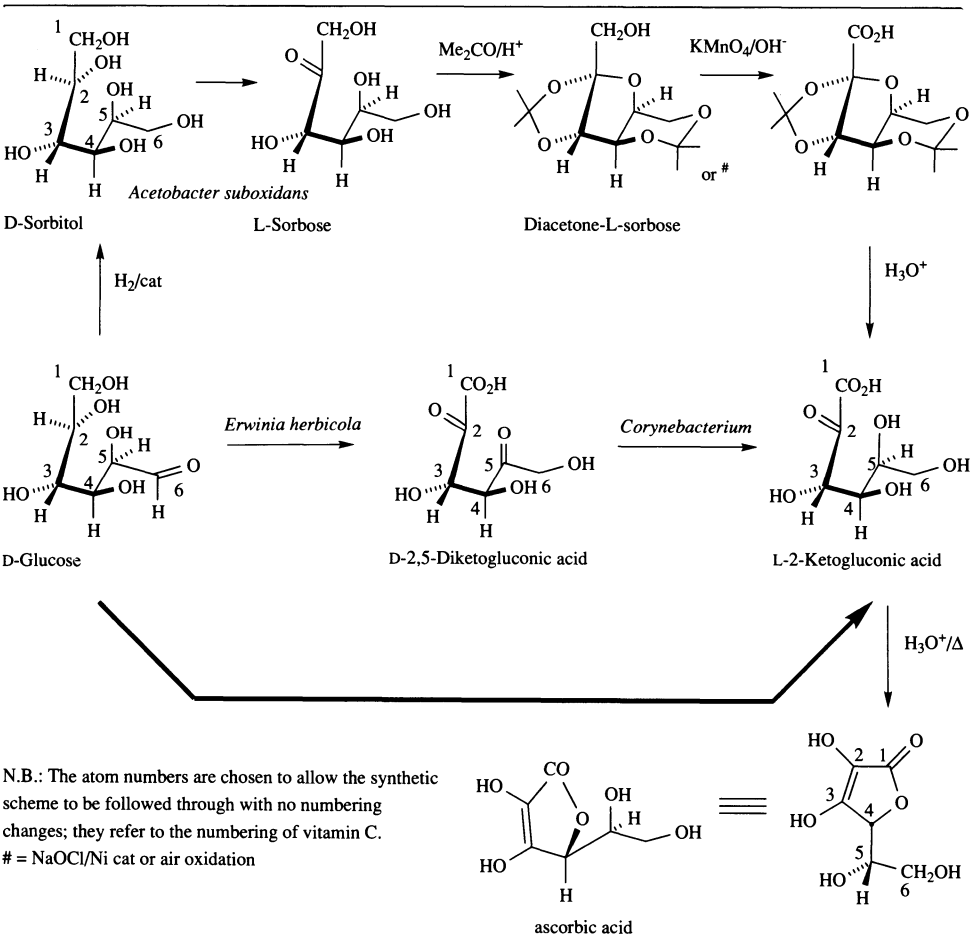
The second example concerns the synthesis of ascorbic acid (Vitamin C) from D-glucose. The conventional process requires reduction to D-sorbitol followed by



Scheme 2.2.



Scheme 2.3. Use of enzymes for production of chiral intermediates for beta-blocking agents. [60,61]



Scheme 2.4. Generations of Vitamin C synthesis (after Pratt).[57]

enzymic oxidation to L-sorbose which yield ascorbic acid after several chemical steps (Scheme 2.4).

Enzymatic processes have now been developed capable of converting D-glucose to L-2-ketogluconic acid via an intermediate or directly. The product readily cyclizes to ascorbic acid.

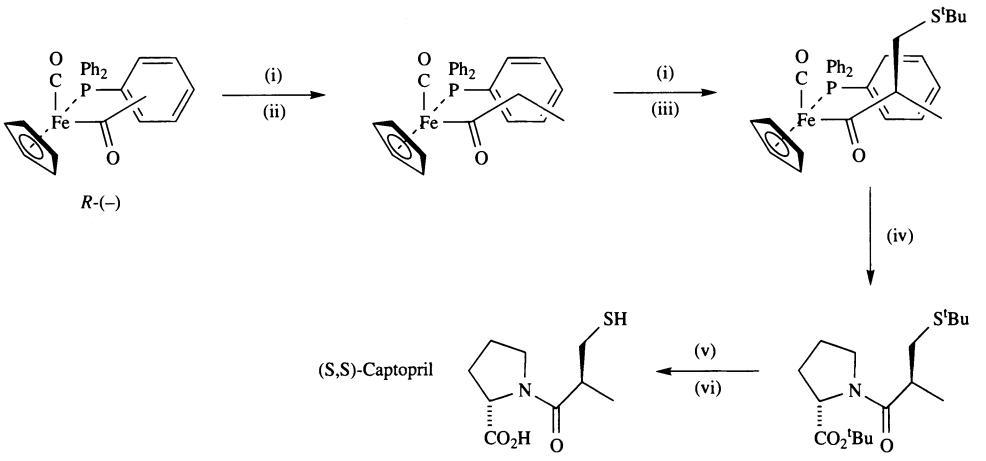
2.5.4. Use of Chiral Auxiliaries[62]

An organic synthon forms one of the ligands of a metal coordination complex in asymmetric catalysis of this kind. The synthon is generally hindered on one side by other ligands and reagents are forced to attack it stereoselectively at its more accessible face. The iron chiral auxilary $[C_5H_5Fe(CO)(PPh_3)]$, for example, exerts powerful stereochemical control over the reactions of attached acyl ligands[63] and has been used in a synthesis of the ACE inhibitor S,S-captopril (Scheme 2.5). Steps in the procedure are as follows:

1. The *R*-(−)-acetyl complex (available in either homochiral form) is methylated to give the propanoyl derivative. Further alkylation with bromomethyl *t*-butylthioether stereoselectively generates a new chiral center with the required absolute configuration.
2. Oxidative decomplexation with bromine in the presence of the *t*-butylester of L-proline produces doubly protected (−)-captopril.
3. Deprotection with trifluoroacetic acid and mercuric acetate gives (−)-captopril in 59% yield.[64]

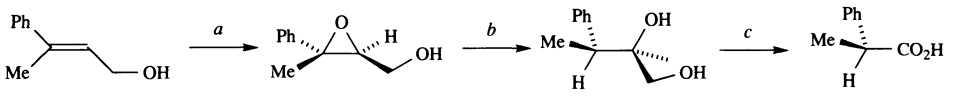
Captopril is 100 times more active than its 2R epimer *epi* captopril as an ACE inhibitor.[65]

The Sharpless procedure of asymmetric epoxidation[66] has had a major impact upon asymmetric synthesis—recently all the L-hexose sugars have been produced by this process.[67] Details of the catalyst are presented in Chapter 5 with regard to its



Reagents: (i) BuLi, (ii) CH_3I, (iii) $BrCH_2S^tBu$, (iv) Br_2, O-^{t}Bu-L-proline, (v) $Hg(OAc)_2$, TFA, (vi) H_2S

Scheme 2.5. Stereoselective synthesis of S,S-captopril (after Davies).[63]



41 **42** single diastereoisomer by NMR *S*-2-phenylpropanoic acid (52%) yield

[a] catalytic epoxidation: catalyst prepared by mixing (+) diisopropyl tartrate and $Ti(OPr^i)4$ at -20 °C, adding the allylic alcohol and stirring for 30 min. The last reagent, *t*-butylhydroperoxide (TBHP), was then added.

[b] H_2 10% Pd-C, -45 °C; inversion at benzylic center.

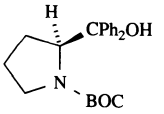
[c] RuO_4-$NaIO_4$

Scheme 2.6.

use in preparing homochiral forms of propranolol and its congeners. Another application is the synthesis of antipodes of 2-arylpropanoic acids related to the anti-inflammatory agent ibuprofen.[68] The allylic alcohol **41** was subjected to the catalytic epoxidation procedure of Sharpless.[69] with (+)-diisopropyltartrate as the chiral part of the coordination complex. The levo epoxide **42** resulted of predictable absolute configuration which, after catalytic hydrogenation (epoxide ring opening with inversion) and oxidation, gave *S*-2-phenylpropanoic acid (Scheme 2.6).

The oxazaborolidine **44** has proved valuable for the stereoselective reduction of carbonyl groups.[70] It is made by treating the *N*-BOC methyl ester of *S*-proline with an aryl Grignard reagent and then heating the *t*-alcohol **43** product with three equivalents of borane. Treatment of α-chloroacetophenone with the borane in the presence of **44** gave the *S*-(+)-*sec* alcohol **45** in 96.5% ee. Recently, a related oxazaborolidine has been employed to synthesize *R*-(−)-isoprenaline[71] as shown in Scheme 2.7.

The reduction step is considered to proceed via the molecular assembly **46**



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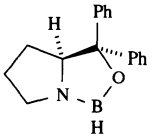

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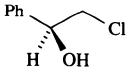

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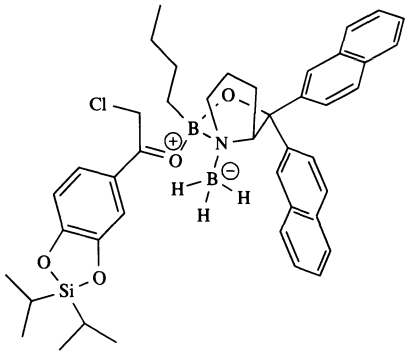
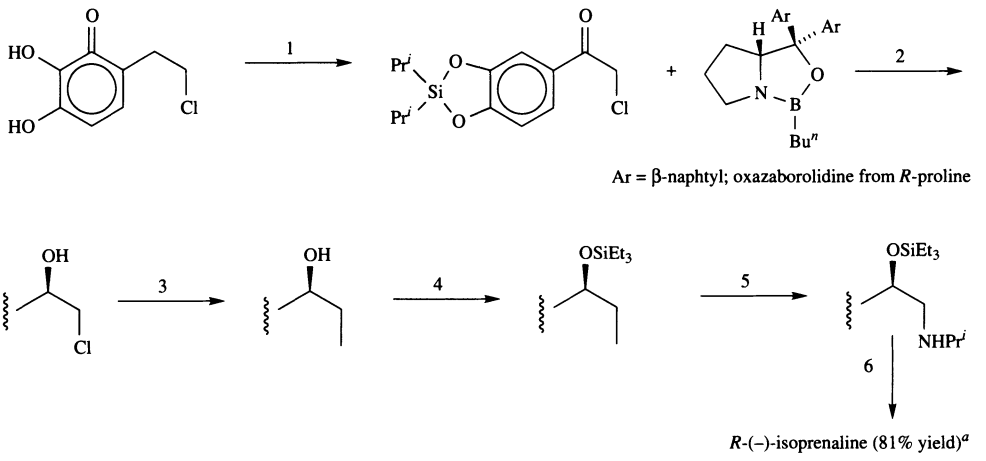


46 (after Corey and Link)[71]

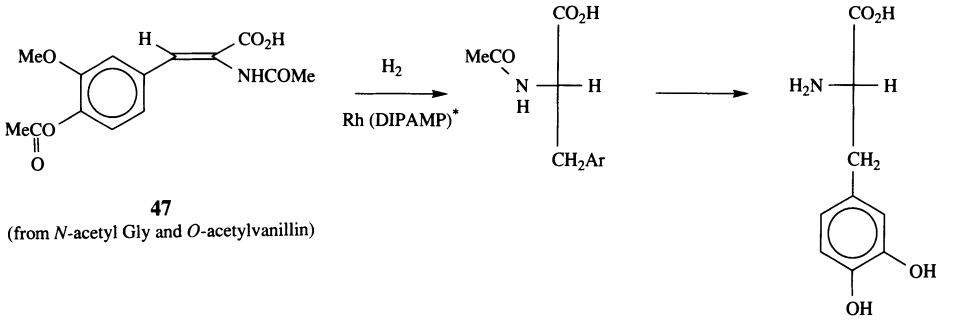


Reagents: 1. $Pr^i_2Si(OTf)_2$, 2,6 lutidine. 2. BH_3 in THF. 3. NaI, acetone, reflux. 4. Et_3SiCl, imidazole, DMF. 5. Pr^iNH_2, sealed tube, 80° C. 6. KF, HCl, MeOH.
[a] established from 500-MHz ^{1}H-NMR spectrum of MTPA (Mosher) ester obtained from *R*-(+)-MTPA chloride (see p. 33).

Scheme 2.7.

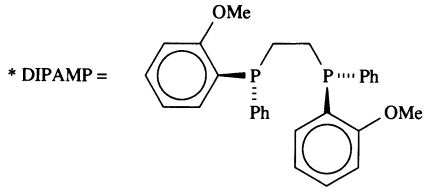
which directs reductive attack to the *front* of the carbonyl feature. An oxazaborolidine reduction step is also employed in the stereoselective synthesis of the *S*-enantiomer (eutomer) of MK-0417, a carbonic anhydrase inhibitor.[72]

As a final example, the formation of levodopa (**48**, L-DOPA), used in Parkinson's disease, is presented. Only the L-antipode undergoes enzymatic decarboxylation to enhance dopamine levels in the brains of patients suffering from this disease. When the enamide **47** is hydrogenated in the presence of a catalyst obtained by complexing rhodium chloride with two chiral phosphine ligands, a protected form of L-DOPA results in 94% ee.[73]



47
(from *N*-acetyl Gly and *O*-acetylvanillin)

L-DOPA **48**



2.6. Chiral Analysis[141]

The quotient obtained by dividing the specific rotation [α] of resolved material by that of material known (or usually assumed) to be fully resolved is termed the *optical purity* (often expressed as a percent). Other terms in use are *enantiomer purity* and *enantiomeric excess* (ee). If the (−)-isomer preponderates over the (+)-form.

$$\% \text{ enantiomer purity} = \frac{\text{moles of } (-) - \text{moles of } (+) \times 100}{\text{moles of } (-) + \text{moles of } (+)}$$

Enantiomer and optical purities have the same numerical value, e.g., 0.9 for a 95:5 mixture.

Optical purities based on polarimetry derive from measurements of optical rotation, values of which are the net result of contributions from each member of the antipodal pair (a sophisticated procedure based on achiral HPLC with dual optical rotation/UV absorbance detection has been described for determining the enantiomeric purity of ephedrine and pseudoephedrine).[74] Methods of analysis which measure the *separate population* of each antipode provide the actual isomeric ratio—a value of greater biological significance than optical purity, especially in cases when the antipodes differ by an order of magnitude or more in their affinities for target sites.

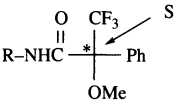
A review of enantiospecific analytical methodology with special reference to drug metabolism and pharmacokinetics has been published by Hutt.[142]

2.6.1. Chromatographic Methods*

Over the last decade interest in the development of methods of chiral analysis by chromatography, especially HPLC, has grown enormously and the literature on this topic is now substantial.[75–82, 137] Most procedures depend on either

1. the conversion of analytes to diastereoisomeric mixtures followed by separation on achiral stationary phases, or
2. direct separation of antipodal mixtures on chiral stationary phases.

Regarding *derivatization*, it is important that the chiral reagent be optically pure and also that reactions proceed to completion, otherwise artefacts are introduced. Reactions used should not be associated with racemization or epimerization. Reagents used for amine analytes include *S*-2-methoxyl-2-trifluoromethyl-phenylacetyl chloride (MTPA-Cl) giving diastereoisomers of structure **49** [83–85] and (−)-menthyl chloroformate giving products **50**[86]; these may also be employed for



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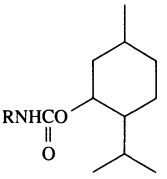

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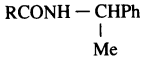
* For recent papers, see the report of the "Symposium on Drug Analysis" (Liège, May 1992) in the Oct.–Dec. issue of *J. Pharm. Biomed. Anal.*

the analysis of chiral alcohols. A capillary GC method for establishing the enantiomeric purity of MTPA (Mosher's acid) has been described following reports of errors in chiral analysis resulting from the use of optically impure reagents.[85] 1-Phenylethylamine may be applied in the case of chiral acids giving **51** (see the example of etodolac, Chapter 3, page 52). Chiral isothiocyanates, which react with antipodal amines to form diastereoisomeric ureas, are also popular, such as those derived from sugars, e.g., 2,6,3,4-tetra-*O*-acetyl-β-D-glucopyranosylisothiocyanate (GITC, **14**, page 60)[87] and its arabinose analogue AITC.[88] Allenmarck[77] and Lough[80] provide a tabulation of chiral derivatization reagents.



51

Chiral stationary phases (CSPs) based on polysaccharides (underivatized, such as cellulose, starch, and cyclodextrins, or derivatized, such as cellulose triacetate) are available. Thus Blaschke[89] separated *rac-N*-methylcyclohexylethylbarbituric acid into its antipodes on a MTCA column (microcrystalline cellulose triacetate) with baseline resolution. Sorbents based on chirally substituted synthetic polymers are also available—polyacrylamide and polymethacrylamide derivatives where the chiral substituents originate from an optically active amine or amino acid. Protein-derived CSPs employ proteins available from blood serum, such as albumin and α_1-acid glycoprotein (AGP) immobilized upon a silica support. AGP, present in human plasma in a concentration of 55–240 mg per 100 ml, is believed to be the main cationic binding protein—hence it is useful for separating chiral amines. Columns packed with this sorbent are marketed under the name "EnantioPac."

2.6.2. Chiral Recognition via Host–Quest Complexation

Columns of this kind depend on the formation of a complex in which the analyte host is enclosed withing the guest molecule which is covalently bound to a silica support. If the stabilities of diastereoisomeric complexes differ, the possibility of chiral resolution exists. Macrocyclic polyethers, known as crown ethers because molecular models of them often resemble a crown in shape, were the first guest molecules to be applied in this way. Amino acids have been so resolved.[90]

Cyclodextrin-bonded (Cyclobond) columns also operate by host–guest complexation and their use has attracted much attention. These compounds are formed of normal β-1,4-D-glucosides cyclized to rings of 6–12 units: the smallest are α(6 units), β(7) and γ(8).

The conformation of a cyclodextrin in an aqueous system is generally assumed to approximate a truncated cone possessing a hydrophobic internal surface at which hydrophobic molecules such as benzene and cyclohexane are absorbed. Enantioselectivity is likely to be associated with the chiral structure at the entrance to the cavity caused by exposed 2- and 3-hydroxy groups of the monosaccharide units (Fig. 2.5). Armstrong and his co-workers have contributed much to this field.[91,92] In a 1988 paper they present antipodal separation parameters for a

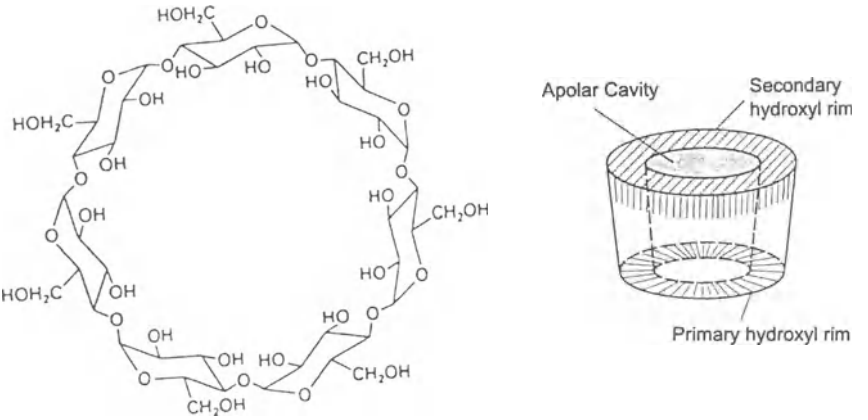
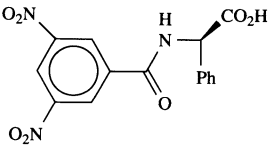


FIGURE 2.5. The structure of β-cyclodextrin with a 3-D diagrammatic representation on the right (after Pagington).[140]

variety of chiral depressant drugs, obtained by use of two 25-cm β-cyclodextrin columns in series; good resolution ($R_S > 1.0$) was obtained for most of the compounds.[93]

2.6.2.1. Donor – Acceptor CSPs

These act by attractive interactions between nonionic functionalities, optimized by rearrangement of hydrogen-bonding, π-donor–acceptor, dipole stacking, and steric interactions between CSP and analyte. The development of such columns owes much to Pirkle and are often so-named. The best known CSPs of this type (available commercially) are those based on *N*-(3,5-dinitrobenzoyl)-phenylglycine **52** and leucine[94] covalently bonded to 3-aminpropylsilica. Figure 2.6 shows Pirkle's recognition model for the interaction of the CSP with a chiral 1-(9-anthryl)ethanol.



52

Lloyd and Goodall[95] advocate the use of polarimetric detection in chiral HPLC procedures. Polarimeters of the demanding degree of sensitivity required for such work (peak-to-noise level of less than 30 μ°) are now available—an intense (laser) light source is a vital element of their design. A fundamental advantage of such detectors is that they give evidence of whether or not chiral separation has been achieved, and the order of elution of antipodes. Armstrong reported that in his experience perhap 10% of new chiral separations produced peak doublets which were in fact due to the separation of the sample and an impurity rather than a separation of enantiomers.[96]

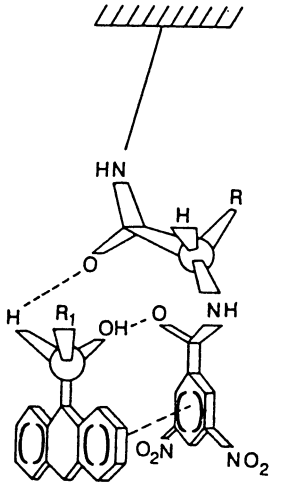
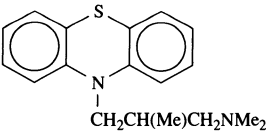


FIGURE 2.6. Pirkle's chiral recognition model. The charge-transfer complex formation causes simultaneous additional enantiomer-dependent contacts between the partners (after Allenmark).[77]

Figure 2.7 shows the chromatograms of D- and L-tryptophan, demonstrating the positive response to the D- and the negative response to the L-amino acid.

In similar vein, circular dichroism (CD) also has potential for on-line detection of antipodes eluting from an HPLC system.[98,99] CD instruments operating with conventional light sources have the required degrees of sensitivity and provide responses of either positive or negative sign.

Recently, examples have been reported of methods in which a chiral additive forms part of the mobile phase—the resultant diastereoisomeric complexes may then be separated on conventional stationary phases. Thus Cooper and Jefferies achieved the separation of enantiomers of trimeprazine **53** on a semipreparative



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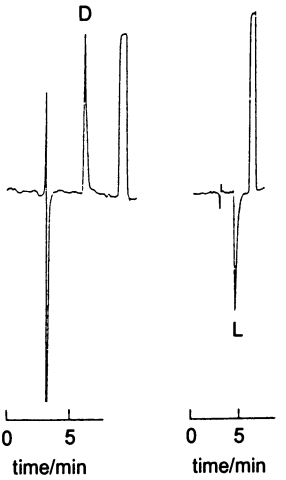
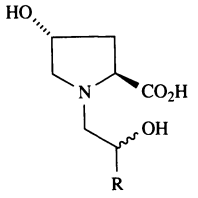


FIGURE 2.7. Chromatograms of D- and *L*-tryptophan. Large signal at the beginning of the D-Trp trace is due to beam defocusing during elution at the void volume. Calibration peaks of 1.6 mdeg appear after the Trp signals. Conditions: column, Spherisorb S5 ODS; solvent, 50:50 methanol:water; flow rate 1 ml min^{-1} (after Lloyd *et al.*).[97]

scale by using a silica-C8 column and a mobile phase containing β-cyclodextrin and buffer.[100]

2.6.2.2. Thin Layer Chromatography (TLC)

Martens and Bhusban[101] advocate TLC as a direct and inexpensive method for the control of enantiomeric purity. Various chiral materials have been used to form the thin layer, such as cellulose and silica gel impregnated with brucine. Commercial plates are available (Chiral plate) composed of resins attached to alkyl derivatives of α-aminoacids, such as (2*S*, 4*R*, 2′*RS*)-*N*-(2′hydroxy dodecyl)-4-hydroxyproline **54**.



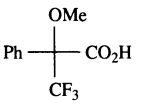
54

The ligand exchange TLC method is based on the stereoselectivity of the Ca^{2+} complex of **54** for L-amino acids. Accurate spotting techniques and use of sensitive densitometers are essential requirements of the TCL method. The authors' examples are restricted to amino acids.

2.6.3. NMR Procedures[18]

NMR methods for the differentiation of antipodal pairs have been known for some time.[102] The sensitivities of magnetic resonance methods have improved following the introduction of high-frequency spectrometers, but usually fall below that of HPLC procedures. However, detection of 1% or less of the minor antipode of a resolved product may often be possible. Procedures involve:

a. Prior Conversation of Antipodal to Diastereoisomeric Mixtures by Use of a Homochiral Reagent. Reagents employed for chiral HPLC separations, discussed above, may serve also for the NMR technique. The frequently used reagent α-methoxy-α-trifluoromethylphenylacetic acid (**55**, MTPA), available in both *R* and *S* antipodal form (Aldrich), has the advantge of (1) marked stability toward racemization (*cf* use of *O*-methylmandely chloride **56**),[103] and (2) good separation of both ^{1}H- and ^{19}F-NMR signals of diastereoisomers (even better at operating frequencies of 270 MHz and above—the original work was carried out at 60 and 100 MHz).



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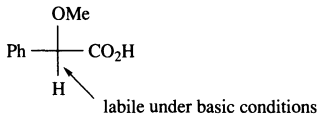
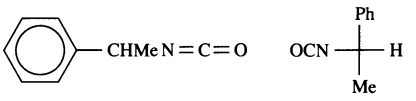


56

MTPA may be applied to chiral analysis of both amines and alcohols. The reagent *S*-(−)-α-methylbenzyl isocyanate **57** is now popular in regard to chiral amines. Thus Rice and Brossi[104] measured the optical purity of the tetrahydrobenzylisoquinoline **58**a by reacting mixtures with *S*-(−)-**57** and examining the ^{1}H-NMR spectra of the diastereoisomeric ureas **58**b; the isomeric methyl signals showed a separation of 0.25 ppm (55 Hz) at 220 MHz. The authors claimed that less than 1% of a minor antipode could be detected in a mixture by this means.



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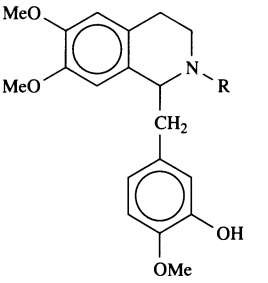
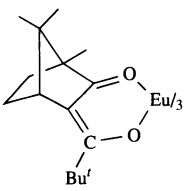


(a) R = H; (b) R = CONHCH(Me)Ph

58

b. Use of a Chiral Shift Reagent (Line-Broadening Effects Restrict Use to Low Frequency Spectrometers). The original example of this kind is that of the resolution of antipodal resonances of *rac*-α-phenylethylamine when the spectrum was run in the presence of the reagent **59** obtained from europium(III) trichloride and *t*-butylhydroxymethylene-(+)-camphor.[105] Many chiral shift reagents of this type have subsequently been introduced.[106,107] The method is practicable and reasonably quantitative if the chemical shift differences between related resonances are great enough at 60 or 100 MHz to allow base-line resolution. Unfortunately, the line-broadening effects of reagents containing lanthanide elements (due to paramagnetic relaxation) are directly proportional to the strength of the operating field and hence resolution is generally lost in spectra recorded above 100 MHz. Cobalt-derived shift agents do not suffer from this disadvantage and cobaltous ATP chelate is of special value, since it may be applied to aqueous (D_2O) solutions.[108] Thus the 360 MHz spectrum of the *rac* imidazoline **60** hydrochlorides in D_2O containing this reagent showed duplicate aromatic signals which were sufficiently resolved to allow estimates of the ee (>98%) of resolved materials (Fig. 2.8).[109]



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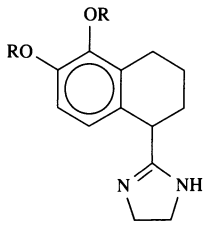


(a) R = H; (b) R = Me

60

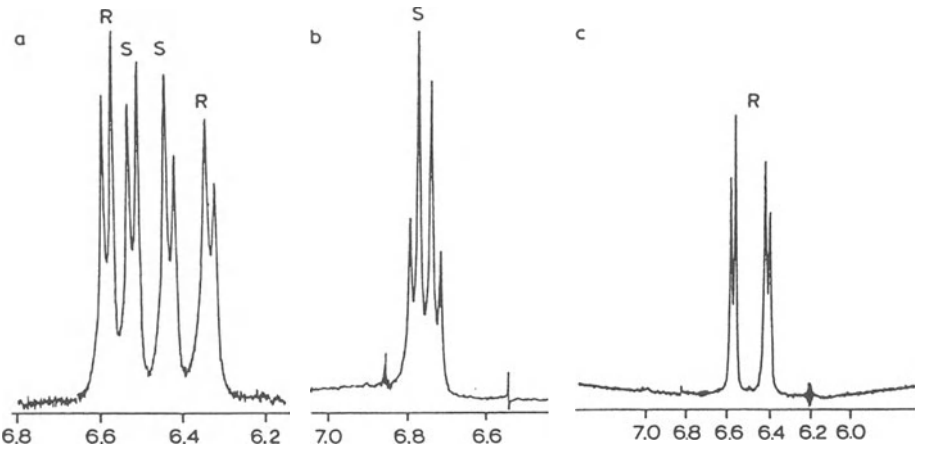


FIGURE 2.8. Part of the 360-MHz ^{1}H-NMR spectra of (a) *rac*-**60**a in D_2O + 200 µl Co-ATP, (b) (−)-*S*-antipode in D_2O + 300 µl Co-ATP, and (c) (+)-*R*-antipode in D_2O + 150 µl Co-ATP. Resonances are due to the aromatic protons of **60**a (after Bernardis *et al.*).[109]

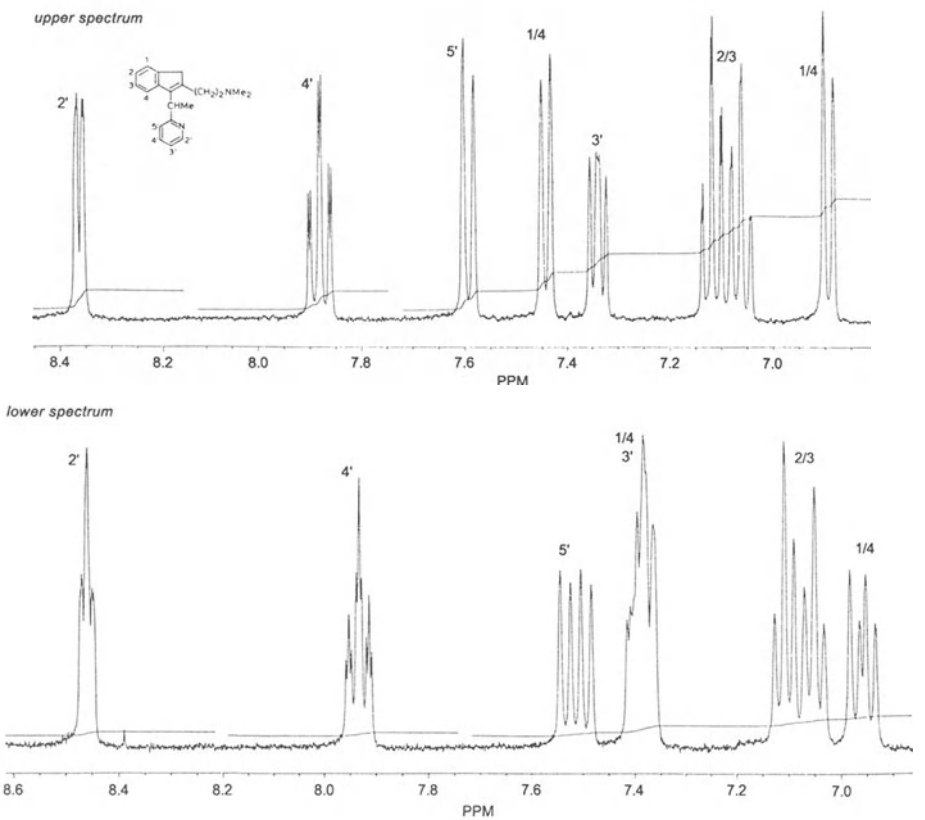


FIGURE 2.9. Low-field region of the 400-MHz ^{1}H-NMR spectrum of *rac*-dimethindene maleate in D_2O. Upper spectrum, no additive; lower spectrum, after addition of 1 molar proportion (approx) of β-cyclodextrin. Clear duplications of 5′ and 1 (or 4) proton signals are evident, while most of the other aromatic resonances become more complex after addition of β-cyclodextrin (after Casy and Mercer).[111]

c. Use of Spectra of Inclusion Complexes Formed with Cyclodextrins. ICI workers first showed that certain ^{1}H-NMR features of antipodes of propranolol included in β-cyclodextrin differed sufficiently to allow separate quantification of the two complexes.[110] This observation was followed up at Bath and the phenomenon observed for a variety of chiral antihistamines and analgesics.[111] The ^{1}H-NMR spectrum of *rac*-dimethindene maleate before and after addition of a molar proportion of β-cyclodextrin is a good example of how antipodal resonances may be resolved by this means (Fig. 2.9). While effects are seen chiefly among aromatics protons, the methine proton signal adjacent to the pyridyl substituent is also duplicated in the presence of the host molecule.

The pyridyl 5′-H resonance provides good evidence of optical purity.[112] It appears as a pair of well-resolved doublets of separation 15 Hz in the 400 MHz spectrum of the *rac*-maleate complex (Fig. 2.9). The 5′-H signals in the corresponding 270 MHz spectra of resolved material formed nearly symmetrical doublets with no evidence of antipodal impurity (no distortion of the slope of the lower field edge of the *dextro* signal, or the higher field edge of the *levo* signal). Spiking experiments were performed by recording inclusion spectra of the (+)-antipode mixed with increasing amounts of the (−)-isomer and monitoring the appearance of the 5′-H doublet. The limit of detection of the minor antipode was found to be between 0.76 and 1.96%.

Another clear-cut example is that of *rac*-fenoldopam (Fig. 2.10) in which the single proton resonance of ring A and the higher field doublet of ring B are duplicated after inclusion in β-cyclodextrin—components of the former signal are of notably high separation (37 Hz at 400 MHz) giving base-line resolution.[113]

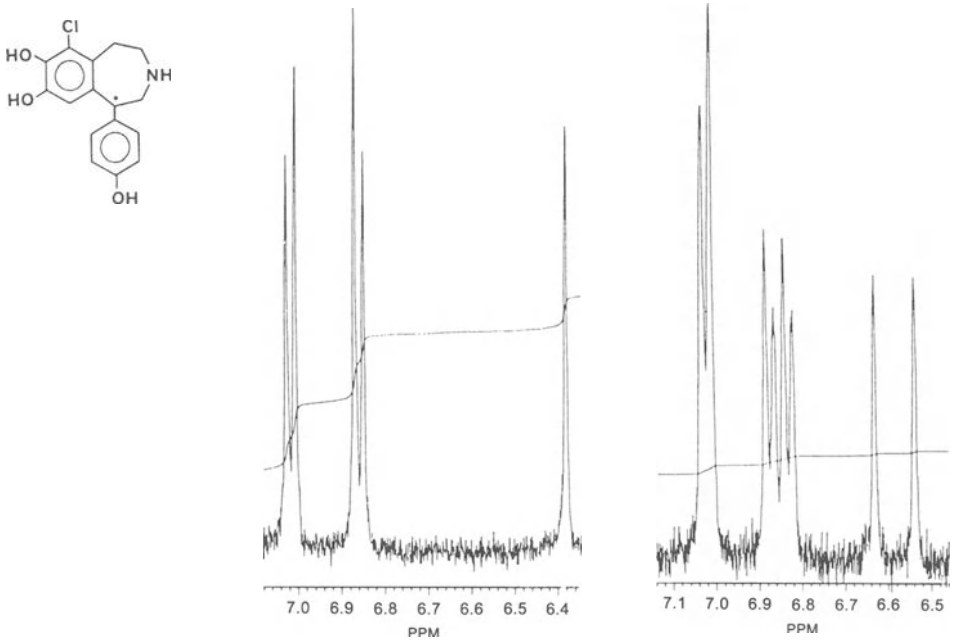


FIGURE 2.10. Aromatic proton region of the 400-MHz ^{1}H-NMR spectrum of fenoldopam mesylate: LHS in D_2O alone; RHS in D_2O + 1 mole proportion of β-cyclodextrin. Chemical shifts in ppm. Singlets near 6.6 ppm of RHS spectrum are separated by 37 Hz (spectra recorded at Bath).

Antipodal analysis by NMR employing cyclodextrins has several advantages over derivatization and chiral shift reagent methods: application to water-soluble materials, absence of broadening effects on ^1H resonances, and narrow range of ^1H resonances due to the host.

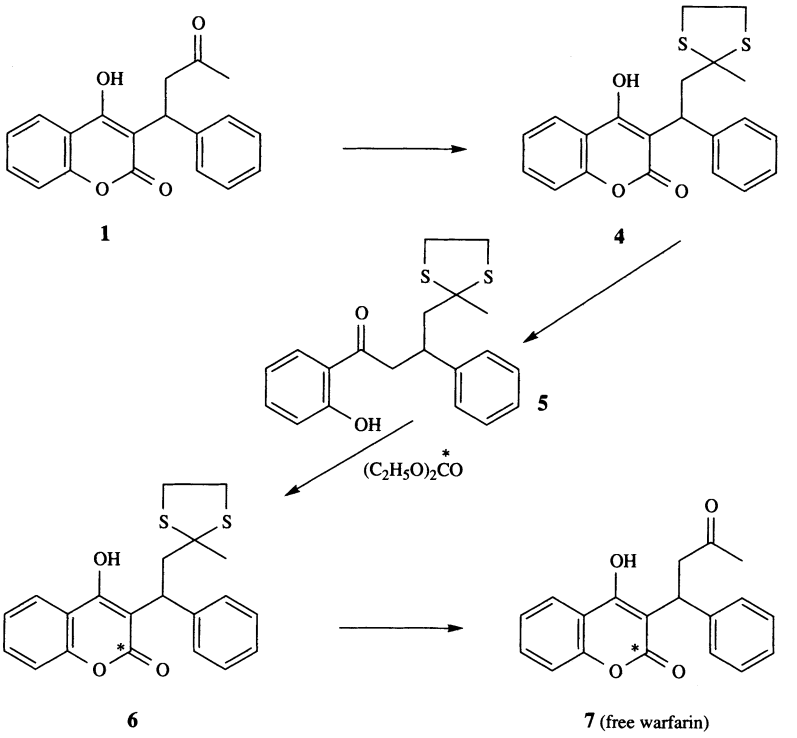
2.7. Pseudoracemates

Mass spectrometry normally has no role in chiral analysis unless coupled to GC after chiral derivatization of the antipodal analytes, e.g., analysis of *R*- and *S*-1-methyl-1,2,3,4-tetrahydroisoquinoline.[114] However, it is a valuable tool in work where "pseudoracemates" are employed. This term is applied to 1:1 mixtures of antipodes in which one member carries a site or sites were ^1H or ^{12}C have been replaced by ^2H or ^{13}C.[115] Such mixtures are valuable aids to the study of the pharmacokinetics of racemic mixtures. After drug administration plasma or urine samples are subjected to GC-MS analysis; ion ratio intensities (e.g., $M : M + 1$ for a mass difference of one) recorded for intact drug and its metabolites provide evidence of stereoselectivity in regard to both distribution and biotransformation of the racemic mixture. The method has the further advantage of permitting concurrent analysis of *R* and *S* antipodes, thus eliminating day-to-day biological variations which introduce differential factors if antipodes are analyzed on separate occasions in the same individual. Furthermore, interactions between antipodes will also be revealed by this procedure. Browne,[116] in a general review of the use of stable isotopes in pharmacokinetic investigations, has drawn attention to both the advantages and disadvantages of the technique. When the original isotope is replaced by a heavier one, a stronger bond results. If this bond is broken in a metabolic process the reaction will proceed more slowly. This so-called metabolic isotope effect is most significant for deuterium-labeled drugs because of the twofold mass difference involved, but becomes of less importance when heavy forms of carbon, nitrogen, and oxygen are employed. The enzymatic N-dimethylation of morphine and N-CD_3 normorphine provides a case in point—the deuterated analogue was a poorer substrate for rat liver microsomal enzymes than the natural alkaloid (Km 3.95 for morphine, 5.66 for the N-CD_3 analogue) and required a higher energy of activation.[117] The examples of warfarin and hexobarbitone are described here.

Optically active, isotopically labeled warfarin was obtained by a process of ring opening and decarboxylation followed by recarboxylation with suitably labeled (^{13}C, ^{14}C) diethyl carbonate and ring close (Scheme 2.8). The procedure was carried out on the ethylene dithioketal of warfarin to protect its side-chain carbonyl function.

Isotopically labeled antipodes of phenprocoumon were obtained in the same way (no derivatization was required). The warfarin pseudoracemate was prepared by mixing *R*-[^{12}C]warfarin with *S*-[2-^{13}C]warfarin in a 1:1 ratio.[120] Relative and absolute quantitation of the warfarin antipodes and their metabolites was obtained by measurement of ion ratios with reference to internal standards by GC-MS analysis.[121]

In the case of hexobarbital the pseudoracemate consisted of a 1:1 mixture of *S*(+)-hexobarbital and ($^2H_3$)-*R*-(−)hexobarbital.[122] The deuterated *R*-antipode **62**



Notes

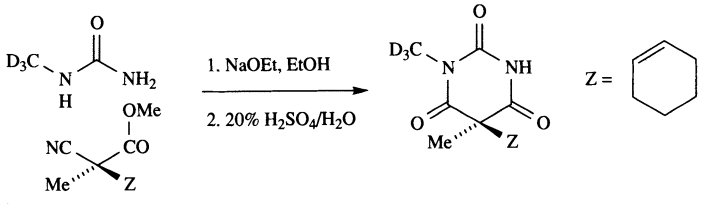
4 → 5 5N NaOH in Na borate solution, heat in oil bath at 155 °C for 24 h under N_2 in sealed stainless-steel container.

5 → 6 90% enriched diethyl carbonate ^{13}C obtained by treating labeled silver carbonate with ethyl iodide and Et_4NI in DMF in cyclohexane-benzene NaH reflux 6 days. [119]

6 → free W mercuric acetate, acetone-water, 55 °C, 45 min.

Scheme 2.8. Synthesis of isotopic labelled warfarin (after Porter *et al.*). [118]

was synthesized by condensing *S*(+)-methyl-2-cyano-2(1-cyclohexenyl)propionate **61** with *N*-trideuteromethylurea (from NCD_3HCl and potassium cyanate). Analysis of pseudoracemic hexobarbital and its major metabolites was by GC-MS with selective ion monitoring. In view of the position of the ^2H label it is fortunate that 3′-hydroxylation rather than N-demethylation is the principle route of metabolism of hexobarbitol (see Chap. 3, p. 55).



S(+)
61

R(−)
62

2.8. Radioimmunoassay

Radioimmunoassay (RIA) techniques, which involve raising antisera that interact stereospecifically with the *R* or *S* form of a drug, provide another means of chiral analysis used in pharmacokinetic investigations. A radiolabeled form of the analyte is required—this is incubated with the antiserum and the uptake of the radioactivity by the analyte–antiserum complex measured in a calibration plot may then be applied to unknown amounts of analyte. The example of pentobarbitone is described in Chapter 3 (p. 56).

2.9. Pharmacological Methods

A wide range of procedures are used to assess and quantitate the biological properties of chiral molecules in both the homochiral and racemic mixture form. These have become increasingly sophisticated over the years in which the present author has followed biological stereoselectivity, emphasis today being placed on the provision of data of direct relevance to the target site, and the biochemical consequences of drug action.

Receptor classification is now well established and often complex in regard to receptor subtypes.[123,124] Much of the evidence rests upon the development of selective ligands of both the agonist and antagonist variety, and the discovery of biological models rich in a specific receptor or receptor subtype (see, for example, Casy,[125] on opioid receptor characteristics of tissues). Procedures may be classified under four headings.

2.9.1. Whole Animal (*in Vivo*) Experiments

Special consideration needs to be given to pharmacokinetic factors in such work (Chapter 2) especially when the route of administration is by mouth. End-points of *in vivo* assays are often subjective, e.g., behavioral tests and data require statistical analysis.

2.9.2. Isolated Tissue (*in Vitro*) Procedures

Application of the drug to its target site is more direct in such experiments, but passage of various barriers may nevertheless be required with potential influence on stereoselectivity.

2.9.3. Binding Experiments[126]

These techniques probably provide the most direct evidence of ligand – receptor interactions, since they involve use of particulate fractions of nervous and other tissue which contain membrane-sited receptor proteins. The procedure is to incubate a radio-labeled ligand with such tissue and, after equilibration, to determine the radioactive uptake of the tissue after its separation from supernatant fluid and washing. This experiment provides a measure of *total* binding, i.e., that due to specific (at receptors) and nonspecific sites. The latter are deemed infinite and the

nonspecific contribution may be assessed by repeating the experiments in the presence of a large excess of nonradioactive (cold) ligand when all specific binding of the radioligand is displaced. Competitive experiments carried out by incubating tissue with radioligand in the presence of first one antipode and then its isomer provides the stereoselectivity of binding of a novel chiral agent, a technique known as stereospecific binding (SSB) and introduced by Goldstein[127] in regard to opioid ligands. Receptor subsite preferences may be gauged by including ligands of known selectivity in the incubation mixture (e.g., study of μ, δ, and κ-opioid subsites). Beer *et al.*[128] determined the selectivity of a range of adrenoceptor ligands for β_1 and β_2 substites by measuring uptake of [^{125}I]IPIN (nonselective) in the presence of excess of first a selective β_2-ligand (ICI 118-551) and then a selective β_1-ligand (CGP20712A) (Chapter 5, page 158).

Criteria in regard to binding assays are shown in Table 2.1.

In general, binding experiments provide measures of binding affinity—not efficacy, hence agonists, antagonists, and partial agonists will not usually be differentiated (see, however, the effect of low concentrations of Na^+ on the ligand affinity of opioid ligands—that of an agonist is decreased while that of an antagonist is increased).[134]

If competitive binding experiments involve a radioligand in racemic mixture form, misinterpretation of the experimental data is possible since it relates to the

TABLE 2.1.
Specificity Criteria and Conditions for Receptor Identification by the Radioligand Binding (after Čarman-Kržan)[126]

1. Radioligand — Radioactively Labeled Receptor Agonist or Antagonist
   Criteria: pharmacologically active
   chemically pure
   specific radioactivity: 5–90 Ci/mmol, preferentially in the range above 20 Ci/mmol for the tritiated products; in the range of 2000 Ci/mmol for iodinated products

2. Binding — Saturability and Affinity of the Receptor-Binding Sites
   Parameters: number of receptor-binding sites (B_{max})
   equilibrium dissociation constant (K_D) for the receptor–radioligand complex, a measure for the affinity

3. Kinetics of Binding Reaction — Reversibility of the Receptor–Ligand Interaction
   Parameters: association rate constant k_1 — determined from the time course of ligand–receptor binding
   dissociation rate constant k_{-1} — determined from dissociation of ligand–receptor complex
   equilibrium dissociation constant $K_D = \frac{k_{-1}}{k_1}$

4. Drug Displacement in Competition Binding Studies — ability of nonradioactive agonist, antagonist, or drug belonging to different chemical and pharmacological classes to compete for the radioligand binding site. To determine affinity, specificity, or stereospecificity of the competitor
   Parameters: $K_i = \frac{IC_{50}}{1 + [L]/K_D}$
   K_i = dissociation constant for the receptor–inhibitor complex, a measure for the affinity
   IC_{50} = concentration of unlabeled ligand (inhibitor) giving half maximum displacement of radioligand
   [L] = concentration of radioligand in the assay
   K_D = dissociation constant for the receptor–radioligand complex

displacement of two radioligands, not a single species. This is particularly serious for high-affinity, but low-activity, radioligands when receptor and radioligand concentrations are well above the K_d values of the radioligand. High-affinity/high-activity radioligands may be used at very low concentrations and, under these conditions, the contribution of the distomer to the overall binding is negligible. Reasons for the avoidance, in general, of *rac*-radioligands have been presented by Hoyer.[129]

The importance of functional correlates between the binding affinity and pharmacological potency has been stressed by Laduron.[130] Artefacts may arise, such as the specific binding of [^{3}H]methylscopolamine to glass fiber filters.[131]

2.9.4. Biochemical Assays

Many biochemical consequences of ligand–receptor interactions are now understood and quantification of these events provides a measure of the efficacy of such interactions. The majority of applications relate to catecholamines, dopamine, and their metabolites and depends on the availability of sophisticated methods of analysis—usually involving HPLC technology. Often, inhibitors of enzymes that would otherwise degrade analytes are added to the system, e.g., addition of the decarboxylase inhibitor 3-hydroxybenzylhydrazine (NSD1015) to allow the accumulation of DOPA (Chapter 6, page 183) or 5-HTP (Chapter 12, page 416).[135] Measurement of *c*AMP, the second messenger of many membrane-sited receptors, is also frequently employed as a criterion of ligand potency, while reports of phosphoinositol (PI) turnover are becoming more common, especially in studies of cholinergic ligands.[132]

2.9.5. General Remark

It is outside the scope of this book to summarize pharmacological methods relevant to each chapter in a systematic manner, although some explanation of tests employed and their significance will be included at appropriate points. As an excellent example of a concise presentation of pharmacological methods in a particular area, the reader is directed to the account of the evaluation of α_2-adrenoceptor antagonists included in the 1986 review of Clark and his colleagues.[133]

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3

Pharmacokinetics

Including Use of Racemic Mixtures in Therapy

3.1. Introduction

When stereoisomers are exposed to a biological system the potential exists for differential phenomena at all levels of interaction in consequence of the dissymmetric nature of the majority of molecules which make up a living organism. Thus if the xenobiotic agents are antipodal, pairs of diastereoisomeric interactions arise. As far as pharmacologically active compounds are concerned, whether these be of endogenous or exogenous kind, such phenomena fall into two classes. The first involves processes which govern the transport of the molecule (ligand) to its site of action, the so-called *pharmacokinetic aspects*. The second concern events at the target site, which is generally macromolecular in nature and may be an enzyme or a membrane-sited pharmacological receptor. Ligand–receptor interactions of this kind are often termed the *pharmacodynamic* aspects of a bioactive molecule.

The primary object of this book is to illustrate the influence of molecular geometry on the interactions of ligands with biological receptors and to show how dissymmetric molecules may act as probes of receptor structure. The relative activities of stereoisomeric sets form an important parameter and yardstick of such studies. These values are dependent, however, on both pharmacodynamic and pharmacokinetic processes. Hence knowledge and appreciation of factors which may differentially influence ligand concentration at its site of action must obtain before experimentally observed, relative activity parameters may be interpreted in terms of receptor events.

The influence of pharmacokinetic processes is liable to be greatest when isomeric potency ratios are assessed under *in vivo* conditions, e.g., in whole animal experiments—this applies whatever the route of administration, although the oral route may lead to the greatest role for differential distribution effects. Due regard to the nonidentical handling of antipodal pairs should also be given following

administration of racemic mixtures which constitute the form in which the large majority of chiral drugs are clinically employed (see page 61).

An outline of pharmacokinetic processes that influence the transport of stereoisomers to their target sites, and of their relative significance, is therefore presented in this chapter. Details of such processes form part of each subsequent chapter, as appropriate to the pharmacological group described.

The 1988 monograph of Wainer and Drayer[1] includes several chapters of relevance to pharmacokinetics, as does the report of a meeting held at Tubingen in the same year (Ref. 20 of Chapter 1).

Processes of *absorption*, *distribution*, *metabolism*, and *excretion* all influence the concentration attained by a xenobiotic at its target receptor and each may show stereoselectivity in regard to isomeric sets. Absorption of drugs from the gastrointestinal (GI) tract generally occurs by a passive process dependent on drug concentration gradients and which handles isomers in the same manner. Stereoselectivity may occur when active processes operate which involve a carrier molecule. Thus absorptions of DOPA and methotrexate take place by active transport which favors the L-enantiomers in each case, e.g., after 30 min the concentration of L-DOPA in mucosal fluid fell to 90 μg/ml as compared to the value of 171 μg/ml for the D-isomer.[2,3] The case of the β-lactam antibiotic cephalexin is an extreme one—only the D-form (7-acylamino side-chain chiral center) is accepted by the dipeptide transport system of rat intestine[4] as confirmed by *in vitro* studies.[5] Thus the L-isomer was not present in serum and urine after oral administration to rats—the D-form was well absorbed (an everted intestinal sac experiment gave the same result). The presence of enzymes in the GI tract wall* that invert (*R* to *S*) the chiral center of nonsteroidal anti-inflammatory drugs (NSAIDs) of the profen type ($Ar^*CHMeCO_2H$) adds a complication to the stereoselectivity of their absorption, as reviewed by Jamali.[6] The longer the *rac*-drug remains unabsorbed, the greater the *S*:*R* AUC ratios observed in the plasma (AUC is the area under the curve of plots of *R* and *S* concentrations against time)[7] Carrier-mediated uptake in blood cells and transport in the kidney, liver, and brain has been reviewed; data relevant to chiral drugs are sparse, however.[8]

3.2. Distribution and Protein Binding

Two factors govern the distribution of intact drug molecules throughout the body: (1) their partition coefficient—this parameter (usually measured in a water–1-octanol system) is important in regard to passive diffusion across the various lipid barriers encountered by the absorbed drug, and (2) plasma and tissue protein binding.[9] Only the latter is expected to be enantioselective, and there are many reports of the protein binding of both chiral acids and bases.[10] Most of the work relates to serum albumin and, less frequently, α-acid glycoprotein.

Although there are a few reports of large differences in the binding affinities of chiral agents such as L-tryptophan 100 times that of D-Trp to bovine mercaptoalbumin,[11] or oxazepam hemisuccinate 35 times that of R to HSA[12], antipodal affinity differences are usually modest in degree. Thus for the extensively bound acids of Table 3.1 (adapted from Jamali *et al.*),[13] most ratios fall below two as

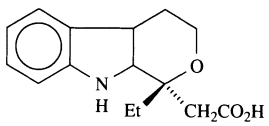
* There is recent evidence that in the rat the inversion site is located in the liver.[5a]

TABLE 3.1.
Plasma Protein Binding of Antipodal Pairs of Some Chiral Drugs (adapted from Jamali *et al.*)[13]

Drug	Percent unbound	Species	Ref.
Acidic			
Acenocoumarol	2:1.8 (*S*:*R*)	human	14
	1.4:1.6 (*S*:*R*)	rat	15
Etodolac	2.2:0.5 (*S*:*R*)	rat	16
Flurbiprofen	0.048:0.082 (*S*:*R*)	human	17
Gliflumide	*R* > *S*	human	18
Hydratropic acid	49.8:44.3 (*S*:*R*)	rat	19
Ibuprofen	*S*:*R* = 1.7	human	20
Mephobarbital	53:66 (−):(+)	human	21
N-Methyl-5-Cyclohexenyl-5-ethylbarbital	63:73 (−):(+)	human	21
Moxalactam	32:47 (*S*:*R*)	human	22
Pentobarbital	26.5:36.6 (*S*:*R*)	human	23
Phenprocoumon	0.72:1.07 (*S*:*R*)	human	24
Warfarin	0.9:1.2 (*S*:*R*)	human	25
	0.8:1.3 (*S*:*R*)	rat	25
Basic			
Amphetamine	84:84 (−):(+)	human	26
Chloroquine	51.5:33.4 (−):(+)	human	27
Disopyramide	22.2:34.0 (*S*:*R*)	human	28
	10.9:36.6 (*S*:*R*)	human	29
Fenfluramine	2.9:2.8 (−):(+)	human	30
Methadone	12.4:9.2 (−):(+)	human	31
Mexiletine	28.3:19.8 (*S*:*R*)	human	32
Nilvadipine	0.9:1.0 (−):(+)	human	33
Oxazepam	(−) > (+)	human	12
Pindolol	45:45 (*S*:*R*)	human	34
Propranolol	10.9:12.2 (−):(+)	human	35
	22.0:25.3 (−):(+)	human	36
Tocainide	83-89:86-91	human	37
Verapamil	11:6.4 (−):(+)	human	38

judged from the percent unbound values. The most extreme example is that of etodolac with a factor of 4.4 that is well below the eudismic ratio of 100 (*S*-eutomer) recorded for the two antipodes of this NSAID.[39]

The plasma concentration of the *S*-eutomer **1** was considerably less that that of the *R*-form (AUC *S*:*R* 2.5:30.9 mg l^{-1} h^{-1}). This difference is considered due to a greater volume of distribution of the *S*-antipode (*S*, 101 l, *R* 25 l for one subject), an explanation which correlates with the greater degree to which *R*-etodolac is bound to plasma proteins and thus more confined to the circulatory system (the



1, *S*-antipode shown

volume of distribution is calculated by dividing the dose by the plasma concentration extrapolated to zero time). Analysis was by derivatization of antipodal acids extracted from plasma with (−)-α-phenethylamine (via mixed anhydride formed by reaction with $ClCO_2Et$) followed by chromatography on silica gel.

The same observations apply to the binding of chiral bases where free drug levels are generally higher than those of acids.

3.2.1. Warfarin (see also page 58, Anticoagulants)

Differences in the degree of binding of antipodes of *warfarin* (**6**a) to HSA, are small (% bound *R*: 99.15; *S*: 99.47) and account for the greater volume of distribution of the *R*-antipode ($V_R:V_S=1.6$). Toon and Trager [40] have revised the eudismic ratio of 5 (*S*-eutomer), a value which took no account of differential plasma binding of warfarin enantiomers[41] to 8 (5×1.6) on this basis.

If warfarin and the uricosuric agent sulfinpyrazone are coadministered, the pharmacological response to the anticoagulant is enhanced.[42] This result may be a consequence of sulfinpyrazone inhibiting the metabolism of *S*-warfarin (the eutomer) while inducing that of the *R*-form.[43] In a later study using a pseudo-racemate,[44] decreased clearance of the *S*- and increased clearance of the *R*-antipode were confirmed, but the latter phenomenon shown to result from the selective displacement of *R*-warfarin from plasma protein binding sites rather than enzyme induction. Sulfinpyrazone brought about a 49% increase in the *R*- and 9.4% increase in the *S*-free unbound fractions of warfarin antipodes. The antibiotic rifampin, which diminishes the anticoagulant action of warfarin, increased the clearance of *R*-warfarin threefold and that of the *S*-form twofold—an effect traced to induction of cytochrome P-450 isozyme(s) responsible for aromatic hydroxylation.[45]

The greater binding of *S*-warfarin has been confirmed by a direct on-line HPLC procedure.[46] The method involved injection of a mixture of *rac*-warfarin and HSA onto a column which excludes macromolecules but retains small molecules. A column switching device then allowed a portion of the eluent containing free warfarin onto a chiral α-glycoprotein (AGP) column capable of resolving the antipodal forms. The free *R*-concentration exceeded that of *S*-warfarin 1.03- to 1.24-fold. Achiral/chiral coupled HPLC studies of *rac*-warfarin have also been reported by Chu and Wainer[47] and McAleer and Chrystyu.[48]

In a series of 14 warfarin derivatives bearing substituents in the $MeCOCH_2CH$*Ph* ring (see **6**a), greater binding up to a factor of 4 was observed in rats for the *S*-enantiomers except for 2′-substituted analogues (*rac*-drug given sc, chiral analysis applied).[49]

Sulfinpyrazone treatment did not affect the hypoprothrombinemia produced by phenprocoumon nor did it significantly alter the plasma elimination kinetics of the individual *R*- and *S*-enantiomers; this study also employed a pseudo-racemate.[50]

3.3. Metabolism

The stereoselective biotransformation of exogenous materials (xenobiotics) is well documented and the phenomenon is a major factor contributing to the

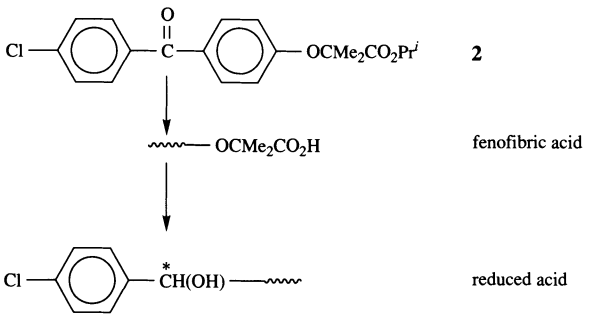
pharmacokinetics of chiral and prochiral molecules. Many reviews are available (see Refs. 13, 51–55, 167).

The significance of drug metabolism is greatest when the drug is administered by the oral route, because all material absorbed from the GI tract is transported via the heptatic portal vein to the liver where it undergoes so-called *first pass* metabolism. When a drug is given by the intravenous or another nonparenteral route, the extent of its metabolism is limited by the rate of blood flow through the liver. Hence differences in plasma levels of drugs and their metabolites after oral compared with IV routes are often encountered, especially in the case of agents that are highly extracted by the liver.

Many varieties of chemical transformation takes place in the liver—mostly with the object of detoxifying the foreign agent and expediting its elimination from the body. Phase I reactions are oxidative (e.g., hydroxylation, dealkylation, deamination), reductive (e.g., aldehyde and nitro reductions), and hydrolytic (e.g., cleavage of ester and amide links) in which changes in substrate functionality take place. Phase 2 reactions mostly involve the linkage (conjugation) of small endogenous molecules of polar character to foreign molecules either in their unchanged form or after they have suffered Phase 1 reactions, e.g., glucuronide and sulfate conjugation, acylation, and methylation. All these reactions carry the potential of stereoselectivity in varying degree, although the extremes seen in certain physiological biotransformations, such as the biosynthesis of catecholamines (stereospecific β-hydroxylation of dopamine, page 78), are rare in xenobiotic metabolism.

In this book we are chiefly concerned with processes of *substrate stereoselectivity* where isomeric pairs are metabolized at different rates and/or by different routes. *Product stereoselectivity* is observed as a result of the creation of chiral centers by the biotransformation of prochiral substrates. When the substrate is achiral, a pair of enantiomers result, when chiral, a pair of diastereoisomers—of variable ratio in either case if stereoselectivity operates.

A recent example is that of the metabolic reduction of the hypolipidemic drug fenofibrate **2**[56] In three animal species the reduction was markedly enantioselective for the levo isomer [(−): (+) ratio 95:5]. This was not due to differences in excretion of the antipodes as established by dosage with *rac*-"reduced"-fenofibric acid



which was recovered in the urine without change in antipodal ratio. HPLC analysis after derivatization with *R*-(+)-[naphthen-1-yl]ethylamine, or directly using a Cyclobond (cyclodextrin) column, was employed.

TABLE 3.2.
Substrate Stereoselectivity in the Metabolism of Chiral Drugs (adapted from Jamali *et al.*)[13]

Drug	Stereoselecivity pathway (dominant antipode)	Species	Ref.
Amobarbital	N-glucuronidation (*S*)		165
Amphetamine	deamination (+)	human	57
Benoxaprofen	inversion (*R*)	human & rat	58, 59
Bromoisovalerylurea	glutathione conjugation (*R*)	rat	60
Bufuralol	1′-hydroxylation (+); 4-hydroxylation (−)	human	61
Cyclophosphamide	C-4 hydroxylation (*S*)	rabbit	62
Disopyramide	N-dealkylation (*S*)	dog	28
	aryl hydroxylation (*R*)	rat	28
Esmolol	ester hydrolysis (−)	dog, rat	63
	ester hydrolysis (+)	monkey, rabbit, guinea-pig	63
Fenfluramine	de-ethylation (−)	rat	64
	de-ethylation (+)	human	64
Fenoprofen	inversion (*R*)	human & rabbit	65, 66
Fenoterol	glucuronidation (+)	rat	67
Flunoxaprofen	inversion (*R*)	rat	68
Flurbiprofen	inversion (*R*)	rat	69
Hexobarbital	3′-hydroxylation: (+) to β-OH, (−) to α-OH	rat	70
Hydratropic acid	inversion (*R*);	rat, rabbit	71, 72
	glucuronidation (*R*)	mouse	71
Ibuprofen	inversion (*R*)	human, rat	73
Indacrinone	hydroxylation (*R*)	monkey	74
Ketoprofen	inversion (*R*)	rabbit, rat, human	75, 76
Mephobarbital	hydroxylation (*R*)	human	77
Mephenytoin	4-hydroxylation (*S*) demethylation (*R*)	human	78, 79
Metoprolol	*O*-demethylation (+) α-hydroxylation (−)	human	80
Mexilitine	glucuronidation (*R*)	human	81
Naproxen	inversion (*R*)	rabbit	82
Nicotine	N-methylation (*R*)	human	83
Nilvadipine	oxidation (−)	human	84
Norgestrel	16α-, 16β-, and 1β-hydroxylation (−); ring A oxidation and 2α-hydroxylation (+)	human	85
Normephenytoin	demethylation (*R*) aryl hydroxylation (*S*)	human	79
Oxazepam	glucuronidation (*S*)	rabbit, dog, human	86, 87
	glucuronidation (*R*)	monkey	87
Penbutolol	4-hydroxylation (+)	human	88
Perhexiline	hydroxylation (−)	human	89
Phenobarbital	N-glucuronidation (*S*)	mouse	166
Phenylethylhydantoin	hydroxylation (+)	dog	90
	hydroxylation (*S*)	human	91
Primaquine	oxidative deamination (−)	rat	92
Propranolol	4-hydroxylation (+)	human	93
	glucuronidation (−), ring oxidation (−)	dog	
	glucuronidation (+)	rat	95
Tocainide	conjugation (*R*)	human	96
Xibenolol	4-hydroxylation (−)	human	97

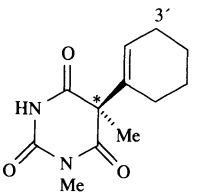
Table 3.2, adapted from Jamila *et al.*,[13] provides a range of examples, some of which are discussed in appropriate chapters of this book. Analytical techniques are crucial in documentation of the stereoselective metabolism of drugs and conclusions must allow for contributions from other pharmacokinetics processes.

3.4. Examples

A few examples, chiefly chosen from therapeutic agents not discussed in later chapters of this book, are presented here to illustrate points made above. Chiral *barbiturates* have attracted much attention in regard to their pharmacokinetics.[97]

3.4.1. Hexobarbital (3)

Antipodes of this hypnotic agent have been tested in rats and evidence of a potency difference obtained. Differences were not great and clear-cut ratios were not defined.[98] In man, 400-mg doses of the dextro antipode resulted in a clear central depressant effect which lasted for several hours, while hardly any effect was noticed after the same dose of (−)-**3**. Plots of plasma concentrations of drug

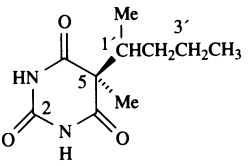


3

following oral dosage of (+)-, (−)-, and *rac*-hexobarbital are shown in Fig. 3.1; analyses were by a nonspecific GC assay. The higher levels and half-life of the dextro eutomer were traced to preferential metabolism of the levo antipode; chief metabolites were the 3′-hydroxy (α-placed in the case of the levo, β-placed in the case of the dextro isomer) and 3′-keto derivatives (see Miyano *et al.*,[99] in regard to α-β-stereochemistry). Later, a study employing a stereospecific assay was made in rats [pseudoracemate method: *S*(+)-N−Me: *R*-(−)-N−CD_3] (p. 41).[100] In contrast to humans, rats (and other species) metabolize *S*-(+)- faster than *R*-(−)-hexobarbital; a plasma clearance of *S*-(+)-**3** sevenfold greater than that of the (−)-antipode was reported.

3.4.2. Pentobarbital (4)

This barbiturate is metabolized by hydroxylation at C-3′ of the *sec*-pentyl side chain. In the dog diastereoisomers in ratio 5 (*SR*) to 1 (*SS*) were obtained from *S*-pentobarbital while *R*-**4** produced at 1:1 mixture.[101] Analysis was by GC-MS of trimethylsilyl ethers.[102]



4

In man 3′-hydroxylation of the 2-thio analogue of **4**, thiopental, was substrate-stereoselective for the *S*-enantiomer and the prime product was the 1′-*S*, 3′-*S* isomer. Species differences in this regard were considered due to differences in the active sites of isozymes present in man and dog.

A more recent pharmacokinetic study of pentobarbital antipodes employed a radioimmunoassay (RIA) technique (see Cook[103] for a review on competitive binding methods by RIA and receptor binding assays). Individual antipodes were N-alkylated with N-crotonic acid and then coupled to bovine serum albumin (Scheme 3.1). Immunization of rabbits with the conjugates led to formation of antisera that selectively bond to the predicted enantiomer with 1.0–1.4% cross-reaction. A radioligand for competitive binding study was synthesized by catalytic (Pd–C) reduction of *R*- and *S*-secobarbital with tritium (secobarbital is the 5-allyl analogue of pentobarbital—the presence of a 5-propyl rather than 5-ethyl substituent did not impair its use in RIA). In humans, the volume of distribution was

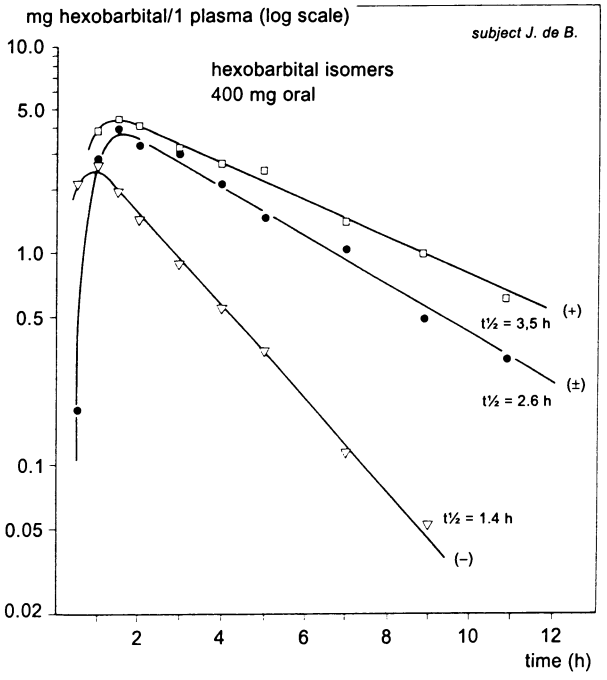
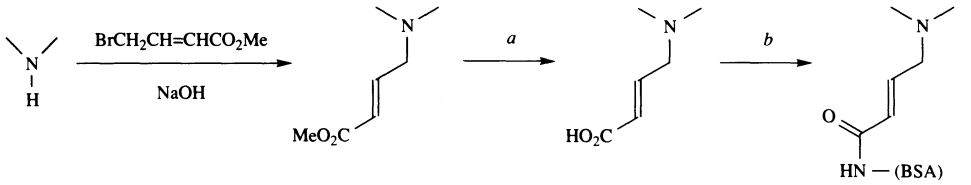


FIGURE 3.1. Blood-concentration time curves of (+)- and (−)-hexobarbital in the same volunteer; 400 mg hexobarbital enantiomers or racemic mixture were given orally on separate occasions (after Breimer and van Rossum).[98]



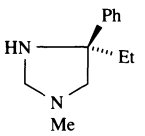
a: HCl in H_2O-dioxane; *b*: $ClCO_2Bu^i$, $^nBu_3N$, BSA

Scheme 3.1. Details of RIA technique.

12% greater for the *R*-enantiomer which also showed a 14% greater rate of elimination. As a result the median clearance of the *S*-antipode (1.96 l h^{-1}) was 25% less than that of the *R*-isomer (2.58 l h^{-1}). The *S*-antipode was also more strongly bound in plasma (73.5% vs. 63.4% for the *R*-form); protein binding was determined by equilibrium dialysis at room temperature. The authors drew attention to the fact that *S*-antipodes of pentobarbital, phenprocoumon, warfarin (page 52), and glifumide, all with chiral centers of the same kind, are the stereoisomers more strongly bound to serum proteins.

3.4.3. Mephenytoin

The example of mephenytoin **5**, an anticonvulsant related to barbiturates, is of importance in regard to polymorphisms of drug metabolism involving cytochrome P-450 isozymes.[52] Such enzymes are often enantioselective in their oxidative action

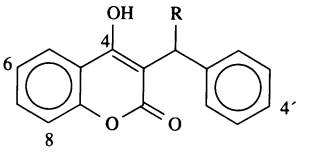


5, *S*-(+)-isomer shown

upon substrates. As a result of genetic variation, individuals of a population usually fall into two classes—those capable of the ready metabolism (extensive metabolizers, EM) and those who biodegrade drugs at slow rates (poor metabolizers, PM). If stereoselectivity is a feature of EM metabolism, it is often of insignificant degree in the PM class. Thus while only the *S*-antipode of mephenytoin **5** is rapidly *p*-hydroxylated, the *R*-form suffers slow N-demethylation in the EM class. In the PM type (2.5% of Caucasian and over 20% of Japanese subjects) N-demethylation is the only significant metabolite route.[104] Under *in vitro* conditions, liver microsomes from EM subjects *p*-hydroxylated *S*-**5** ten times faster than *R*-**5**; the *S*-**5** rate of this reaction was reduced to that of the *R*-isomer when PM microsomes were employed.[105] In a recent Japanese stidy[106] the *R*/*S* ratios of *p*-hydroxylated metabolites of 14 samples were less than 0.3, and the value for 5 others ranged from 0.71 to 2.47. Subjects who showed rations above 0.7 were defined as poor metabolizers. Content of P-450 human-2 enzyme assessed by Western blots was correlated to microsomal S-(+)-**5** 4′-hydroxylation. (See also related studies on adrenergic β-blockers, Chapter 5, page 143.)

3.4.4. Anticoagulants

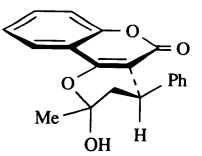
Studies of the metabolic hydroxylation of antipodal anticoagulants are of interest, especially when acted upon by cytochrome P-450 enzymes induced by β-naphthoflavone (BNF).[107] In the case of warfarin (**6a**) 8-hydroxy (major) and 6-hydroxy derivatives were the chief products, and an *R*/*S* ratio of 4.5 was observed. Antipodes of phenprocoumon **6b** were similarly hydroxylated but with a regio preference for the 6-position, and a *reversed* stereoselective index (*R*/*S*=0.2).



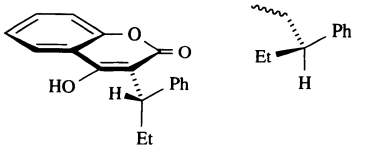
(a) R = CH_2COMe; (b) R = Et

6

Kaminsky *et al.*,[108] obtained similar results after induction with 3-methylcholanthrene. Both groups employed HPLC analyses. Heimark and Trager[109a] rationalize the inverse stereoselectivities of the two anticoagulants in terms of the hemiketal form of warfarin (see **7**), in which the *R* and *S* antipodes have the same relative orientations of the phenyl and coumarin rings. The α-orientation of the ethyl substituent shown in *S*-phenprocoumon (**8**) avoids nonbonded interactions with the coumarin ring and is believed to aid binding to the catalytic site of the enzyme when so positioned (Trager, personal communication).

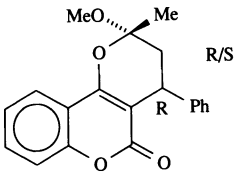


7
R-warfarin



8
S-phenprocoumon

The *R*-enantiomer of cyclocoumarol **9**, the cyclic ketal analogue of warfarin, was likewise selectively hydroxylated. In contrast the *S*-enantiomer of warfarin 4-methyl ether (**6a** 4-OH→OMe) in which hemiketal formation is precluded (hence it is directly analogous in structure to phenprocoumon) was the more extensively metabolized antipode.[109b] It is of interest that eutomeric forms of warfarin and



9

phenprocoumon are both of *S*-configuration—evidence that the open-chain tautomer of warfarin is the active inhibitory form of the drug at the vitamin K dependent site.

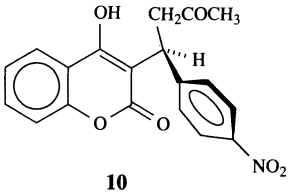
Hemiketal and open-chain forms of warfarin alike in configuration gave rise to CD curves of a near mirror image relationship.[110]

Metabolic profiles of warfarin antipodes measured under *in vitro* conditions using human liver microsomes proved distinctly different from those of the *in vivo* studies.[111] This was traced to the presence of a high-affinity (relevant to the low substrate concentrations of the *in vivo* work) and low-affinity subset of isozymes (important when the substrate concentration is high as in the *in vitro* experiments).

In a series of warfarin derivatives with substituents in the phenyl ring of the side chain, the stereoselectivity of hepatic clearance in rats depended on the position of substitution (warfarin and 3′ $R>S$; 4′ and 2′ *S*-*R*; 4′-OMe/Me $R=S$).[49]

3.4.5. Acenocoumarol (10)

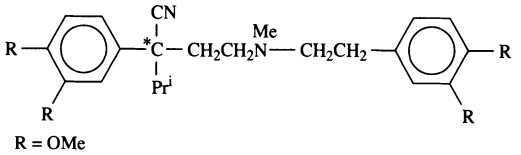
The *R*-antipode of acenocoumarol (**10**, *S* shown) is several times more effective than its antipode as an anticoagulant. This difference was found to be due to the greater rate of clearance of the *S*-distomer from plasma (about tenfold). No difference in binding to plasma proteins was detected. Differences in the potency (and plasma levels) of the antipodes were satisfactorily accounted for by differential metabolic rates (selective for *S*-**10**, reduction of 4-NO_2 → 4-NH_2 → NHCOMe).[112]



10

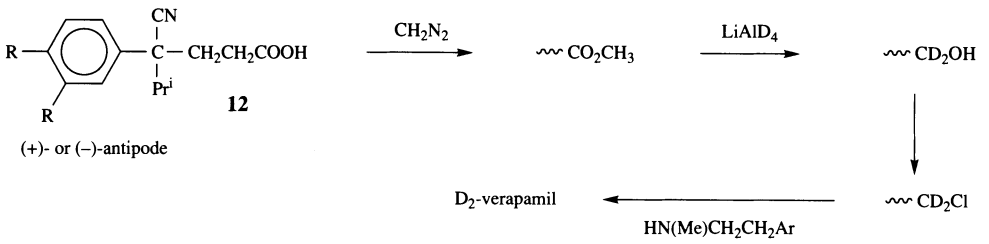
3.4.6. Verapamil (11)

This agent is a Ca^{2+} channel blocking agent marketed as a racemic mixture. Its clinical effects in man were found to be 2–3-fold less after oral than after iv doses when amounts were given which produced equal plasma concentrations as measured by a nonstereoselective method. The answer to this paradox followed application of the pseudoracemate technique when it was discovered that clearance of the more active levo antipode (18.1 ml min^{-1} kg^{-1}) was greater than that of the distomer (10.2 ml min^{-1} kg^{-1}). The difference in clearance was found to be due to stereoselective first-pass biotransformation with preferential elimination of (−)-**11**.[113] In a test on the heart (dromotropic activity, i.e., changes in conduction)



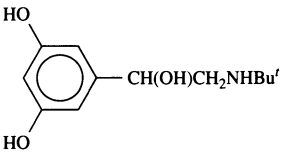
11

Echizen *et al.*,[114] found levo verapamil to have 8 times the activity of the dextro isomer. The ^{2}H-labeled component of the pseudoracemate was made by reactions involving the intermediate **12** prepared as shown. Exactly the reverse situation is found for propranolol (discussed on page 143)—here stereoselective first-pass metabolism of the *less active* form of propranolol results in oral dosage being more effective than nonparental.[115]



3.4.7. Terbutaline (13)

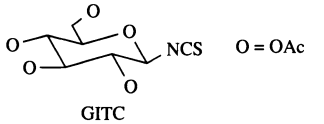
Clearance values (l h^{-1} kg^{-1}) of 0.186 for (+)-terbutaline and 0.125 for the levo antipode (selective β_2-agonist, eutomer, see page 77) have been reported in man).[116]



13

These results, taken with the fact that 49.6% of a given dose of (+)-**13** was absorbed while the degree of absorption of (−)-**13** was 74.8%, allow prediction of a (−):(+) plasma concentration of 2.2.[117] The mean ratio obtained experimentally (using a chiral assay based on preseparation of both antipodes on an achiral column followed by transfer to a Cyclobond column)[118] was 1.87. The difference was ascribed to the combined influence of (−)- on the absorption of its (+)-antipode and the influence of (+)-**13** on the elimination of (−)-**13**.

Walle and Walle[119] evaluated the stereoselective sulfation of terbutaline using the phenolsulfotransferase enzyme of rat cytosol. The sulfate conjugates were determined by HPLC after chiral derivatization with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (**14**, GITC).[120] Dextro-**13** was found to be conjugated to



14

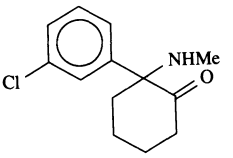
seven times the extent of the eutomeric (levo) isomer. A similar ratio was found when *rac*-**13** was the substrate. Preferential sulfation of (+)-**13** also occurred in man but with a lower factor (2).

3.5. Therapeutic Use of Chiral Drugs in Racemic Mixture Form[52,115,121,126]

The clinical use of racemic mixtures as opposed to the more potent enantiomeric forms (eutomers) is a matter of some controversy as witnessed by the many reviews and discussions of the topic (references as above). Some authorities, notably Ariëns, strongly advocate the use of homochiral agents and regard the distomer component of a racemic mixture as "isomeric ballast" which acts as a pollutant and, as such, should not be present. There is much to be said for this viewpoint, especially in cases where distomers have been shown to have adverse clinical effects, and it may well be that regulatory authorities will look askance at future applications for the product licensing of novel racemic mixtures. At the least it is probable that evidence of the biological properties of both components of a *rac*-mixture will be required when applications are submitted. In view of the large number of racemic mixtures on the market and the lack in general of evidence of problems arising from the presence of distomers, it appears to some that the blanket rejection of a *rac*-mixture is unreasonable and would entail a mostly unnecessary increase in the cost of chiral medicinal agents.[52, 126]

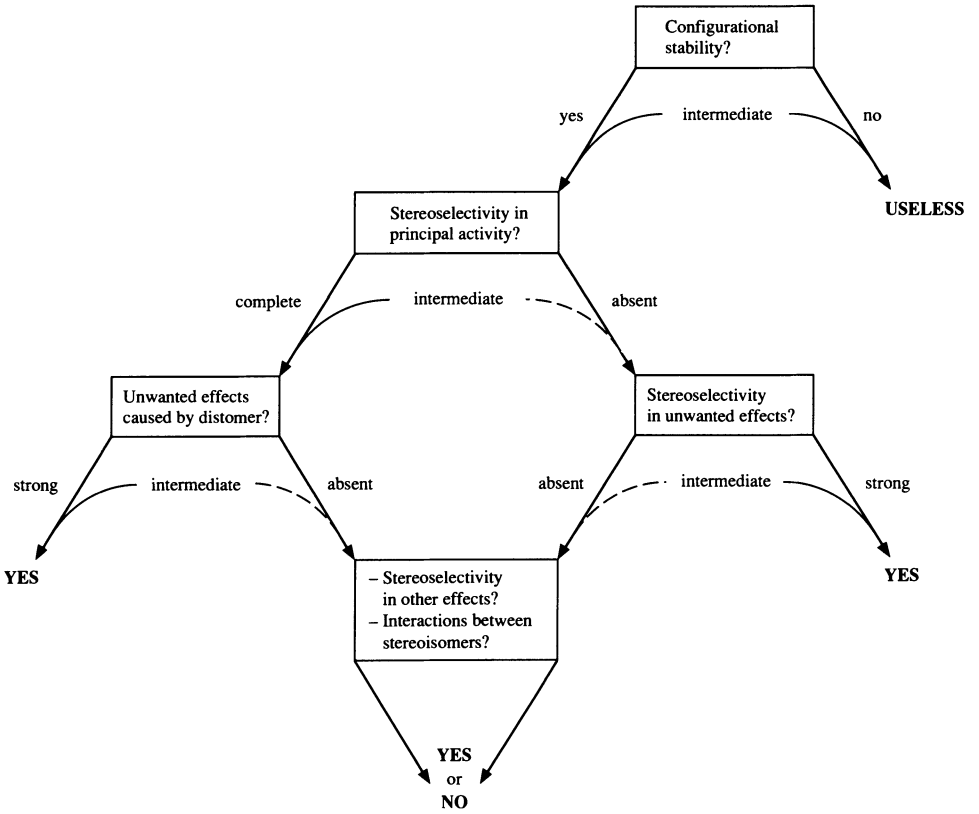
Testa has proposed a logical scheme addressing the question: "Should chiral drugs (and more generally drugs existing in stereoisomeric forms) be used in therapy as the pure eutomer (Scheme 3.2) in which the evaluation of pharmacokinetics and pharmacodynamic data enables a logical decision to be made".[127] In the latest version of this "decision tree," considerations of configurational stability have been appended.[126]

A few examples of cases when information on both components of a *rac*-mixture in clinical use is known are now described. First some cases where it is disadvantageous to employ the racemic mixture. The clinical usefulness of the parenteral anesthetic *Ketamine* (**15** used as the *rac*-mixture) is limited by its postoperative side effects which include restlessness, combativeness, and disorientation. White *et al*,[128] found that use of the dextro antipode gave a more adequate anesthesia and produced considerable less disturbing side effects than either *rac*- or levo-**15**. Induction doses were 1(+), 2(*rac*), and 3(−) mg/kg.



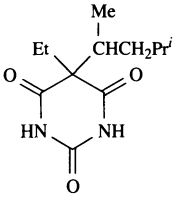
15

In certain agents the desired pharmacological effects of the eutomer may be counteracted by those of the distomer. Thus in certain chiral barbiturates the depressant actions of one antipode (generally the *S*-isomer) are diminished or reversed by the convulsant actions of its optical partner. An extreme case is



Scheme 3.2. Logical decision scheme offered to answer the question: "Should the eutomer or the racemate of a given chrial drug be developed and marketed?" (after Testa and Trager).[126] Several papers discussing the regulatory aspects of chiral therapeutic agents have now been published.[125, 168, 169]

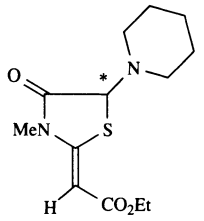
that of 5-ethyl-5-(1,3,dimethylbutyl)barbital **16**.[129] In mice, *S*-isomers of pentobarbital, secobarbital, thiopental, and thioamytal are more toxic than the *R*-isomers or racemic mixtures (however, all forms have similar therapeutic rations LD_{50}/ED_{50}).[130]



S-(–): depressant
R-(+): convulsant
rac: convulsant

16

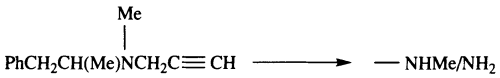
Another case is that of the loop diuretic etozoline **17**, where the diuretic action of the levo antipode (metabolized to the active free acid) is inhibited by the (+)-isomers.[131]



17

The occurrence of opposed antipodal properties is not alway disadvantageous, as seen in the clinical value of certain central analgesics in which one component is an agonist and the other an antagonist, such as picenadrol and viminol (Opioid Chapters 13 and 14, pages 469 and 543).

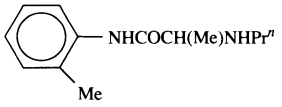
Liability of the distomer to be metabolized to a toxic product provides another reason for its exclusion. Both antipodes of the MAO inhibitor deprenyl **18**, used as



18
MAO inhibition (–) >> (+)

an antidepressant, are metabolized by removal of the propargyl substituent. Antipodal *N*-methylamphetamines and amphetamine result with undesirable central effects, most pronounced for (+)-amphetamines which are produced from the distomeric (+)-form of deprenyl. Mean percents of dose metabolized in 6 subjects were methylampthetamine 63.3 and amphetamine 15. Deprenyl is now marketed as the levo antipode (*Selegilin*).[132]

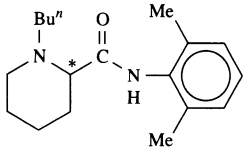
The local anaesthetic *rac*-prilocaine **19** is another agent best employed in homochiral form because the *R*-(−)-isomer is especially prone to metabolism to toxic *o*-toluidines which may lead to methaemoglobinemia.[133] *S*-(+)-prilocaine



19

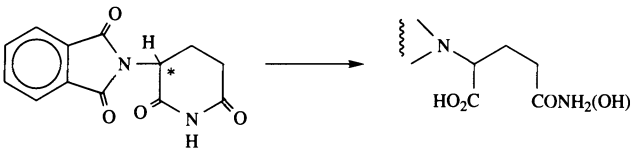
proved more active than the *R*-(−)-isomer in wheal and nerve block experiments and on surface application,[134] but effects of the two compounds *in vitro* on the impulse propagation in isolated nerve were of the same order. *In vivo* differences were attributed to differences in the rate of penetration of the two isomers.

In the case of another local anaesthetic, bupivacaine **20**, the levo antipode acts as a vasoconstrictor, a property which is advantageous in such agents.[135]



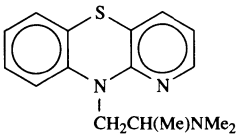
20

The teratogenic consequences of thalidomide **21** might have been avoided if the *R*-(+)-antipode had been used clinically as a sedative to pregnant mothers, since it has now been shown that only the *S*-(−)-antipode is metabolized to toxic products (levo *N*-phthaloyl-glutamine and glutamic acid) in rodents.[136] Antipodes were separated by resolution on a chiral polyamide column.[137]



21
Derivatives of natural (L) Glu are toxic, those of D are nontoxic

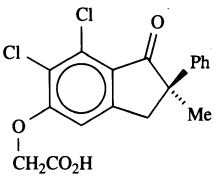
Isothipendyl **22** is a case where the racemic mixture exceeds the potency of either antipode after oral administration. All forms of isothipendyl were equieffective antagonists of histamine in the GP ileum assay. However, in an *in vivo* test for protection against histamine, an oral dose of *rac*-**22** was 1.4 times as effective as the levo and 2.5 times that of the dextro isomer.[138] The reasons are probably pharmacokinetic, perhaps mutual inhibition of metabolism.



22

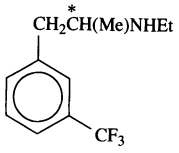
As regards indacrinone **23**, the diuretic action of *rac*-indacrinone is chiefly due to the (−)-antipode. Both antipodes promote the elimination of uric acid but the uricosuric action of *rac*-**23** is inadequate clinically. It was found, however, that a 10 mg (−):80 mg (+) mixture provided an agent that was clinically advantageous in both respects.[139] A similar example has been reported by Fanelli *et al.*[140]

In a pharmacokinetic study[141] AUC plasma values for the *S*-(+)-distomer were 7 times those of the *R*-(−)-isomer, while renal and plasma clearances of the latter were correspondingly the larger. Radiolabeled forms of antipodes of indacrinone were used in the study.



R-(−)-antipode shown
23

In animals, the *S*-(+)-enantiomer of the anorexic drug fenfluramine **24** is 4–5 times as effective as the (−)-isomer but only twice as toxic. In man, side effects of dizziness, drowsiness, and sedation have been attributed to the *R*-(−)-distomer.[142] This is a clear-cut case for the clinical use of a single isomer and, in fact, fenfluramine has recently been marketed as the *S*-(+)-form (Adifax).[143]



Fenfluramine
24

The preferred use of the *distomeric* form of timolol is discussed in Chapter 5 (page 151).

Some chiral clinical agents owe their therapeutic properties to differential contributions of their component isomers. Cases in point are the heart stimulant agent dobutamine (*rac*-form used: dextro antipode is a β-agonist, the levo form an α-agonist) and the antihypertensive agent labetalol (a mixture of diastereoisomers, one with α- and the other with β-blocking activity), (See pages 84 and 88 for details).

3.6. Metabolic Interactions between Enantiomers[144]

Competitive interactions between antipodes are to be expected when both have sufficient affinity for the same enzyme or isozyme. An illustration is provided by the rabbit liver microsomal metabolism of *para*-chloroamphetamine.[145] When incubated alone the rate of oxidation of the *R*-(−)-antipode [0.329 nmol (mg protein)$^{-1}$-min^{-1}] was somewhat above that of the *S*-(+)-isomer (0.258, units as before). However, when the racemic mixture was incubated and the enantiomers monitored by a stereospecific method (chiral derivatization with MTPA-Cl, page 33) both rates fell, that of *R* ninefold and that of *S* twofold. It may be concluded that *S-para*-chloroamphetamine is a slightly poorer substrate but has a higher affinity than the *R*-isomer for monooxygenases.

A similar example is provided by the reaction of antipodes of nicotine with an N-methyltransferase; only the natural *R*-(+)-alkaloid is N-methylated while the *S*-(−)-base acts as a strong competitive inhibitor.[146] Cooper and Anders[147] report that the analgesic activity of levomethorphan (page 448) is enhanced and prolonged by the corresponding dextro isomer, again due to metabolic inhibition.

In vitro experiments showed that *S*-propranolol (eutomer species) is a noncompetitive inhibitor of *R*-propranolol glucuronidation in dog liver microsomes (the reverse situation obtained in rabbit liver)—this may be true also in humans where only the eutomer is subject to conjugation with glucuronic acid.[148]

Sometimes, the metabolism of antipodal forms of a drug are influenced differentially by a chemically unrelated agent. For example, the metabolism of *R*(+)-

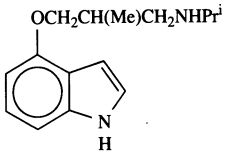
warfarin was inhibited by cimetidine (a H-2 histamine antagonist) but that of the *S*(−)-isomer was unaffected).[149] The same phenomenon was seen in regard to the influence of phenylbutazone and sulfinpyrazone on warfarin metabolism.[150,151]

3.7. Biliary and Renal Excretion

Relatively few studies have been made in which the excretion rates of antipodal pairs have been compared. Jamali *et al*[13] summarize available data in their review. Differences in antipodal levels observed in bile and urine often reflect differential plasma levels and other pharmacokinetic factors rather than demonstrate true stereoselective elimination. A case in point is that of the excretion of acenocoumarol (**10**) in rat bile.[152] Although the *R* concentration represented 0.8% and the *S* 0.2% of the dose, the same workers had previously found that after administration of *rac*-acenocoumarol to the rat, the AUC of the *R*-antipode was four times higher than that of the *S*-form,[153] hence the higher amount of *R*-antipode in the bile may simply reflect the greater plasma concentration of this isomer. There is evidence for stereoselective bilary elimination, however, for antipodes of ketoprofen.[76]

Stereoselective *renal clearance* may arise as a result of active transport, renal metabolism, and active tubular secretion processes, but not by passive glomerular filtration. Evidence for stereoselective renal clearance has been obtained for pindolol,[154] chloroquine,[155] disopyramide,[156] quinine and quinidine, [157] and ketoprofen glucuronides.[159]

Notterman *et al*,[157] compared the clearance of unbound quinidine and quinine (diastereoisomers) with that of creatinine in seven subjects and found that of quinidine to be 6.1 ± 2.2 times, and of quinine to be 1.5 ± 0.6 times that of the standard agent. The fact that the two diastereoisomers possess similar physical constants (pK_a, lipid partition in octanol/pH 7.4 buffer) and fractions bound to serum proteins led the authors to attribute clearance differences to stereoselective renal secretion. The question of differences in metabolism was not addressed. The renal clearance of the β-blocker levo pindolol **25** exceeded that of the dextro isomer in six subjects after 20 mg oral dosage of the racemic mixture (a stereospecific HPLC assay was applied with derivatization with *S*-α-methylbenzylisocyanate).[154] Again, this difference was attributed to renal transport (antipodes bind to plasma proteins in a similar degree). The authors cite evidence against renal metabolism but do no consider heptatic metabolism.

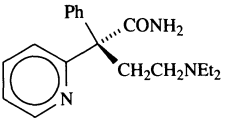


25

The renal clearance of ketoprofen glucuronides in elderly patients is reported to stereoselectively favor the *R*-isomer after both single and multiple oral doses.[158] These workers found more than 80% of the dose in urine, present in the form of

conjugates; the *S*/*R* ratio was 1.19 after single and 1.17 after repeated doses. The influence of enzymatic inversion upon the *R*:*S* ratio in urine was considered small, since the total amount of conjugated *S*-antipode recovered did not exceed 25 mg (50% of *rac*-dose)—if the extent of *R* to *S* inversion were high, more than 25 mg *S*-ketoprofen would have been recovered. A stereospecific HPLC assay was employed.[159] The authors speculate that the higher level of *S*-antipode in the urine is a result of stereoselective conjugation followed by preferential *biliary* excretion of the *R*-conjugate.

In the case of the antiarrhymic agent disopyramide **26** (S-eutomer),[160] renal clearance in man of the *S*-antipode was almost three times greater than that of the *R*-form.[156] Since the unbound renal clearance of both enantiomers was higher than the filtration clearance expected, the authors concluded that disopyramide undergoes tubular secretion by an active process that stereoselectively favors the *S*-form.



Unbound renal clearance
S 338 ml/min
R 182 ml/min

26

3.8. Significance

As pointed out by Tucker,[115] the most important pharmacokinetics parameter is unbound clearance, the product of free fraction in the plasma and total drug clearance. If typical enantiomer ratios for free fraction in plasma (up to 1.5) are multiplied by typical ratios for total clearance (1.5–2), this suggests that enantiospecificity in pharmacokinetics is generally a factor of less than 2–3.

Thus while pharmacokinetic differences between antipodal pairs may have important clinical consequences in regard to dosage regimens and routes of administration, their contributions to eudismic ratios, with values of, say, 10 to 20 or above, may generally be assumed to be minor. An instance where pharmacokinetics have a particularly large effect on the eudismic ratio is that of the isomeric inversion of the profen class of NSAIDs. Because *R*-ibuprofen is inverted to the active *S*-form *in vivo*, this factor has a huge damping effect on the eudismic ratio when *in vitro* potency is compared to *in vivo* potency with regard to inhibition of prostaglandin synthesis.[161, 162]

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4

Adrenergic Ligands

Chiefly Agonists

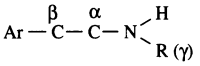
4.1. Introduction

In this and subsequent chapters reviews are presented of the stereochemical aspects of the major classes of pharmacological receptors and their ligands. A useful guide to receptor nomenclature has recently been published as a supplement to the January 1990 and 1991 issues of TiPS. This provides details of nomenclature, potency order in regard to endogenous ligands, selective agonists and antagonists, radioligands and effector pathways, plus gene and structural information in cases where receptors have been isolated and/or cloned.

Steric influences among adrenoceptors and their ligands are manifold but often difficult to analyze on two particular counts. First, at least four subclasses of adrenoceptor have now been defined, namely α_1, α_2, β_1, and β_2 as a result of the discovery of selective agonists and/or antagonists for each variety.[1] Second, there is a need to differentiate adrenergic agents which act *directly* at receptor sites from those that achieve their effects *indirectly* by displacing noradrenaline (NA) from storage sites (some agents act in both manners). As an additional complication, considerations of distribution, *in vivo* synthesis, metabolism, and uptake need to be addressed.

Useful reviews are those of Patil *et al.*,[2] Ruffolo (general coverage),[3] Rutledge *et al.* (uptake and conformation),[4] and Timmermans (α-ligands).[5]

Most adrenoceptor ligands (certainly agonists) are derivatives of 2-phenethylamine **1** which may be chiral at the α- or β-carbon centers (or both); the chirality locus may be within the N–R substituent, designated γ in this chapter for ease of comparison (it is unfortunate that stereochemical and adrenoceptor subspecies

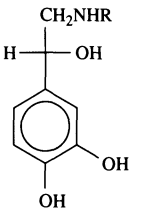


1

nomenclatures employ the same symbolism and care must be taken not to confuse the two aspects). Chiral agents of these types with *direct* actions will now be considered.

4.2. Natural Catecholamines and Synthetic Agents with Direct Actions

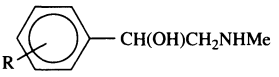
Adrenaline **2b** (epinephrine) is the prototype sympathomimetic amine with β-carbon chirality; it occurs naturally as the homochiral levo isomer. Abderhalden and Müller's examination of (+)- and (−)-adrenaline in 1908 probably represents one of the first pharmacological studies of an antipodal pair.[6] They found that the levo isomer was 15 times more potent than the dextro form in raising the blood pressure of dogs. Numerous similar studies of adrenaline and NA have been



(a) R=H; (b) R=Me; (c) R=i-Pr*; (d) R=t-Bu

2

reported subsequently in which a variety of test animals were employed; in general the levo enantiomers were the more active by factors of 15–40, higher values being noted for tests on lung tissue[7,8] (see also Table 4.4). Stereospecificity of action is also noted for enantiomers of *N*-isopropyl (**2c**) and *N*-*t*-butyl (**2d**) analogues of adrenaline (see below) and for the monophenolic derivatives *p*-synephrine **3a** and

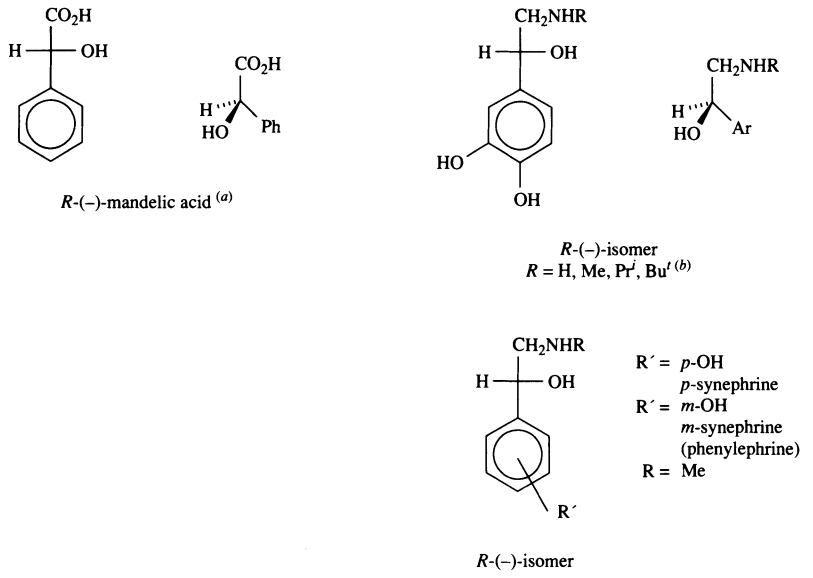


(a) R = 4-OH p-synephrine; (b) R = 3-OH phenylephrine (*m*-synephrine)

3

phenylephrine (*m*-synephrine) **3b**,[9–11] and for the β-agonist salbutamol **5**.[12] The configurations of antipodes of adrenaline and its congeners were not established until the late 1950s, the delay in this fundamental work probably being due to the high chemical lability of phenolic amines. Pratesi[13,14] related a variety of optically active catecholamines to (−)-mandelic acid of known (*R*) absolute configuration and showed that the more active antipodal forms of adrenaline, NA, isoprenaline, and the monophenolic amines *p*-synephrine and *m*-synephrine all have the *R*-configuration (Scheme 4.1). Some of the reactions employed are presented in

* A stereoselective synthesis of *R*- and *S*-isoprenaline has been reported (p. 31 of Chap. 2).

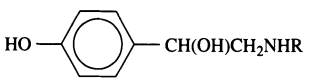


[a] Fregda's 1940 proof of configuration (Ref. 15, p. 99) now confirmed by X-ray methods. [16]
[b] 2 × *RS* as β-agonist. [17]

Scheme 4.1. Configurational relationships between *R*-(−)-mandelic acid and the more potent antipodal forms of chiral sympathomimetic agents.

Scheme 4.2. Initially, the validity of the assignments rested on Fregda's work on the configuration of mandelic acid enantiomorphs carried out in the 1940s[19,20] but in 1973 an X-ray crystallographic analysis of (−)-adrenaline–(+)-hydrogen tartrate by Carlstrom[21] confirmed the *R*-configuration of natural catecholamines. In the Swedish work the *relative* configurations of (−)-adrenaline and the (+)-hydrogen tartrate counterion were determined. Since the *absolute* configuration of (+)-tartaric acid has been established by the X-ray technique of anomalous dispersion,[22] that of (−)-adrenaline automatically follows. In an earlier examination of (−)-noradrenaline (achiral counterion) no attempt was made to determine the absolute configuration by anomalous dispersion.[23]

Papers dealing with the absolute configurations of adrenergic amines, several of which present conflicting data, have been reviewed by Midgley *et al.*[24] These authors report an X-ray analysis of (−)-*p*-synephrine (−)-3-bromocamphor-8-sulfonate which establishes the absolute configuration of the levo base as *R* by both refinement of the chirality parameters (via anomalous dispersion) and comparison with enantiomers of 3-bromocamphor-9-sulfonic acid and 3-bromocamphor of known absolute configuration. Because (−)-*p*-synephrine and (−)-adrenaline have been linked by chemical arguments (Pratesi, Scheme 4.2), these results clinch the issue of the absolute configuration of natural catecholamines. The CD curves of hydrochloride salts of (−)-*p*-synephrine **4b** and (−)-*p*-octopamine **4a** showed Cotton



(a) R = H; (b) R = Me

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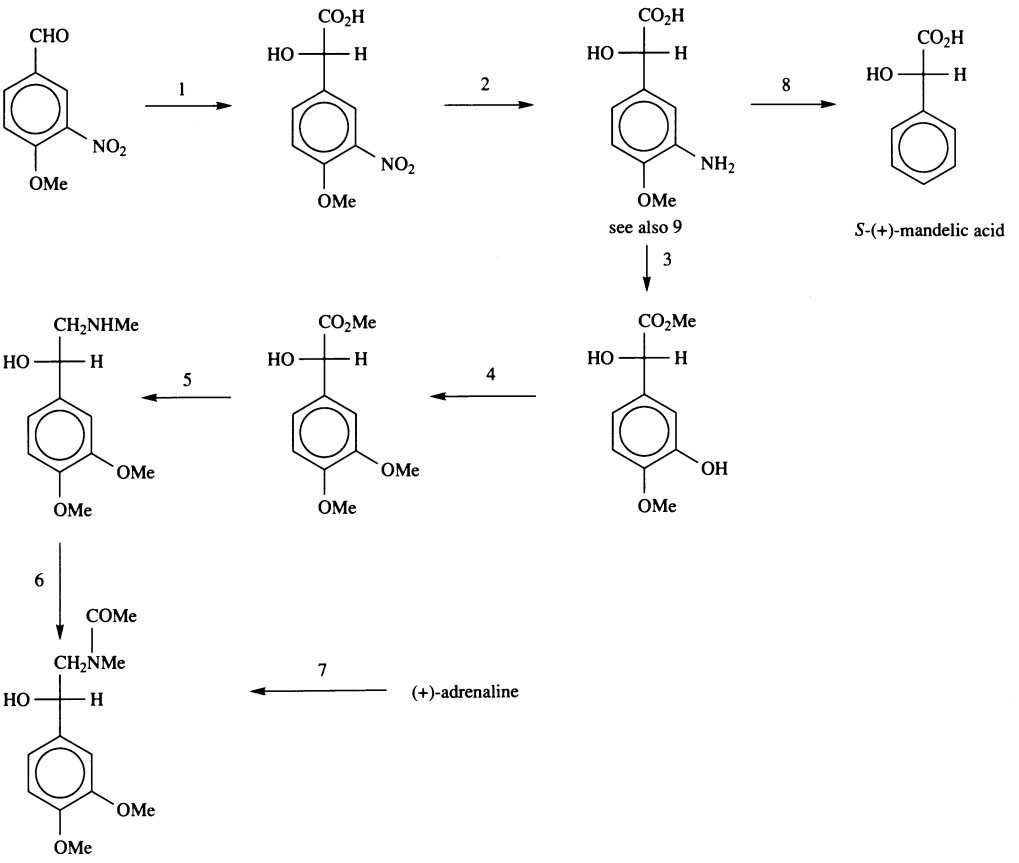


Notes

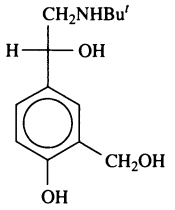
1. HCN followed by H_3^+O, then resolve the resultant hydroxy acids with cinchonine and brucine.
2. Catalytically reduce the corresponding methyl ester ($ArNO_2 \rightarrow ArNH_2$).
3. Diazotize with $NaNO_2$-HCl, then heat with $CuSO_4$-H_2O.
4. Methylate *m*-OH with diazomethane.
5. $MeNH_2$ ($CO_2Me \rightarrow CONHMe$); reduce amide with LAH.
6. N-acetylate.
7. Reactions on adrenaline: N-acetylate, then methylate phenolic OH groups with diazomethane (actually performed on the levo isomer to give the optical antipode of the dimethoxy compound shown).
8. Related to *S*-(+)-mandelic acid by transformations not affecting the chiral center.
9. The mono *p*-methoxy derivative derived from this intermediate was linked to (+)-*p*-synephrine by similar methods; *m*-synephrine (phenylephrine) was correlated to this series by converting its levo antipode via a *m*-methoxy, *p*-nitro intermediate to *O*,*O*-dimethyl-(–)-adrenaline (Ref. 18).

Scheme 4.2. The configuration of (–)-adrenaline.[14]

effects near 270 nm of negative sign that were superimposable—evidence of like configuration. However, the Cotton effect signs of antipodal forms of *m*-synephrine and *m*-octopamine (also levo rotatory at 589 nm) were positive. This result suggests that levo isomers of the *meta* and *para* series differ in configuration. However, pharmacological data (see page 98 which establish the superior potency of levo antipodes in *both* series makes this conclusion improbable. It may be that electronic transitions of *m*- and *p*-phenolic chromophores differ sufficiently to give rise to Cotton effects which are not comparable.[25]

Optical comparisons are cited as evidence that the (–)-*t*-butyl derivative **2d**

(ORD)[13] and (−)-salbutamol **5** (CD comparisons with levo octopamine) also belong to the *R*-steric series.[12] Octopamine (**4a**, the nor analogue of *p*-syenphrine **3a**) is further discussed later.

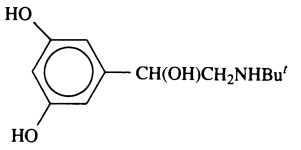


R:*S* potency ratio 70 at β_2-site (guinea-pig trachea)

Both isomers feeble at β_1-site (guinea-pig atrium)

5

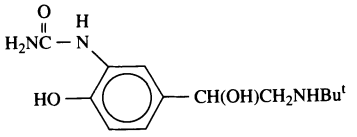
Terbutaline **6** and carbuterol **7**, β_2-selective agonists related to salbutamol **5**, have also been resolved.[26]; their activity at both β_1 and β_2 sites is largely confined to the levo antipode (see legend alongside formulas).[27,28] Pharmacokinetic factors favor the levo isomer; 4 h after the morning dose of a 7-day b.i.d. regimen (7.5 mg of *rac*-drug) mean plasma levels were 9.5 nmol/l for (+)- and 17.5 nmol/l for (−)-terbutaline[29] (see Chapter 3 for further details).



6 Terbutaline

pD_2 values:	Tracheal muscle relaxation (β_2)[a]	Soleus muscle depression (β_2)	Papillary muscle ionotropy[b]
(−)	7.28	7.28	5.03
(+)	3.76	undefinable	nil

[a] contracted with carbachol; [b] partial agonist, $\alpha = 0.74$.

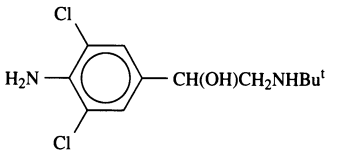


7 Carbuterol

ED_{50} *M* values:	GP trachea	GP atria
(−)	6.7×10^{-9}	1.3×10^{-7}
(+)	2.7×10^{-6}	9.4×10^{-5}

Racemic orciprenaline (the N-isopropyl analogue of terbutaline) and fenoterol (see page 83) have been resolved by LC on α_1-acid glycoprotein but comparative

pharmacological data on the antipodal pairs have not been published (S.N. Allen, Boehringer Ingelheim, private communication). In tests on β_2 preparations (guinea-pig trachea and soleus muscle), (−)-clenbuterol **8** had agonist potencies 2–3 orders above those of the dextro isomer. Both isomers were competitive inhibitors of isoprenaline on the trachea (levo 100× effect of dextro).[30] Two further chiral β-agonists are isoetharine (page 96) and trimetoquinol (page 103).



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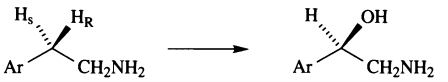
4.3. Aspects of *in Vivo* Synthesis[31,32]

The *in vivo* synthesis of the naturally catecholamines *R*-(−)-noradrenaline and *R*-(−)-adrenaline involves several stereospecific enzymes (Scheme 4.3). Tyrosine hydroxylase requires L-tyrosine as its substrate for the formation of levo DOPA, while dopamine β-hydroxylase converts achiral dopamine exclusively to *R*-(−)-NA as recently demonstrated by use of a chiral HPLC column.[35]

The stereospecificity of hydrogen removal in the hydroxylation step has been established by use of DA substrates tritiated at the β-carbon.[36] Since tritium was retained in NA derived from the 2*S*- and lost in that from the 2*R*-substrate, the added hydroxyl must occupy the prochiral H_R site (see **9**). The synthesis of the tritiated **R**-substrate **11** involved the reduction of the tritiated benzaldehyde **10** with liver alcohol dehydrogenase which gave the *S-sec*-alcohol (steric course established[37,38]) and conversion of the alcohol to **11** by reactions that included an inversion step and a Curtius degradation.



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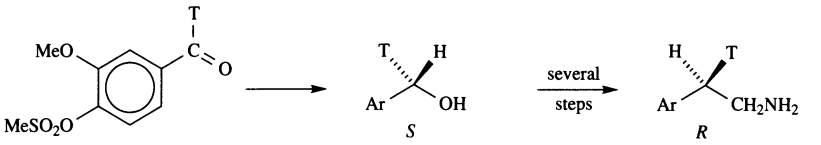
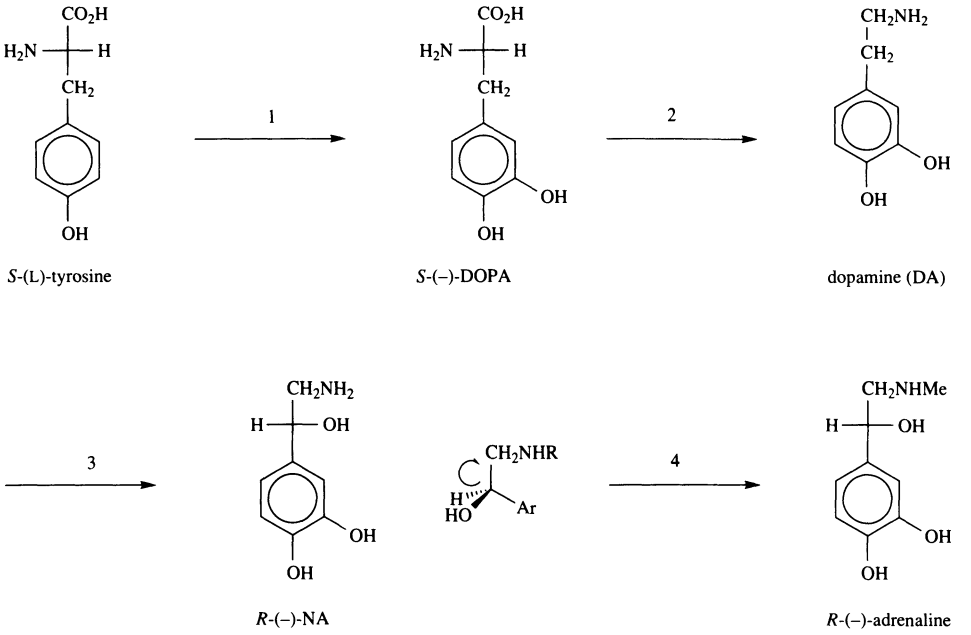


10 **11**

Inhibitors of catecholamine biosynthesis are also subject to stereospecificity, notably in regard to DOPA decarboxylase, an enzyme for which most aromatic amino acids of L-configuration serve as substrates.[39] The compound α-methyl DOPA inhibits the enzyme but its action is limited to the L-(−)-isomer.[40] The levo



Notes

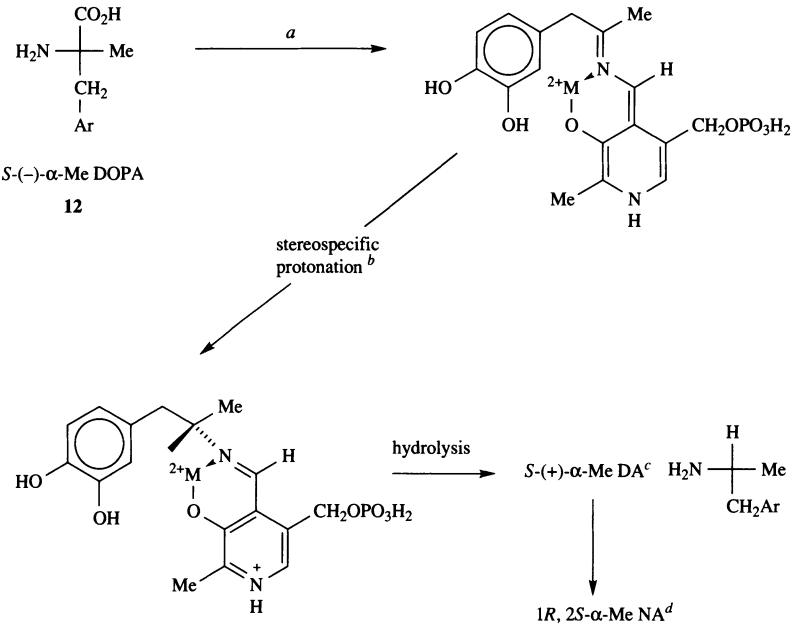
1. Catalyzed by tyrosine hydroxylase; *in vitro* the enzyme requires Fe^{II}, oxygen, and a tetrahydropteridine cofactor, and can be inhibited reversibly by some structurally related compounds such as α-methyltyrosine.
2. Catalyzed by DOPA decarboxylase, an enzyme that acts generally on L-aromatic amino acids and is pyridoxal phosphate-dependent.[33]
3. Catalyzed by dopamine-β-hydroxylase; the reaction requires ascorbic acid and Cu^{2+} and is therefore sensitive to chelating agents. It is inhibited by other substances that act as substrates for the enzyme, such as tyramine. The reaction is stereospecific.[34]
4. *N*-methylation of NA occurs in mammals chiefly in the adrenal medulla. The enzyme, an *N*-methyl transferase, catalyzes the transfer of a methyl group from active methionine to NA and to other amines, such as 2-phenethylamine.

Scheme 4.3. *In vivo* synthesis of catecholamines.

isomer proved more than 40 times as effective than its D-antipode in preventing the decarboxylation of 5-hydroxytryptophan, and in depleting NA in mouse heart.[41] Levo α-Me DOPA (**12**, Aldomet) is marketed as an antihypertensive agent. Its mode of action, initially linked to its inhibition of DOPA decarboxylase, is now thought to be due to the effects of its metabolite, 1*R*,2*S*-α-methyl NA (see Scheme 4.4). α-Methyl NA acts as a false transmitter in displacing NA from storage sites and also has direct action with selectivity for central α_2 receptors (see page 94). The antihypertensive effects of α-methyl DOPA in both cats[45] and man [46] are confined to the L-isomer.

L-α-Methyl DOPA is separated from the racemic acid by a process that does not require a resolving agent.[47] The technique is one of selective crystallization in which a supersaturated solution of the bisulfite salt of the racemic mixture is seeded with either of the pure antipodal salts. Success of the process requires critical attention to detail. The configurational assignment of the L-antipode, originally based on rotational comparisons with D-(+)-DOPA of known geometry,[48] has been confirmed by X-ray analysis.[49]

Another inhibitor of aromatic amino acid decarboxylase is L-(−)-carbidopa



Notes

[a] Aromatic L-amino acid decarboxylase - requires pyridoxal-5-phosphate and bivalent metal ($^{2+}$M).

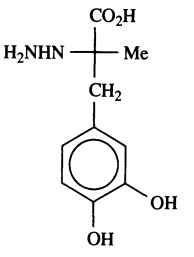
[b] Shown to be stereospecific in regards to *S*-tyrosine → *R*-1-tyramine. [42]

[c] Identified in urine of patients given *S*-(–)-α-Me DOPA. [43]

[d] α-Me NA found in hearts of mice given *S*-(+)-α-Me DA [none detected after *R*-(–)-α-Me DA]. The 1R-configuration is tentative [44] (see also p. 94).

Scheme 4.4. Metabolic interconversions of *S*-(–)-α-Me DOPA.

13, the hydrazine analogue of L-α-methyl DOPA to which it has been stereochemically correlated by a variety of chemical interconversions (Scheme 4.5).[50] Of the two carbidopa antipodes, only the L-(−)-form had the ability to potentiate the effects of L-DOPA in reserpine-treated mice.[51] L-(−)-Carbidopa cannot penetrate the CNS and is used to prevent peripheral decarboxylation of levodopa in Parkinsonism patients treated with combinations of the two agents. The stereochemistry of benserazide **14**, a decarboxylase inhibitor derived from *RS*-serine



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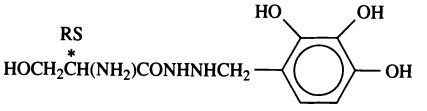
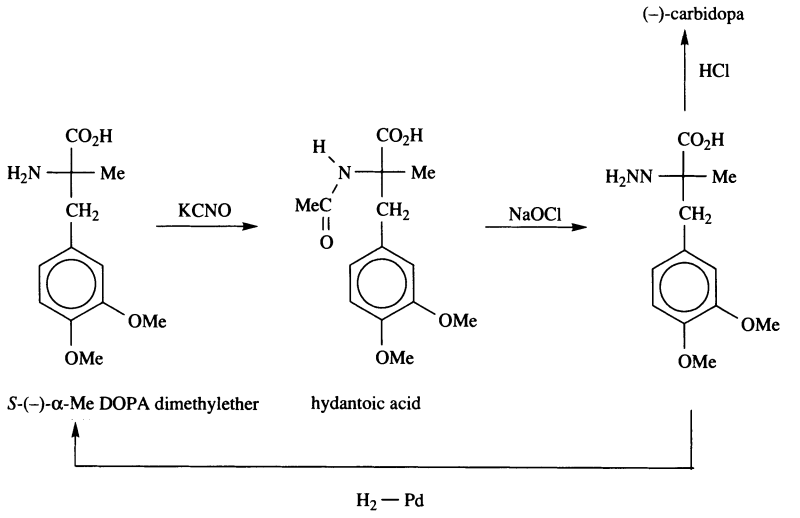


14

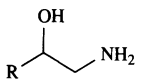
and administered as the racemic mixture (*Martindale* **29**, page 1010), does not appear to have been reported.



Scheme 4.5. Chemical correlation of *S*-(–)-carbidopa and *S*-α-Me DOPA .[50]

The last steps in the biosynthesis of adrenaline, i.e., the N-methylation of NA, involves the enzyme phenylethanolamine N-methyl transferase (PNMT) with *S*-adenosyl-L-methionine (AdoMet) serving as the methyl donor. PNMT is chiefly located in the adrenal medulla but its presence has also been demonstrated in cell bodies within the CNS.[52]

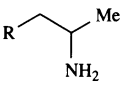
PNMT proves to be stereoselective toward both substrates and inhibitors (the last developed to establish the role of PNMT and adrenaline in hypertension) but generally only in modest degree. In the biochemical assay relevant to these studies a mixture of phosphate buffer (pH 8.0), AdoMet (unlabeled and tritiated), substrate, inhibitor (if required), and enzyme (from bovine adrenal gland) is incubated for 30 min at 37° C, the reaction terminated with borate buffer (pH 10), and an organic solvent extract counted for radioactivity. Axelrod[53] and Fuller[54] found that natural *R*-(–)-NA was methylated twice as fast as the *S*-(+)-antipode. Grunewald's group[55] observed a similar preference for the *R*-antipode as substrate in the case of the phenylethanolamine **15**a (× 1.8) and its saturated analogue **15**b (× 1.3) as judged by V_{max}/K_m ratios. Antipodes of the cyclooctyl analogue **15**c, overall a poor substrate although a potent inhibitor of PNMT,[56] had similar parameters (V_{max}/K_m × 100: levo 0.88, dextro 0.67).



(a) R = Ph; (b) R = C_6H_{11}; (c) R = C_8H_{15} (cyclooctyl)

15

Chiral amphetamine **16** and methylbenzylamine **17** analogues behaved as inhibitors, with the *S*-antipodes a few-fold more effective (lower K_I values) than *R*-isomers.[55] *S*-(+)-Amphetamine **16**a itself inhibits the N-methylation of NA



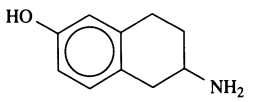
(a) R = Ph; (b) R= C_6H_{11}; R = C_8H_{15}

16

K_i
R = (+) 412 μM
S= (−) 44 μM

17

three times more effctively than the *R*-(−)-antipode.[57] Absolute configurations were tentatively assigned on the basis of order of elution of *N*-(trifluoracetyl)-*S*-prolinamide derivatives.[58] In further papers,[59,60] 2-aminotetralins and other conformationally restricted analogues of amphetamine were examined. The derivative **18** (K_i 4.7 μM) had 40 times the inhibitory activity of *p*-tyramine **19**. Of the geometrically isomeric pair **20** the *cis*-racemic mixture was a substrate for PNMT (K_m 22 μM, V_{max} 0.15 nmol product/mg/min) while its *trans*-partner was an inhibitor (K_i 9.4 μM).[61]



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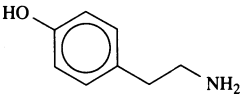

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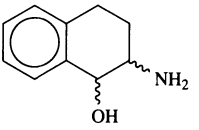
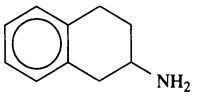


20

Later all four isomers **20** were examined.[62] Only the IR, 2*S*-antipode of the *cis*-mixture was a substrate (K_m 4.5 μM, V_{max} 0.16, units as above), while the inhibitory action of the *trans*-mixture was confined to the 1*S*,2*S*-isomer (K_i 4.6 μM). Hence a 2*S*-configuration promotes optimal activity for both substrates and inhibitors in accord with findings for amphetamine and its analogue presented above, and also for 2-aminotetralin itself (see **21**). Substrate activity requires a 1*R*-hydroxyl orientation, as seen in NA and its analogues—however, this feature is a far greater determinant of activity in the restrained tetralins than in the acyclic substrates.



	K_i μM
2*S*	4.1
2*R*	10.0

21

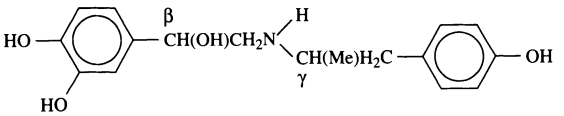
The absolute configurations of antipodes of *cis*- and *trans*-**20** were established by Zymalkowski and Dornhege[63] by synthesis of 2-aminotetralins from *R*- and *S*-aspartic acid (see also Chapter 6, page 175) and conversion of the 1-hydroxy derivatives **20** to **21** by catalytic hydrogenolysis.

Grunewald and his colleagues have analyzed their results in terms of active site models of the PNMT enzyme.[64]

4.4. Isoprenaline and Other Agents of Primarily β-Agonist Character

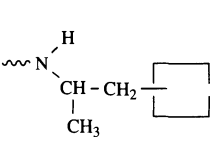
Isomeric potency ratios for isoprenaline reported in the 1950s are significantly greater than those reported for adrenaline and NA, even when allowance is made for the possible presence of levo impurity in the less active dextro isomer.[65,66]

Isoprenaline has activity at β-receptors; hence, since adrenaline and NA interact at both α- and β-sites (preferentially α- for NA), β-adrenoceptors were assumed to be more stereoselective toward their ligands than the α-variety. Ariëns[67] found that adrenaline and NA exhibited (−)/(+) potency ratios of 4 and 8 respectively at an α-receptor (rat vas deferens) while larger ratios (20 and 50) were noted at a β-site (guinea-pig atrium). With isoprenaline the isomeric ratio exceeded 500 at the β-preparation. *R*-(−)-Isoprenaline was a weak agonist at the α-site but the *S*(+)-analogue proved to be an antagonist, a fact first brought to light by the failure of the racemic material to effect a maximal contraction of the rabbit aorta strip, as produced by the *R*-(−)-isomer. Differing α/β stereospecificities are also illustrated by the NA analogue (Cc 25, **22**) with a chiral N-substituent (levo isomer derived from *R*-NA, dextro from *S*-NA, configuration at C-γ unknown). At an α-site (rat vas deferens) both diastereoisomers of **22** were antagonists with the *S*-form the more potent by a factor of 10; however, at guinea-pig atrium β-sites the *R*-isomer was over 1000 times more active as a sympathomimetic agonist than its antipode.[67] Claassen reported lower *R*:*S* ratios of 50 and 17 for β-sites in uterus and tracheal muscle, respectively.[68] The related 3,5-dihydroxyphenyl analogue of **22** is marketed as the racemic mixture (fenoterol) for use as a bronchospasmolytic.



22 Cc25

The compound Cc25 (**22**) may be regarded as an analogue of isoprenaline in which one of the isopropyl methyls has been substituted by an aromatic group (see **23**). Goodman and his colleagues prepared some related derivatives by attachment of heptanoic acid *p*-(trifluoromethy) anilide to NA giving **24**.[69] In tests on



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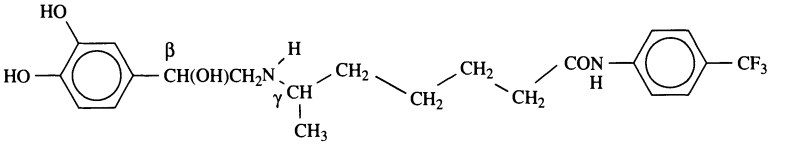
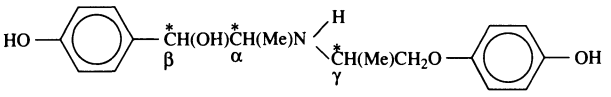


24

stereoisomeric mixtures substantial increases in potency were observed over that of the parent catecholamine.[70] When the four separate isomers were examined the *R*(β)*R*(γ)-form had the greatest binding affinity for both β_2 (S-49 mouse lymphoma cells) and β_1 (turkey erythrocyte membranes) sites followed by *RS*=*SR*>*SS*. The same order of potencies for stereoisomers was found for increasing myocardial contractile force in isolated guinea-pig left atria.[69] Details of chemistry are included in a related study on β-adrenoceptor blocking agents (Chapter 5, page 155).[71]

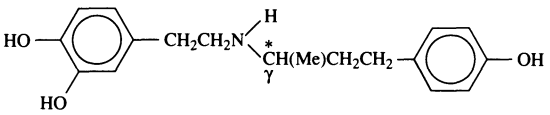
In the case of the derivative **25**, with three chiral centers, one of the four



25

possible racemates (isoxsuprine, Defencin) was formerly marketed as a combined β_2-agonist–α-antagonist for arrest of preterm labour (*Martindale* **29**, page 1505). It is remarkable that agonist properties are associated with one member of the antipodal pair and antagonist actions with the other. The levo antipode (β*S*:α*R*:γ*R*) was the more effective—antagonist (vas deferens), while the dextro isomer (β*R*:α*S*:γ*S*) was 30–50-fold the more potent as a β-agonist.[68] These results correlate with those found for antipodes of the compound Cc25 (**22**)[67]—in both cases the more potent β-agonists are related in configuration to *R*-(−)-isoprenaline (see also discussion of α-methyladrenaline, p. 94). Configuration methods are outlined in Scheme 4.6. An *R*-configuration at the γ-chiral center appears to be associated with receptor blockade, as is seen also in the case of labelatol isomers (page 88).

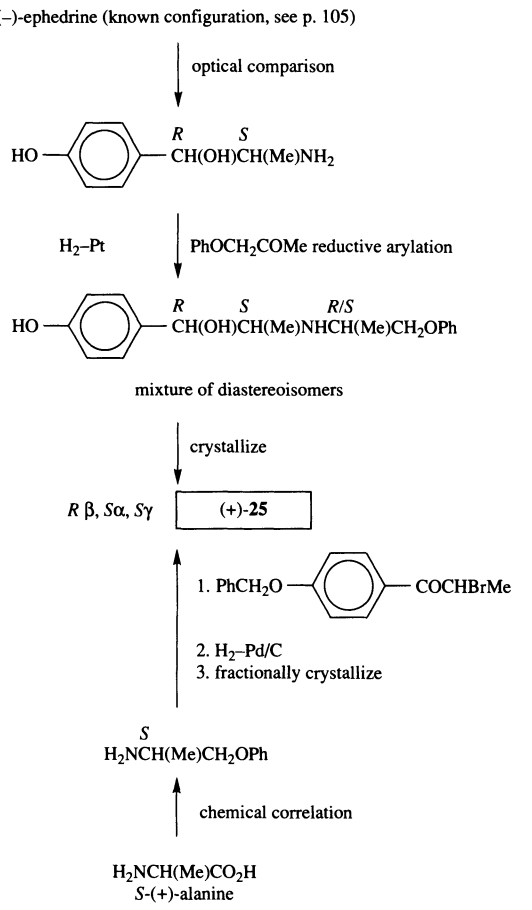
The chirality of *dobutamine* **26** (the racemate is used as an ionotropic agent) lies solely in a carbon of the γ-type. The levo antipode is an agonist (α, potent; β, weak) while the dextro forms combines strong β-agonism with α-antagonism



26

(Table 4.1). Antipodal potency differences were 6.3-fold (levo eutomer) at α-sites (rat aorta), and 3.6–14-fold (dextro eutomer) at β-sites (cat papillary muscle and atrium). Antipodal affinities at rat aorta (*vs m*-synephrine) differed little, hence the potency difference between (+)- and (−)-forms may be attributed to differences in efficacy. This parameter, measured by the receptor inactivation technique of Furchgott and Burztyn,[74] proved to be 0.327 for (−)-dobutamine and 0.009 for the (+)-isomer relative to a value of 1 for *m*-synephrine.

In the pithed rat the levo isomer possessed potent pressor activity (direct action at α-1 adrenoceptors)—its effects were potentiated when β-sites were blocked by propranolol (β-effects oppose those of α-effects). The (+)-antipode was only a weak pressor agent.[75] Both isomers produced β-effects in pithed rats with vascular tone maintained by infusion with angiotensin II; the (+)-antipode had the greater action as it did also in regard to tachycardia. Enantiomers of 3-0-methyldobutamine, a metabolic product of dobutamine, lacked α- and β-agonist effects.



Scheme 4.6. Reaction employed to establish the absolute configuration of antipodes of iosoxsuprine (**25**). [72]

TABLE 4.1.
Biological Properties of Dobutamine Antipodes[73]

Compound	Rat aorta (α) – log ED_{50}	Relative potency	– log K_D [a]
(–)-Dobutamine	7.72 (0.6)[b]	2.29	7.02
(+)-Dobutamine	6.92 (0.03)	0.36	7.07
(–)*m*-Synephrine	7.36 (1.0)	1.00	

Compound	Cat papillary muscle (β) contractility – log ED_{50}	Cat right atria rate (β) – log ED_{50}
(–)-Dobutamine	5.82 (0.74)	6.10 (0.92)
(+)-Dobutamine	6.38 (1.15)	7.24 (0.93)
(–)-Isoprenaline	6.91 (1.0)	8.19 (1.0)

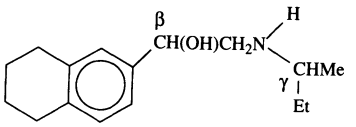
[a] Dissociation constant versus (–)-*m*-synephrine.
[b] Maximum response relative to standard values of 1.0.

However, both were competitive α_1-antagonists with the (+)-isomer about 10-fold more potent than the (−)-form in guinea-pig aorta or displacement of [^{3}H]-prazosin binding in rat cerebral cortex (pA_2 7.33, $-\log K_i$ 7.72 for dextro isomer).[76] Antipodal activity differences of the same kind were found in the cardiovascular system of the pithed rat.[77]

In tests on cardiac function in pithed rats, (−)-dobutamine showed the greater ionotropic selectivity (increased cardiac output and stroke volume) while the (+)-isomer had the greater influence on heart rate consistent with its characterization as a β-agonist.[78]

Antipodal configurations have now been established (D. W. Robertson, private commun.). It turns out that the levo isomer (α-1 agonist) has the *S*- and the dextro isomer (α-antagonist) the *R*-configuration. These results form part of a correlation presented later (p. 98).

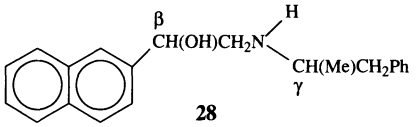
Study of the four isomers of butidrine (**27**, also known as butedrine), used clinically as a β-blocker (isomeric mixture?), shows that the most potent antagonist is the *R*(β) *R*(γ)-antipode (Table 4.2) in stereochemical accord with previous results (see above).[79] Again, a chiral N-substituent of *R*-configuration leads to the most effective receptor blockade. The *RS* isomeric form of **27** was less effective than *RR*



27

while *SS* and *SR* isomers had very low levels of activity. Stereochemical assignments were based on syntheses using the optically active intermediates *R*- and *S*-*sec*-butylamine (rotational evidence)[19] and 5,6,7,8,-tetrahydro-2-naphthyl-glycolic acid—the (+)-antipode of this acid was related to *S*-mandelic acid by ORD comparisons of their thiourea derivatives.[80]

The four isomeric 2-naphthyl derivatives **28**, analogues of pronethalol (p. 140) have also been examined as β-antagonists.[81] Configurations are known from ORD



28

TABLE 4.2.
Blocking Activities of Butidrine Isomers[79]

Isomer (β, γ)	ED_{50} mg/ml	
	Rabbit atrium[a]	Rat uterus[b]
RR	0.57	0.1
RS	0.85	0.7
SS	9.5	6.0
SR	6.5	7.5
Butidrine	1.5	0.35

[a] Stimulated by levo adrenaline (0.5 μg/ml).
[b] Stimulated by isoprenaline (0.003 μg/ml).

TABLE 4.3.
Stereoselectivity of Noradrenaline (NA) in α-Preparations[83, 84]

Tissue[a]	pD_2		R/S ratio
	R-(−)-NA	S-(+)-NA	
Rabbit spleen	6.59	4.16	274
Rabbit vena cava	6.37	3.96	251
Rabbit ileum (α_2)	6.08	3.68	319
Rabbit vas deferens (α_1)	6.62	4.14	302
Rabbit seminal vesicle	6.07	3.63	275
Rabbit aorta (α_1)	8.18	5.71	293
Rat aorta (α_1)	8.93	6.70	170
Cat aorta (α_1)	6.74	4.52	166
Guinea-pig aorta (α_1)	6.34	3.95	251

[a] Treated with cocaine, tropolone and sotalol.

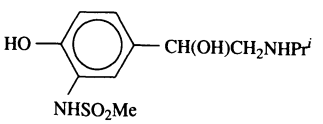
data and use of R- and S-amphetamine in the synthetic procedures. The potency ranking βR, $\gamma R > \beta R$, $\gamma S \gg \beta S$, $\gamma R > \beta S$, γS corresponds with that found for isomers of butidrine.

In 1969 Patil[82] pointed out that isomeric ratios measured under normal experimental conditions are obscured by several factors operating at adrenergic nerve terminals, namely, the stereoselective uptake of ligands, the unequal distribu-

TABLE 4.4.
Stereoselectivity of β-Agonist Action[a] [85, 86]

Compound	Guinea-pig atria (β_1)		Guinea-pig trachea (β_2)	
	pD_2	R/S ratio	pD_2	R/S ratio
R-(−)-NA	7.47	500	7.03	200
S-(+)-NA	4.77		4.73	
R-(−)-Adrenaline	7.35	63	7.98	29
S-(+)-Adrenaline	5.55		6.53	
R-(−)-Isoprenaline	8.67	1000	8.90	500
S-(+)-Isoprenaline	5.66		6.21	
R-(−)-Soterenol[b]	7.78	75	8.30	150
S-(+)-Soterenol	5.92		6.10	
R-(−)-Salbutamol (**5**)	7.70	200	8.44	300
S-(+)-Salbutamol	5.42		5.97	
(−)-Trimetoquinol (**57**)	8.68	40	9.63	4
(+)-Trimetoquinol	7.07		8.08	

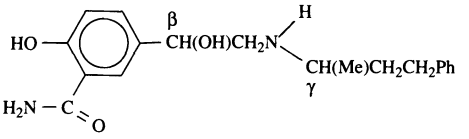
[a] Reserpine-pretreated tissue employed for antipodes of NA, adrenaline, and isoprenaline; normal tissue used for the rest. Tissues pretreated with cocaine, tropolone, and phentolamine (no cocaine used in the case of isoprenaline isomers).
[b] Structure:



tion of antagonistically interacting α- and β-adrenoceptors in the same tissue, and the presence of enzymes which can cause selective degradation of isomers. If all these factors were properly controlled, isomeric ratios recorded may then have magnitudes characteristic of receptor type (α, β or subspecies thereof). Some data recorded in the presence of (a) cocaine (to inhibit neuronal uptake) and tropolone (to inhibit catechol-*O*-methyl transferase), and (b) sotalol (β-antagonist) or phentolamine (α-antagonist) are shown in Tables 4.3 (α-preparations) and 4.4 (β-preparations). It is notable that antipodal potency ratios for a variety of α-receptor tissues are substantially greater than the values reported in the earlier uncontrolled experiments while the narrow range sugests that subspecies populations are similar in all cases (chiefly α_1). In the β-preparations, only the antipodal potency ratios of isoprenaline show a pronounced rise over values found at α-sites.

4.5. Labetalol and Other Combined α, β Blockers

Ariëns[87] has pointed out that introduction of an arylalkyl substituent on the amino group of a catecholamine confers α-adrenergic blocking properties (see above) while modification of the phenolic hydroxyls may induce β-blockade. Labetalol (**29**, *Trandate*) combines both molecular modifications and is used as an



29

antagonist of both α- and β-adrenoceptors (*Martindale* **29**, p. 788). The clinical agent consists of a mixture of approximately equal amounts of all four possibe isomers. When the individual isomers were tested it was found that the *R*(β)*R*(γ)

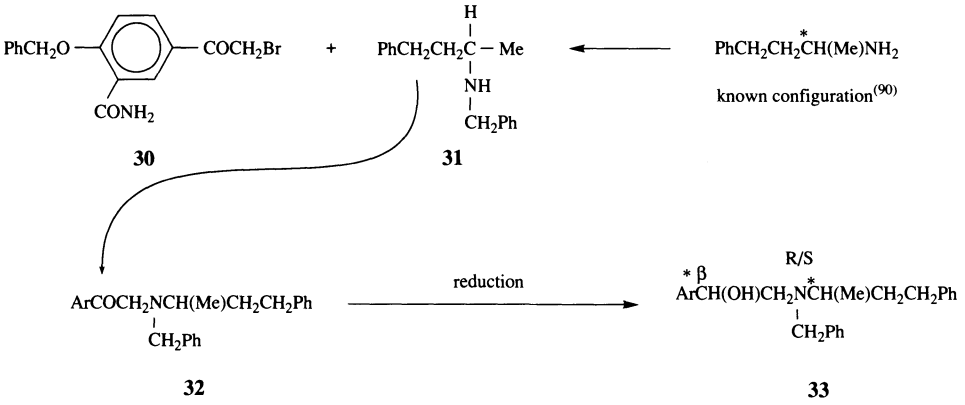
TABLE 4.5.
Activities of Labetalol Isomers[89]

Isomer (β, γ)	β_1-Blockage ID_{50} mg/kg[a]	α-Blockage ID_{50} mg/kg[b]
Labetalol (mixture)	0.25	7.1
**RR*	0.07	35.0
RS	4.1	inactive
**SR*	5.0	1.3
SS	inactive	4.8

[a] Dose required to produce 50% inhibition of the tachycardic response to (±)-isoprenaline (0.1 mg/kg, iv) in anesthetized rat.
[b] Dose required to produce 50% inhibition of the pressor response to phenylephrine (10 mg/kg, iv) in anesthetized rats (*m*-synephrine).

antipode (see **29** for chiral center designations) possessed almost all the β_1-blocking activity but displayed little action at α-sites. In contrast the *S,R* isomer had most of the α-blocking activity. These findings were first reported by a Glaxo group.[88] Some later data of Gold *et al*,[89] are shown in Table 4.5. These results confirm further the need for an *R*-configurated β-chiral center for ligand uptake at β-adrenoceptors, and the fact that blockade of both α- and β-sites is best achieved when the γ-center is also of *R*-configuration (chiral centers of Cc25 **22**, isoxsuprine **25**, butidrine **27**, and labetalol may be compared meaningfully because all the γ- and β-centers are of similar type in terms of the sequence rule).

Chemistry. In the procedure of Gold *et al*,[89] the phenacyl bromide **30** was condensed with the amine **31** of known configuration (*R* or *S*) to give **32**. Reduction of **32** gave separable diastereoisomers **33** epimeric about the β-center. The isomerically pure products were reductively debenzylated to give each of the four



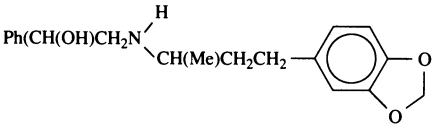
labetalol isomers. The absolute configuration (β-center) of one of these was established by X-ray analysis of a HCl salt, and this proved to be the *RR* isomer. Prior to the X-ray work, CD comparisons were employed; isomer HCls (*RR* and *RS*), and *R*-salbutamol all showed negative Cotton effects. Recently, the single *RR* isomer has been marketed as the β-blocker dilevalol.[91]; its use has resulted in several reports of hepatic reactions, an adverse effect only rarely observed with the mixture of isomers.[92]

Medroxalol **34** is another 4-component (1:1:1:1) mixture. Assays of enriched isomers (stereochemistry unknown) showed that levo isomers displayed greater β_1- and dextro the greater α-blocking activity (Table 4.6).[93] All isomers were equi-

TABLE 4.6.
Blocking Activities of Medroxalol Isomers[93]

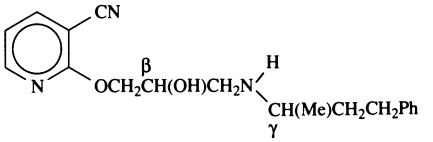
Isomer[a]	pA_2 (α_1) rabbit aorta	pA_2 (β_1) GP atria
(+)-A	6.31	6.99
(−)-A	5.18	8.36
(+)-B	6.44	6.40
(−)-B	5.1	7.7

[a] Optical purity not complete.



34

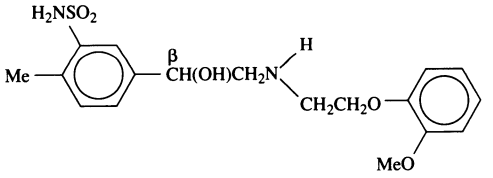
effective in reducing the blood pressure of spontaneously hypertensive rats. In the case of the four isomers of the propranolol analogue **35** (Table 4.7) preference for both α_1- and β-sites was found for a single isomer (*SR*) with *SR* = *SS* at β-sites and 2 × *SS* at α-sites. Note that the *SR* form of the aryloxypropranolamine **35** is the steric equivalent of *RR*-labetalol. The *S*-antipode of the monochiral analogue of **35** (with $NHCMe_2CH_2CH_2Ph$) had similar affinities for α/β sites as *SR*-**35**.



35

Antipodes of the derivative **36** (amosulalol) with a single chiral center differed in their abilities to block adrenergic subsites: the (+)- was tenfold more effective than the (−)-isomer at α_1-sites, while the reverse was true at β_1-sites.[95] In a later paper, blocking activities of *rac* and antipodal forms of amosulalol together with the desoxy analogue (YM-11133, **36** β-OH replaced by H) at four adrenoceptor subsites were reported. (Table 4.8).[96]

Dextro-**36** was the eutomer at α_1-sites (14 × levo) while the levo isomer was the



36

more effective at β_1 (48 ×) and β_2 (47 ×) sites. Both antipodes had reduced activities at α_2-sites and differed little in potency. Binding parameters correlated with the pharmacological data.

TABLE 4.7.
Blocking Activities of Isomers of the Propranolol Analogue 35[94]

	K_i (nM)	K_i (nM)
Isomer (β, γ)	α_1 vs. [^{3}H] prazosin	β vs. [^{3}H] DHA[a]
SR	26	0.67
RR	56	5.40
SS	70	0.73
RS	120	7.8

[a] DHA denotes dihydroalprenolol.

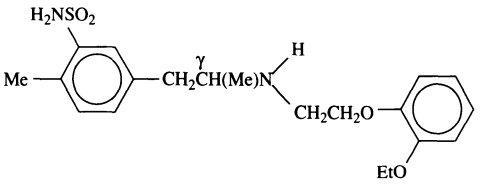
TABLE 4.8.
Blocking Activities of Amosulalol Isomers and the Related Desoxy Analogue at Adrenoceptors[96]

	pA_2 values					
	α-1[a]	α-2[b]	α_1/α_2	β-1[c]	β-2[d]	α_1/β_1
rac-Amosulalol (**36**)	7.97	5.25	525	7.56	7.04	2.6
(−)-Amosulalol (**36**)	7.17	4.92	178	7.71	7.38	0.29
(+)-Amosulalol (**36**)	8.31	5.36	891	6.03	5.71	191
Desoxy analogue of **36**	8.14	6.05	123	6.19	5.76	89

[a] Rabbit aorta vs. NA, β-receptor blocked with propranolol.
[b] Rat vas deferens, electrically stimulated.
[c] Rat right atrium vs. isoprenaline.
[d] Guinea-pig trachea vs. formoterol (β_2-agonist; α-receptors blocked with phentolamine).

The potency of the desoxy analogue approached that of the distomer at β-sites in accord with Easson and Stedman's hypothesis (page 4); at α_1-sites, however, the desoxy derivative had a potency that was only slightly less than that of the eutomer, evidence that a *sec*-hydroxyl function has a less active role at α_1- than at β-receptor sites.

It would be of interest to correlate the configurations of amosulalol isomers with those of the corresponding chiral centers of labetalol diastereoisomers (absolute geometries have not yet been reported). Even higher potencies were achieved in the chiral desoxyanalogue of amosulalol **37** (YM-12617) in regard to blockade of α_1- and α_2- sites.[97] *R*-(−)-**37** was the eutomer at both subsites



37 YM-12617

[pA_2 α_1 (rabbit aorta) 9.98; α_2 (guinea-pig ileum) 6.18]—it exceeded the blocking activity of its distomer 180- (α_1) and 3.5 (α_2) fold. The specificity of *R*-(−)-**37** for α_1- and α_2 -sites (8300) approached that of prazosine (12,300).

Ariëns[87] has tabulated a variety of agents of this kind, some of which await a full stereochemical investigation.

4.6. The Easson and Stedman Hypothesis

During the early 1930s Easson and Stedman[3,98] advanced a three-point attachment theory to account for activity differences between antipodes of adrenaline and NA and their relatives. For *R*-(−)-isomers, groups involved in binding to the receptor were (a) the basic nitrogen (protonated), (b) the aromatic feature (affinity enhanced by *meta* and/or *para* phenolic hydroxy groups), and

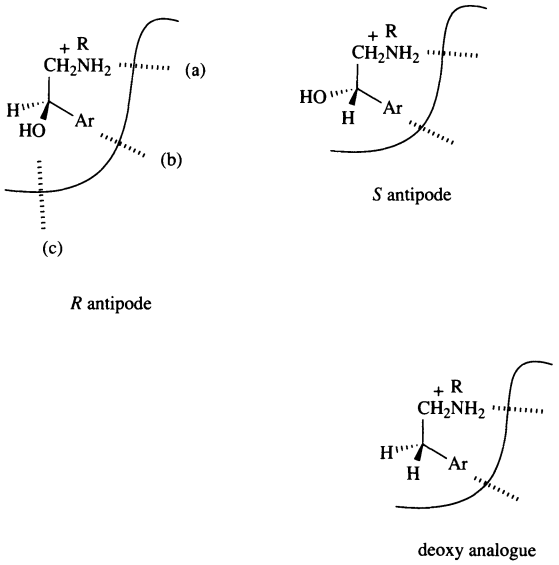


FIGURE 4.1. Demonstration of the Easson–Stedman hypothesis.

(c) the benzylic hydroxyl of the β-carbon. Only in the *R*-antipodes are the three groups arranged in a configuration appropriate to corresponding active sites of the receptor (Fig. 4.1). Thus in *S*-antipodes, binding may occur only at two of the three active sites and ligand affinity is reduced in consequence. If binding interactions common to *R*- and *S*-antipodes are taken to be those of groups (a) and (b), then the β-hydroxyl (c) of an *S*-antipode plays no role in the ligand–receptor association, and its affinity is predicted to be similar to that of the corresponding deoxy analogue (the hypothesis requires that an incorrectly positioned hydroxyl plays a purely passive role and does not impede binding). The relative activities of several *R,S*, deoxy trios of adrenergic ligands have been measured to test these proposals. Most satisfactory support comes from experiments performed on reserpinized tissue to ensure that the potencies recorded reflect direct action only (reserpine displaces NA from storage sites; see Ref. 7 and Appendix to this chapter).

Thus in the data of Table 4.9 which relates to activity at α_1-sites, results for NA and adrenaline triads accord well with the hypothesis when reserpinized tissue is used. In normal tissue the deoxy member was more active than the *S*-(+)-antipode and its potency approached that of the *R*-(−)-isomer. From the data of Table 4.9 all agents appear to act both directly and indirectly (*cf.* $-\log ED_{50}$ values in normal and reserpinized tissue). The fact that the indirect component is greatest for deoxy compounds is evidence that the presence of β-hydroxyl (*R* or *S*) impedes release of stored NA.

Further studies have shown that the Easson–Stedman proposals hold true for all known adrenoceptor subtypes.[3]

Following their earlier work[7] Patil and his colleagues went on to examine the α-adrenoceptor interactions of adrenaline enantiomers in greater depth. Potencies were measured in rat aorta and other tissue models of α-sites, together with

TABLE 4.9.
Relative Potencies (α_1) of Some Antipodal Sympathomimetic Amines and their Desoxy Analogues in the Normal and Reserpine-Pretreated Rat Vas Deferens[7]

Compound	Normal		After reserpine	
	$-\log ED_{50}$[a]	Maximal effect	$-\log ED_{50}$	Maximal effect
R-(−)-NA	5.23	99	4.80	100
S-(+)-NA	4.51	107	4.08	78
Desoxy NA[c]	4.61	107	3.95	67
R-(−)-Adrenaline	5.78	84	5.39	91
S-(+)-Adrenaline	4.51	106	3.96	70
Desoxyadrenaline	4.77	100	4.07	81
R-(−)-Isoadrenaline[d]	4.96	104	4.8	61
S-(+)-Isoadrenaline	4.42	40	*b*	10
Desoxy-isoadrenaline[e]	4.86	89	3.94	32
R-(−)-*m*-Synephrine (**3b**)	5.05	109	5.19	91
S-(+)-*m*-Synephrine	4.16	84	*b*	7
Desoxynor-*m*-synephrine	4.71	100	*b*	6

[a] Molar concentration.
[b] Unobtainable because maximal effect was considerably reduced.
[c] Dopamine.
[d] α-Methyladrenaline (*erythro*) (**40**, R = Me).
[e] Racemic mixture.

dissociation constants(K_d) estimated after partial α-site inactivation with phenoxybenzamine (PBZ)—data so obtained were used to estimate efficacy.[99] Whereas potency, affinity, and efficacy varied between tissues, the eudismic ratio showed only small variations for both potency and affinity. Efficacy was similar for both antipodes, hence potency differences between them must be due principally to differences in affinity. Some data are shown:

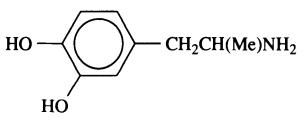
	(−)-NA	(+)-NA	Ratio
Aorta			
pEC_{50}	9.19	7.52	49
pK_d	7.69	6.10	55
Efficacy[a]	42	38	1.4
Efficacy[b]	1.50	1.42	1.1
Portal vein[c]			
pEC_{50}	7.40	5.94	40
pK_d	6.41	5.30	18
Efficacy[a]	12	10	2.4
Efficacy[b]	0.99	0.64	1.5

[a] Efficacy is the reciprocal of fractional occupation at EC_{50}.[100]
[b] Efficacy expressed as (pEC_{50}–pK_d).
[c] Saline medium contained agents to block neuronal uptake, β-adrenoceptors, COM transferase, and extraneuronal uptake.

Results in regard to the rat vas deferens were confirmed in later work, which included receptor binding studies carried out at two temperatures (27 °C and 0 °C).[101] In the binding experiments (displacement of [^{3}H] WB4101, see Chapter 5, page 130) two affinity sites were detected, that of lower affinity correlating with values obtained in the functional test. Affinity constants at 0 °C were slightly greater than those at 27 °C. Thermodynamic parameters for low-affinity sites at 27 °C calculated from these differences were: (−)-NA $\Delta G°$-8.1,$\Delta H°$-0.9; $T\Delta S°$ 7.2; (+)-NA $\Delta G°$ -6.2, $\Delta H°$ -0.2, $T\Delta S$ 6.0(all kcal/mol).[102] Parameters for the high-affinity site differed from those of the low chiefly in regard to entropy, suggesting that the receptor undergoes some energy-requiring conformational change when it is coupled to produce a functional response. The small difference in the isomeric $\Delta G°$ values (-1.9 kcal/mole) is consistent with an energy change associated with formation of a hydrogen bond between the correctly placed benzylic hydroxyl of *R*-(−)-NA and the receptor.[103]

4.7. Phenethylamines with α-Carbon Chirality

The key compound of this class is α-methyldopamine (α-Me DA) **38**, which has been resolved and the configurations of its antipodes established by chemical methods that include a synthesis from *R*-(−)-alanine by a reaction sequence that does not disturb the α-chiral center[104] (Scheme 4.7) (see also Pratesi *et al.*[105]). *S*-(+)-α-Methyldopamine is a metabolic product of *S*-(−)-α-methyl DOPA in man (see Scheme 4.4).



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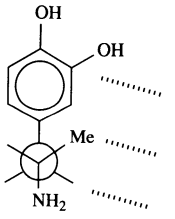
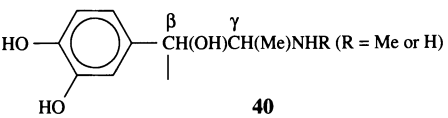


39

The S-(+)-isomer is the more potent agonist at guinea-pig tracheal (reserpinized) β_2[107] and ileal (α_2) sites.[108] At α_1-sites (rat vas deferens and guinea-pig aorta, both reserpinized) both antipodes (also dopamine for aorta) had similar low orders of activity. Selectivity for α_2 over α_1 sites was 23-fold for the *S*-(+)- and 2-fold for the *R*-(−)-antipode.

Ruffolo argues[3] that *S*-(+)-α-methyl DA binds to α_2 receptors by a three-point attachment (involving α-methyl of optimal *S*-configuration, see **39**), while its *R*-(−)-antipode and DA itself interact in a two-point manner. In contrast, two-point binding operates at α_1-sites for all members of the triad. This extension of the Easson–Stedman hypothesis requires that analogues of α-Me DA likewise exhibit optimum activity when the methylated carbon α to nitrogen has an *S*-configuration. A test case is that of α-methyladrenaline (iso-adrenaline) **40** and α-methyl NA.



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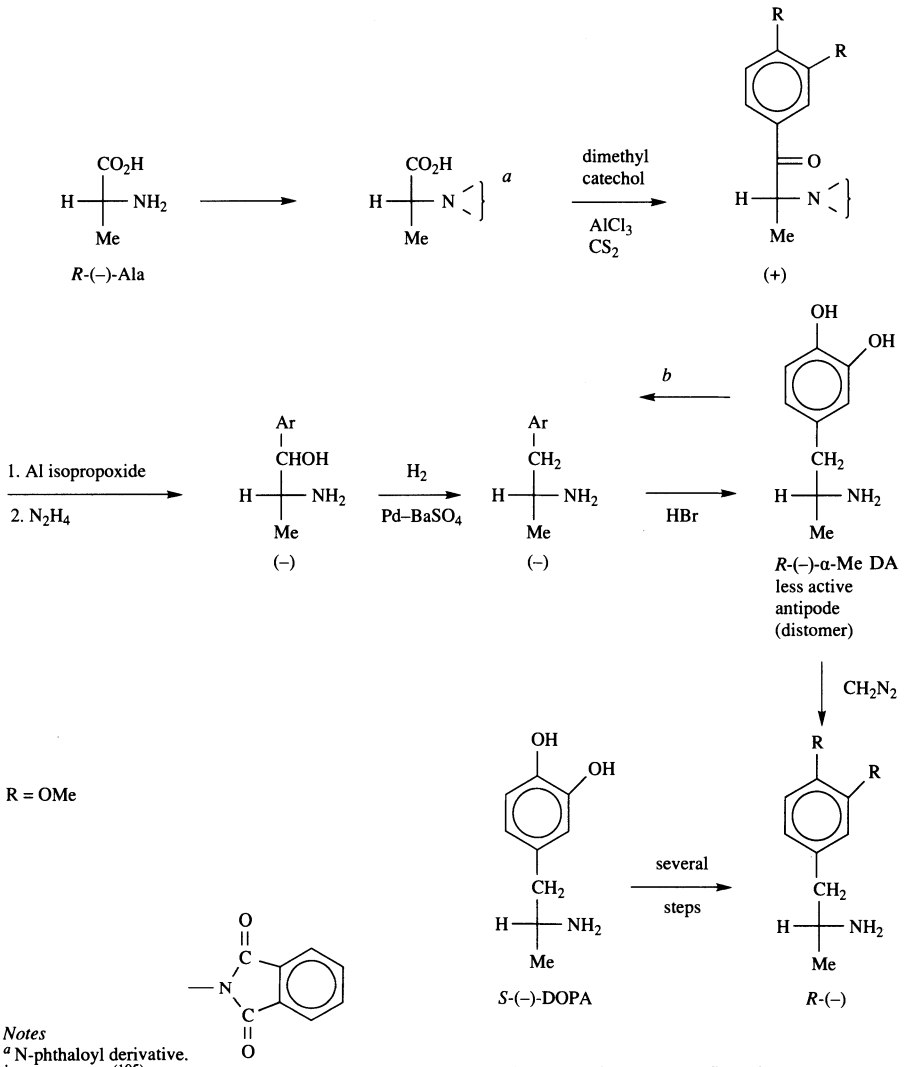


Notes

[a] N-phthaloyl derivative.

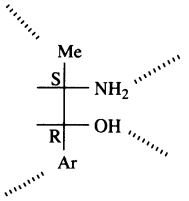
[b] Pratesi *et al.* [105] converted *R*-(–)-α-Me DA to the *O,O*-dimethyl derivative of known *R*-configuration through its chemical correlation with *S*-(–)-DOPA (ref. 106).

Scheme 4.7. The configuration of α-methyldopamine (α-Me Da).[104]

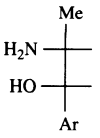
Although α-methyladrenaline exists as a mixture of diastereoisomers, a synthesis is reported from which only the *erythro*-racemate may be separated.[109] Its relative configuration is based on the likely steric course of its preparation (catalytic reduction of a related propiophenone) and on the magnitude of spin–spin coupling between the 1-H and 2-H protons (the value $J = 4$ Hz is characterisitic of *erythro*-isomers of this kind; see ephedrine p. 106). Comparisons of the CD spectrum of resolved *erythro*-**40** (R = Me) with those of R-NA, R-adrenaline and 2*S*-*erythro*-α-methyl NA[110] showed that the levo antipode had a 1*R*,2*S* configuration.[111]

In tests on tissues selectivity-rich in each of the four classes of adrenoceptor,

the 1*R*, 2*S*-(−)-*erythro*-isomer **41a** proved the more potent in all experiments, in support of the above ideas regarding both α(2)- and β(1)-carbon chirality.[112]



41a

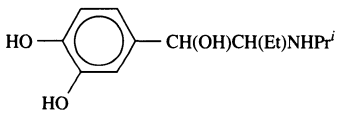


41b

The fact that the potency difference between levo **41a** and dextro **41b** was much greater at α_2 (× 550) than at α_1 (× 60) sites correlates with the demonstration (above) that α_1-sites are relatively insensitive to the presence of α-methyl, and Ruffolo[3] considers that the (−)-isomer **41a** binds to α_1-sites by 3-point, and α_2-sites by 4-point attachment. In support 1*R*-(−)-NA is equipotent or slightly more potent than 1*R*, 2*S*-(−)-**41a** at α_1-sites[108,112] while the latter isomer is significantly more potent than (−)-NA at α_2-adrenoceptors[112,113] since its binding is enhanced over that of (−)-NA by the α-methyl contribution.

Binding sudies of Goldberg *et al*[114] correlate with pharmacological data in that both (−)-*erythro*-α-Me NA and (−)-*erythro*-α-Me adrenaline were shown to have high affinities for α-2 sites and to bind much less effectively to β- and α-1 sites of rat forebrain (Table 4.10). It is also noteworthy that (−)-1*R*,2*S*-**40** and the β-agonist levo isoxsuprine **25** share the same configurations at their α- and β-chiral centers (page 84).

Isoetharine (**42**, Dilabron) is an N-isopropyl analogue of α-Me NA with α-Me



42

replaced by α-Et. The *erythro*-racemate is used as a bronchodilator and is selective for β_2-sites. A Beecham group[115] examined all four isomers. Some pharmacologi-

TABLE 4.10.
Ligand Binding Data at Adrenoceptors of Rat Forebrain[114]

	K_1[a]		
	α-1[b]	α-2[c]	β[d]
(−)-NA	460	6.8	390
(−)-*erythro*-α-Me NA	3600	3.0	220
(+)-*erythro*-α-Me NA	2.3×10^6	866	9.8×10^4
(−)-adrenaline	230	1.9	280
(−)-*erythro*-α-Me adrenaline	11,200	8.5	200
(+)-erythro-α-Me adrenaline	14.6×10^4	2900	3.1×10^5

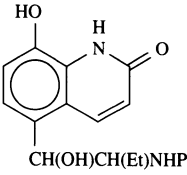
[a] $K_1 = EC_{50} \div 1 + L/K_d$, where K_d is taken from Scatchard plots and L is ligand concentration (see Table 2.1 of Chap. 2).
[b] vs. [^{3}H] prazosine.
[c] vs. [^{3}H] clonidine.
[d] vs. [^{3}H] dihydroalprenolol.

TABLE 4.11.
β-Adrenoceptor Activities of Isoetharine Isomers[115]

Isomer	Dose ratios (isoprenaline = 1) in guinea pigs	
	Trachea (β_2)	Atrial (rate) (β_1)
racemic-*erythro*	7.2	370
(−)-*erythro*	1.9	310
(+)-*erythro*	1,200	8,900
racemic-*threo*	780	25,000
(−)-*threo*	180	6,400
(+)-*threo*	940	4,400

cal data are shown in Table 4.11. The levo *erythro*-isomer stands out as the most potent β-agonist. Only relative configurations, based on $^3J(H_1H_2)$ values, are known.

Procaterol **43**, a close analogue of isoetharine based on carbostyril, is also a selective β_2-agonist when used in its *erythro*-racemic mixture form.[116] Equipotent doses for inhibiting histamine-induced bronchonconstriction in dogs were: *erythro*-**43** *rac* 2.03, (−) 1.41, (+) 18,500, (−)-isoprenaline 1.0. *Threo*-isomers were far less effective (dextro eutomer 566), as observed for isoetharine analogues. *Rac*- and (−)-*erythro*-**43** were almost 25 times more selective for β_2 than for β_1 (rat heart) sites.



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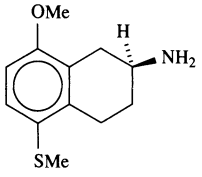
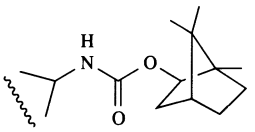
Reduction of a keto precursor of **43** with $NaBH_4$ gave the *erythro*-product which, after functional group protection, was inverted to the *threo*-form by replacement of hydroxyl by chlorine and subsequent hydrolysis. Stereochemical assignments were based on dimensions of the C*H*OH ^{1}H-NMR signal (4–4.2 Hz for *erythro*, 8.2–8.3 Hz for *threo*).[117]

The 2-aminodecalin **44** (*S*-antipode shown) may be regarded as a cyclized form of α-methyldopamine. Unlike this agent, however, it is a selective agonist for α_1



44

rather than α_2 adrenoceptors.[118] The *S*-antipode was about 20 times more potent than the *R*-form in producing a constrictor response in the isolated rabbit ear artery (α_1-test) and 80 times effective than the standard α_1-agonist methoxamine. It had no inhibitory effect on [^{3}H] NA release in the rabbit ear artery even at the high concentration of 3 μM. Hence eutomers of **44** and α-methyl DA correspond in configuration but differ in their selectivities for α_1- and α_2-sites. *S*-**44** was made from one of the diastereoisomers **45** obtained from the corresponding 2-aminotetralin and (−)-isobornylcholoroformate, and its configuration established by X-ray analysis.



45

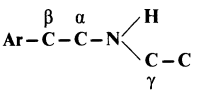
Now that a variety of adrenoceptor ligands with multiple (2 or 3) chiral centers have been described in this chapter, a presentation of the data has been provided in Table 4.12 to aid analysis of configurational influences on activity. The following points may be made:

1. Uptake at β-adrenoceptors requires β-(*R*)-chirality for both agonists and antagonist (items 1, 2, 4, 5, 6, 8, and 10).
2. Blockade of α-adrenoceptors is best achieved with β-(*S*)-chirality (items 1, 2, 6).
3. Blockade of α-adrenoceptors is best achieved with γ-(*R*)-chirality (items 2, 4, 6)—the same applies to β-blockade (items 4, 5, 6).
4. Both α- and β-agonism is best achieved with α-(*S*)-chirality [items 2 (β only), 7, 8].
5. One may predict that the antipodes of Cc25 and dobutamine which exhibit α-antagonism have γ-(*R*)-chirality [now confirmed for (+)-dobutamine] (private commun. Robertson 1990), and also that (−)-*erythro*-isoetharine has the β-(*R*)-, α-(*S*)-configuration.

4.8. Octopamines and Synephrines

In 1952, a novel neurotransmitter was identified in the posterior salivary gland of the common octopus and named *p*-octopamine on account of its source.[119] The compound **4**a was subsequently found to be a widely distributed invertebrate neurotransmitter[120] and to occur in the mammalian sympathetic nervous system where its function is unknown. MS analytical techniques have disclosed that the *o*- and *m*-octopamines and *N*-methyloctopamines (*m*- and *p*-synephrine) also occur in mammals.[121] Recently, antipodal forms of the *m*- and *p*-octopamines and synephrines have been evaluated extensively in biological tests. The compounds were far weaker agonists at β_1-sites (GP atria) than (−)-NA and even feebler at tracheal (β_2) sites.[122] Apart from antipodal *p*-synephrines (both isomers were almost inactive) the potency of levo exceeded that of dextro isomer. In the *m*-series, secondary

TABLE 4.12.
Summary of Configurational Influences on the Activity of Adrenoceptor Ligands with Chiral Centers of the β-, α-, and γ-type[a]



Item	Compound	Configuration of designated centers and potency ranking	Pharmacological profile	Reference
1	Cc 25 (**22**)	β(*S*):γ(?) > β(*R*):γ(?) β(*R*):γ(?) ⩾ β(*S*):γ(?)	α-antagonist β-agonist	67
2	Isoxsuprine (**25**, Cc 40)	β(*S*):α(*R*):γ(*R*) > β(*R*):α(*S*):γ(*S*) β(*R*):α(*S*):γ(*S*) > β(*S*):α(*R*):γ(*R*)	α-antagonist β-agonist	68
3	Dobutamine (**26**)	(−)-γ(*S*) > γ(+)-(*R*) (+)-γ(*R*) > γ(−)-(*S*) (+)-γ(*R*) > γ(−)-(*S*)	α-agonist α-antagonist β-agonist	75 and private communication
4	Butidrine[b] (**27**)	β(*R*):γ(*R*) > β(*R*):γ(*S*) > β(*S*):γ(*R*) > β(*S*):γ(*S*)	α-antagonist	79
5	2-Naphthyl analogue (**28**)	β(*R*):γ(*R*) > β(*R*):γ(*S*) ⩾ β(*S*):γ(*R*) > β(*S*):γ(*S*)	β-antagonist	81
6	Labetalol (**29**)	β(*S*):γ(*R*) > β(*S*):γ(*S*) ⩾ β(*R*):γ(*R*) > β(*R*):γ(*S*) β(*R*):γ(*R*) ⩾ β(*R*):γ(*S*) > β(*S*):γ(*R*) > β(*S*):γ(*S*)	α-antagonist β-antagonist	89
7	α-Methyldopamine	α(*S*) > α(*R*)	α-2 agonist β-2 agonist	107, 108
8	α-Methyl-NA and α-methyladrenaline	β(*R*):α(*S*) > β(*S*):α(*R*)	α- and β-agonist	108, 112, 113
9	Isoetharine (**42**)	(−)-*erythro* > (+)-*erythro*	β-2 agonist	115
10	Goodman's *N*-heptanoyl-anilides (**24**) (page 83)	β(*R*):γ(*R*) > β(*R*):γ(*S*) = β(*S*):γ(*R*) > β(*S*):γ(*S*)	β-agonist and binding to β-sites	69

[a] The various α-, β-, and γ-chiral centers are of similar kind in terms of the *R*/*S* nomenclature convention, and thus may be compared in a meaningful manner.
[b] Same rank order for β-antagonism except β(*S*):γ(*S*) ⩾ β(*S*):γ(*R*).

amines (NHMe) were more potent than primary (NH_2) (Table 4.13). Maximal effects were less than that attained by (−)-NA.

The monophenols **4** and their *meta* analogues were more effective at α_1- (rat aorta and anococcygeus muscle) than β_1-sites, and all attained effects close to the NA maximum (Table 4.14). Although the same potency variations in regard to sign of rotation (at 589 nm) were observed, primary and secondary amines had similar levels of activity. The potency ratios between antipodal *m*-synephrines (420) and *p*-syneprhines (75) were notably high.[123] Similar variations among the pD_2 values of these isomers were found in the rat anococcygeus preparation, but lower potency levels were found. In the rat saphenous vein α_2 preparation (treated with cocaine to block neuronal uptake of sympathomimetic amine) all isomers were far less effective than (−)-NA with levo isomers the more potent of the antipodal pairs; e.g.; pD_2 values were: (−)-NA 7.6, (−)-*m*-synephrine 5.45, (+)-*m*-synephrine 3.85, ×40 potency difference.

Binding experiments confirmed the superior affinity of levo isomers for

TABLE 4.13.
Adrenergic Activities of Octopamine and Synephrine Antipodes at β_1-sites (GP Atria)[122] [a]

Compound	pD_2 NA	Relative potency	Fraction of (−)-NA maximum
(−)-NA	8.62	1.00	1
(−)-*m*-Synephrine	6.68	0.01	0.66
(+)-*m*-Synephrine	5.65	0.001	0.83
(−)-*m*-Octopamine	4.84	0.0001	0.68
(+)-*m*-Octopamine	<4.00	very weak	0.27
(−)-*p*-Synephrine	<4.00	very weak	0.48
(+)-*p*-Synephrine	<4.00	very weak	0.11
(−)-*p*-Octopamine	4.78	0.0001	0.68
(+)-*p*-Octopamine	<4.00	very weak	0.28

[a] Isolated from reserpinized GP, hence direct action parameters are recorded.

both α_1- and α_2-sites over their antipodes (rat cerebral cortex: competition *vs* [^{3}H]prazosin for α_1-, and [^{3}H]yohimbine for α_2-sites) but differences between antipodes were less than those anticipated from the pharmacological assays. All ligands bound more effectively to α_2-sites. Illustrative data (negative logarithm of test compound concentration that displaces 50% of specific radioligand binding) are: (−)-NA 5.04 (α_1), 6.07 (α_2); (−)-m-synephrine 4.05 (α_1), 5.60 (α_2); (+)-*m*-synephrine 3.33 (α_1), 4.61 (α_2). Antipodal affinity ratios were not great—the highest value was 17 observed for (−)- and (+)-*m*-octopamine at α_1-sites.

Tests have also been carried out at specific octopamine neurones identified in invertebrate nervous systems[120] which respond only feebly to (−)-NA. Subspecies

TABLE 4.14.
Adrenergic Activities of Octopamine and Synephrine Antipodes at α_1-sites (Rat Aorta)[123]

Compound	pD_2 NA	Relative potency	Fraction of (−)-NA maximum
(−)-NA	8.30	1.00	1
(+)-NA	6.78	0.03	1
(−)-*m*-Synephrine[a]	7.50	0.16	0.98
(+)-*m*-Synephrine	4.88	0.0004	0.60
(−)-*m*-Octopamine	7.50	0.16	0.98
(+)-*m*-Octopamine	6.61	0.02	0.98
(−)-*p*-Synephrine	5.38	0.001	0.89
(+)-*p*-Synephrine	3.50	very weak	0.49
(−)-*p*-Octopamine	5.34	0.001	0.85
(+)-*p*-Octopamine	4.66	0.0002	0.80

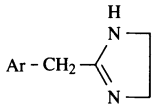
[a] Rat vas deferens data: pD_2 normal tissue (−) 5.05, (+) 4.16; after reserpine (−) 5.19, (+) not measurable.[7]

of receptors characterized are E_{2A} (presynaptic) and E_{2B} (postsynaptic) whose effects are mediated by increases in *c*-AMP, and E_1 which may act by releasing Ca^{2+} from intracellular stores. At E_{2A}, E_{2B}, and E_1 sites of the extensor-tibiae muscle of locust hind leg, *para* derivatives **4** were more potent than *meta* analogues. Some results at E_{2A} receptors are as follows ($EC_{50} \times 10^{-7}$M): *p*-synephrine (−)0.84, (+)32; *p*-octopamine (−)5.7, (+)1500; *m*-octopamine (−)8400, (+) inactive; (−)-NA 280.

The absolute configurations of (−)-*p*-octopamine and *p*-synephrine are known to be *R*, i.e., identical with that of (−)-NA (see above), hence their superior action over corresponding (+)-antipodes may be understood. Although CD evidence points to (−)-isomers of *meta*-octopamine/synephrine and *para*-octopamine/synephrine having opposite configurations, this seems unlikely on the basis of the pharmacological demonstration that the higher actvities of levo over dextro antipodes hold also in the *meta* series.

4.9. Imidazolines

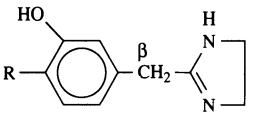
Imidazoline derivatives are well recognized as an important class of drugs that interact with α-adrenoceptors behaving either as agonists, e.g., naphazoline **46**a, or antagonists, e.g., tolazoline **46**b. It may be argued that such derivatives are active



(a) Ar = 1-naphthyl; (b) Ar = Ph

46

because they include the 2-arylethylamino backbone of catecholamines. However, it is unlikely that the two classes of ligand interact with adrenoceptors in the same manner. Thus insertion of hydroxyl at the β-carbon of the deoxy analogues **47**a *decreased* agonist activity while the rank order of potency in the (**47**b) series was deoxy $> R(-) > S(+)$ in contravention of the Easson–Stedman hypothesis. At α_2-adrenoceptors the $R(-)$-form was only 6-fold more potent than the $S(+)$-isomer (as in α_1-tests) and, again, the deoxy analogue (a potent partial agonist) was the most active of the three compound.[124] Absolute configurations were established by X-ray crystallography of a (+)-dibenzoyltartaric acid salt.[125] Similar results were found for the β-OH analogue of tolazoline itself.[126]

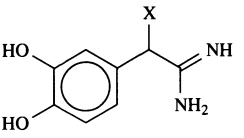


(a) R = H; (b) R = OH

47

The rank order deoxy $\geqslant R\text{-}(-) > S\text{-}(+)$ was confirmed for the **47**b series at α-sites of rat aorta: pEC_{50} **47**b (deoxy) 8.59; β-OH analogues *R*-(−) 8.59,

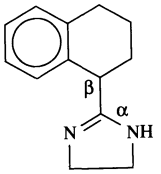
S-(+) 7.67.[127] These results together with dissociation constants (K_d) measured after partial receptor inactivation with PBZ gave measures of efficacy (see page 233), and it was shown that differences in antipodal potencies were due solely to differences in affinity. Similar results were found for the related amidine triad (**47**c).



47 c

X	pEC_{50}
H	7,68
R–OH	6,99
S–OH	6,61

Tetrahydrozoline (**48**), a chiral analogue of naphazoline in which the β-carbon is linked to the phenyl ring, is in clinical use as a vasoconstrictor (α-agonist). The affinity of its (−)-antipode (measured versus phenylephrine on rat aorta—a tissue to which it binds but lacks efficacy) was ten times that of the dextro form.[128] Chiral analogues of the agonist naphazoline have also been examined. Insertion of methyl or benzyl at *C of **49** yielded weak antagonists, and antipodal pairs differed little in affinity, e.g., pA_2 5.6 (*R*), 5.8 (*S*) for methyl derivatives.[129] The chiral derivative **50** (another 4-substituted imidazoline) proved to be a much weaker α_1 agonist (rat aorta) than 2-(3,4,-dihydroxybenzyl)imidazoline with an *R*/*S* potency ratio dose close to unity.[130]



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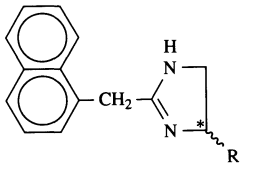

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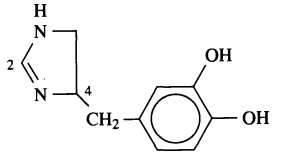
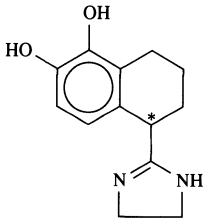


50

Greater interest lies in the 3,4-dihydroxy analogue of tetrahydrozoline. This agent **51** is a very potent α-agonist (12–13 × NA), selective for α_2-sites.[131] Activity and binding parameters for *R*-(+) and *S*(−), shown in Table 4.15, demonstrate the



51

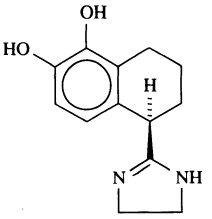
TABLE 4.15.
Pharmacological and Binding Parameters for the Imidazoline 52[132]

	ED_{50} NA/ED_{50} test compound		Binding K_i (nM)[b]	
	Rabbit aorta (α_1)	PBZ – DSV[a] (α_2)	α_1	α_2
R (+)	29	248	523	0.55
S (−)	1.0	15.4	817	28
RS	13	184	400	6.5
NA	—	—	398	37

[a] Phenoxybenzamine (inactivates postsynaptic α_1-receptors) – pretreated dog saphenous vein (postjunctional α_2).
[b] Displacement of [^{3}H] prazosin from rat liver (α_1), and [^{3}H] rauwolscine from rat cortex (α_2).

antipodal potency ratios (*R* 30 × *S*, α_1, *R* 16 × *S*, α_2) and ligand preference for α_2-sites. Although the affinities of *R*- and *S*-**51** for α_1-sites are similar, antipodal efficacies differ substantially. This phenomenon is not uncommon.[133] The stereochemical requirements of presynaptic α_2-receptors were identical to those of postsynaptic sites—evidence that proteins of similar kind are involved.

Resolution of *O*,*O*-dimethyl **51** was monitored from ^{1}H-NMR spectra of respective HCl salts in D_2O containing the chiral shift reagent CoATP (see Chapter 2, page 38). The antipode isolated as a crystalline salt with (+)-di-benzoyl-D-tartaric acid was shown by X-ray crystallography to have the *S*-configuration. The more active antipodes of **51** and NA correlate nicely on paper (see **52** and **53**), and it would be of interest to find out if (−)-tetrahydrozoline fits the same pattern.



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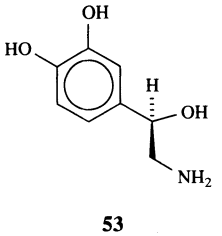

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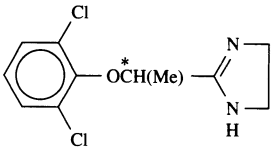
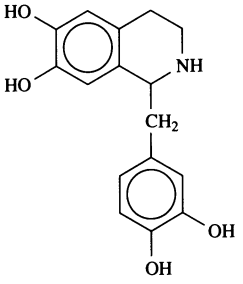


54

Lofexidine **54**, an imidazoline derivative related to both clonidine and tymazoline, is an α-agonist—its levo antipode produced pressor effects at doses 20-fold lower than those of the dextro form.[134]

4.10. Trimetoquinol (TMQ)

Interest in the adrenoceptor activities of tetrahydroisoquinolines (THIs) arose as a result of their detection in the urine of Parkinsonian patients after L-Dopa administration.[135] The compounds **55** and **56** (salsolinol) are believed to form by *in vivo* condensation of DA with 3,4-dihydroxyphenylacetaldehyde (a DA metabolite) and acetaldehyde, respectively; Holz[136] has demonstrated the enzymatic formation of **55** from DA.



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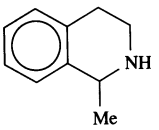

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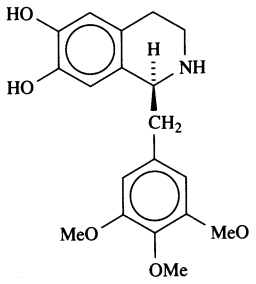
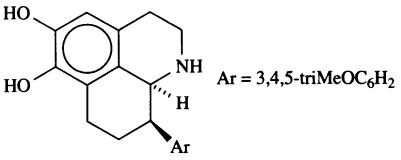


57 *S*-(−)-TMQ

Trimetoquinol (**57** TMQ) proved the most potent of a series of THIs examined as β-agonists.[137] In test on antipodes the *S*-(−)-isomer proved to be the more active by factors of 100 (GP trachea), 59 (lipolysis), and 20 (GP atria),[139]—selectivity of action at β_2-sites[140] was not confirmed although β_2- exceed β_1-potencies (see also Table 4.4). TMQ antipodes were equieffective as antagonists of NA-induced contractions of GI aorta (α-site). The absolute configurations of enantiomers of TMQ and its relatives were established from ORD data[137] and X-ray crystallography.

Analogues of TMQ methylated at benzylmethylene have been examined.[141] Potency rank orders as β_2-agonists (GP atria) were *threo* > TMQ > *erythro*, and as β_1-agonists (GP atria) TMQ > *threo* > *erythro* (all racemic mixtures). Diastereoisomeric assignments were based on ^{1}H-NMR and IR (Bohlmann bands) evidence.

Among other variants of TMQ, diastereoisomers of hexahydrobenzo[d,e]-quinoline **57a** were examined; the *trans*-isomer (pD_2 6.03) proved 78 times more potent than the *cis* (pD_2 4.14) as a relaxant of GP tracheal muscle.[138] Only the *t*-isomer presents its two aryl features in an *anti*-orientation, as obtains in the solid state conformation of TMQ.

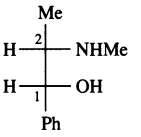


57a (*trans*-isomer shown)

Recently, attention on TMQ has moved to its ability to inhibit platelet aggregation induced by thromboxane A_2-mimetic agents.[142] It does this in a stereoselective manner with the *R*-(+)-antipode 40 times more effective than the *S*-(−)-isomer in a platelet function test employing the stimulant U46619 (a stable prostanoid). The fact that the eutomer in this test is the distomer in β-adrenoceptor tests is evidence that different mechanisms are involved. TMQ does, however, block U46619-induced phosphoinositol breakdown, again with *R*-TMQ the eutomer[144]—an effector pathway of α_{1B} adrenoceptor (α-receptors are blocked by antipodes of TMQ, see above). Further work has confirmed the potent and stereospecific blockade of thromboxane A_2 (TXA_2) receptors by TMQ analogues.[144a] Data on the 4-imidazoline analogue of **47b** has also been reported in this regard. The *S*-antipode **50** was 66 times more effective than the *R*-isomer in preventing NA-induced platelet aggregation.[143] Antipodes of **50** were obtained from *R*- and *S*-DOPA.

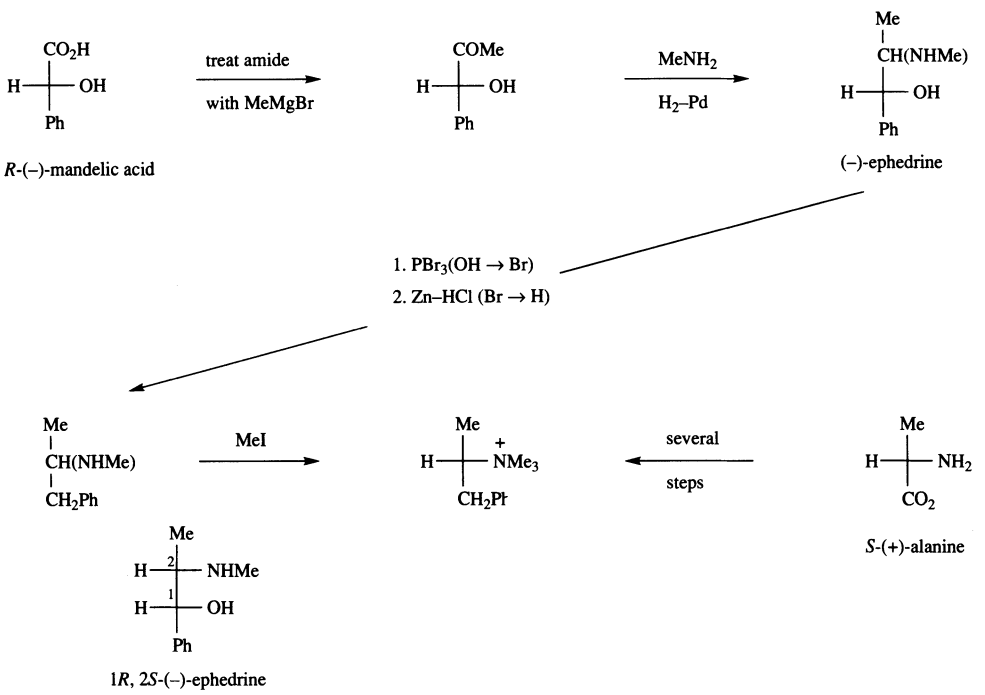
4.11. Adrenergic Agents with Indirect and Mixed Actions (False Transmitters)

A variety of 2-phenethylamine derivatives that lack the 3,4-diphenolic hydroxyls of natural catecholamines are in clinical use. Some, especially those with an *m*-phenolic hydroxyl or a β-*sec*-hydroxyl function, display a significant degree of direct adrenergic action, while those lacking these features chiefly act indirectly by displacing NA from intraneuronal storage granules. Indirect agents are of much reduced potency after reserpine (see above and Appendix to this chapter). α-Effects predominate, as anticipated if action is mediated via NA, but β-sympathomimetic effects are also shown which may be result of the direct component of their activity profile. The *ephedrine* group is a well-known example; formula **58** depicts natural (−)-ephedrine. The full isomeric set comprises a pair of *erythro* (ephedrines) and *threo* (pseudoephedrines) racemates.

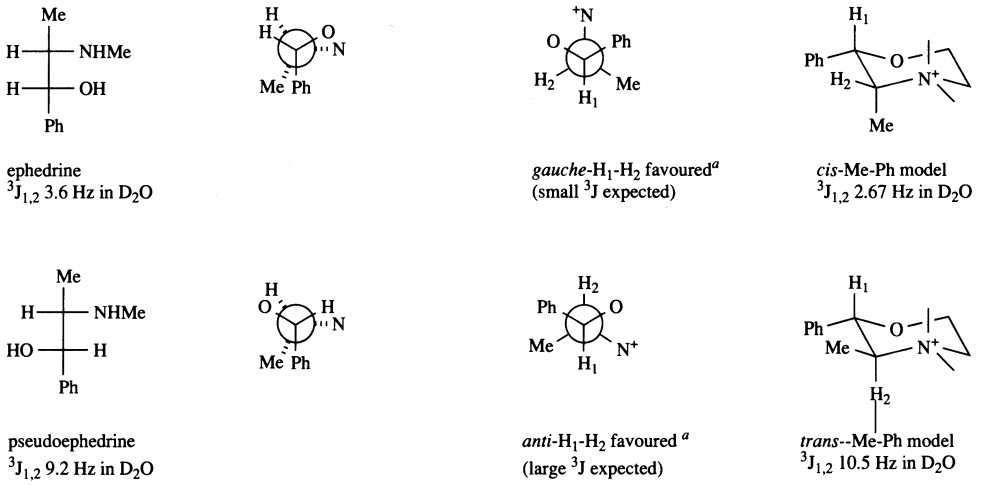


1*R*,2*S*-(−)-ephedrine
58

Freudenberg established the stereochemistry of these diastereoisomer amines in the 1930s (Scheme 4.8).[145]



Scheme 4.8. Absolute configuration of (−)-ephedrine.[145]



ephedrine
$^3J_{1,2}$ 3.6 Hz in D_2O

gauche-H_1-H_2 favoured[a]
(small ^3J expected)

cis-Me-Ph model
$^3J_{1,2}$ 2.67 Hz in D_2O

pseudoephedrine
$^3J_{1,2}$ 9.2 Hz in D_2O

anti-H_1-H_2 favoured [a]
(large ^3J expected)

trans--Me-Ph model
$^3J_{1,2}$ 10.5 Hz in D_2O

[a] Probably stabilized by intramolecular $^+NH \cdots O$ hydrogen bonding.

Scheme 4.9. ^{1}H-NMR evidence for the relative stereochemistry of ephedrine and pseudoephedrine. (147)

(−)-Ephedrine and (+)-pseudophedrine have the same configuration at C-2(α) because removal of β-OH yields (+)-*N*-methylamphetamine in each case.(146) ^{1}H-NMR confirmation of the *erythro/threo* assignments is provided by the greater $^3J(H_1, H_2)$ coupling found in pseudoephedrine compared with that of ephedrine salts, and the close correspondence of th ^3J values with those of *cis*- and *trans*-2-phenyl-3-methylmorpholine models (Scheme 4.9).(147) This principle has served to establish relative stereochemistry in several *erythro–threo* pairs of this kind (e.g., metaraminol, page 108) *In vivo* and *in vitro* (α_1) tests on isomeric sets are shown in Table 4.16. In all cases (−)-1*S*, 2*R*-ephedrine proved the most potent isomer with a β-carbon configuration identical with that of (−)-NA. In the rat VD,

TABLE 4.16.
Pharmacological Comparisons of Isomers of Ephedrine and Norephedrine

	Relative pressor activities		Rat VD − log ED_{50} (α_1)(7)	
			Normal tissue	Reserpinized tissue
	cats(148)	dogs(149)		
Ephedrines				
(−)-1*R*, 2*S*	1.0	1.0	4.73	2.17
(+)-1*S*, 2*R*	0.34	0.33	*a*	*a*
Pseudoephedrines				
(−)-1*S*, 2*S*	0.2	0.2	*a*	*a*
(+)-1*R*, 2*R*	0.3	depressant	*a*	*a*
norephedrines				
(−)-1*R*, 2*S*			4.86	2.46
(+)-1*S*, 2*R*			*a*	*a*

[a] Not measurable.

(−) ephedrine and (−)-norephedrine had -log ED_{50} values which fell sharply when tested on reserpinized tissue, showing their action to be chiefly indirect.

Stereochemical influences on agents of indirect action relate to mechanisms by which they effect release of NA rather than interactions of adrenoceptor ligands as such.[150] The neuronal uptake of catecholamines and their analogues may be studied by fluorescence histochemical or radiochemical methods, both of which allow visualization of uptaken molecules into tissues that have been depleted of catecholamines by reserpine. The overall process is undoubtedly stereoselective as demonstrated by affinity constant K_M (μM) values recorded for NA antipodes in heart tissue; (−) 0.27, (+) 1.39.[151] Details of stereochemical influence are difficult to establish, however, since at least two processes have been defined that bear upon uptake.[3] The first, termed $uptake_1$ or membrane uptake, involves transport of the substrate into the nerve terminal by a cocaine-sensitive (inhibitory) amine uptake pump located on the neuronal cell membrane. A second process governs uptake of amines into the adrenergic storage vesicle themselves. The results of most investigations show that the $uptake_1$ process is only modestly stereoselective (a few-fold preference for *R*-antipodes of β-OH phenethylamines is observed) while vesicular uptake is highly stereospecific.[152] Antipodes of 6-[^{18}F]-fluoro NA have recently become available for PET studies of catecholamine uptake.[35]

The uptake pump displays larger degrees of stereoselectivity for phenethylamines with asymmety at the α-carbon. Thus 2*S*-(+)-amphetamine is transported to a 20-fold greater extent than the 2*R*-(−)-antipode.[153] Similar results, but with only a 3-fold preference for *S*-antipodes, are reported by Marquardt[154] from measurements of the inhibition of NA uptake into rat brain synaptosomes. Some data illustrating these points, taken from Iversen's 1965 review,[150] are shown in Table 4.17.

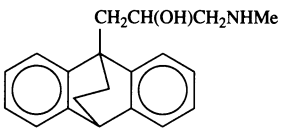
TABLE 4.17.
Inhibition of [^{3}H] NA Uptake by Sympathomimetic Amines in Rat Heart ($Uptake_1$)[150]

Amine	ID_{50}[a]	Relative affinity for uptake site (β-phenethylamine = 100)
rac-NA	6.7×10^{-7}	165
(−)-NA	2.7×10^{-7}	407
(+)-NA	1.4×10^{-6}	79
rac-Adrenaline	1.6×10^{-6}	70
(−)-Adrenaline	1.0×10^{-6}	110
rac-Amphetamine	4.6×10^{-7}	240
(+)-Amphetamine	1.8×10^{-7}	610
(−)-Amphetamine	3.7×10^{-6}	30
rac-α-Me NA	4.3×10^{-7}	256
(−)-α-Me NA	2.0×10^{-7}	550
(−)-Ephedrine	2.2×10^{-6}	50
(−)-Metaraminol	7.6×10^{-8}	1440

[a] Concentration producing 50% inhibition of NA uptake.

In false transmitters with dual α,β-asymmetry, isomers of 1*R*,2*S*-configuration are not necessarily preferred for uptake. In iris from reserpine-pretreated rats both (−)- and (+)-*erythro*-α-methyl NA were substrates for neuronal uptake and in equal degree.[155] *Threo*-isomers were not substrates for uptake in iris, but were so in mouse and rabbit heart although in less degree than *erythro*-α-methyl NA.[156,157]

Many antidepressant act by inhibiting uptake of monoamines including that of NA, and chiral agents exhibit marked stereoselectivity of action in this respect, e.g., oxaprotiline **59** and mianserin **60** (dextro isomers are the more effective).[158,159] In the case of nomifensine **61**, IC_{50} values for inhibition of NA uptake into rat brain synaptosomes were: *rac* 3.2×10^{-8}, *S*-(+) 1.8×10^{-8}, *R*-(−) $\sim 10^{-5}$.[160] Further details are given in the 1989 review of Coutts and Baker.[161]



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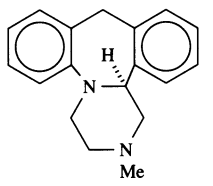

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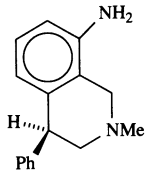
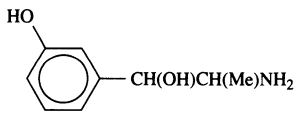


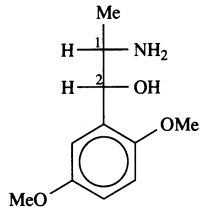
61

In the case of metaraminol **62** and its isomers, since only the 1*R*,2*S*-(−)-isomer functions as a false transmitter[162,163] while all four isomers are substrates for neuronal uptake ($uptake_1$), one may infer that only vesicular uptake is stereospecific in this case.[164] Note that (−)-metaraminol has a significant degree of direct action (rat vas deferens—log ED_{50} value, 4.80; cf. 4.35 after reserpine).[7]



62

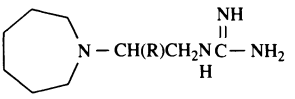
Levo **62** proved the most effective inhibitor of [^{3}H]NA uptake in rat heart among a large group of sympathomimetic agents (relative affinity, 1440; cf. levo NA 407, Table 4.17). The clinical agent Aramine is the *RS*-(−)-diastereoisomer (*Martindale* **29**, page 1468). Relative configurations are based on vicinal coupling (^3J) magnitudes, and absolute on ORD evidence.[165]



63 (*erythro*-isomer depicted)

The antihypertensive action of guanethidine **64**a appears to be related to its being a substrate for the amine pump and its displacement of NA from storage

sites. The chiral analogue **64b** reduced the NA content of mouse heart more effectively than the parent, and the antipodal potency ratio was 4(−):1(+) in this regard.[169]



(a) R = H; (b) R = OH

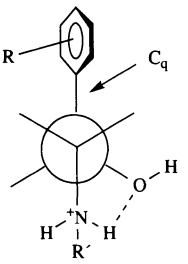
64

The stereochemistry of the α-agonist methoxamine **63** (Vasoxine) does not appear to have been reported.[166] The ^{1}H-NMR features of its 1-H (broad singlet near 3.8 ppm after decoupling Me signal) and 2-H (narrow doublet; $^3J \sim 4$ Hz near 5.16 ppm) signals (HCl in D_2O) are evidence of its *erythro* (*RS*/*SR*) configuration (cf. $^3J_{1,2}$ values of ephedrine and pseudoephedrine, p. 106; 400 MHz spectrum of HCl in D_2O recorded at Bath). Its α-agonist activity may well be a result of indirect action. In β-adrenoceptor preparations, however, it behaves as an antagonist. Patil *et al.*,[167] recorded the pA_2 values 6.25 and 4.37 for levo and dextro methoxamine, respectively, versus (−)-isoprenaline in GP tracheal muscle. Levo antipodes of the *N*-isopropyl (IMA) and *N*-*t*-butyl (butoxamine) analogues were also more effective than their dextro forms in this test.[168]

Finally, mention is made of 1-(phenylthio)-2-aminopropane (**16**, R = PhS), an analogue of amphetamine (**16a**). This compound has indirect sympathomimetic action and behaves as a potent antihypertensive agent as a result of the selectivity of its *S*-antipode for both the catecholamine reuptake transporter and the terminal enzyme of NA biosynthesis (dopamine β-monooxygenase).[168a] Antipodes were obtained by stereospecific synthesis utilizing chiral oxazolines.[168b]

4.12. Conformational Studies

Catecholamines and their relatives have been the subject of numerous conformational investigations by the three well-recognized techniques of X-ray crystallography (solid state), molecular orbital computations, and NMR spectroscopy. Work up to 1980 has been reviewed by Rutledge *et al.*[4] X-ray analysis of over 30 phenethylamines has shown that over 85% of the compounds (chiefly examined in salt form) have the nitrogen atom and aromatic substituent in an antiperiplanar conformation **65** (ArCq−C−C−N dihedral angle, τ_2, 180°) with evidence of a



65

weak H-bond between the OH and $^{+}NH_2R$ functions. Racemic phenylpropanolamine (HCl salt) exists in both antiperiplanar and synclinal ($\tau_2 = 60°$) conformations in the solid state—evidence for their near-equal energies.[170]

Most molecular orbital and empirical potential calculations applied to isolated molecules in vacuo (i.e., no interactions with solvent considered) lead to the conclusion that antiperiplanar conformers are preferred over synclinical forms, but only to small degrees. Thus Kier's group, using extended Huckel theory, calculated that rotamer **65** should be preferred for ephedrine, NA, adrenaline, and isoprenaline.[171,172] Pullman *et al*,[173] using the more refined technique of perturbative configuration of interaction localized orbitals (PCILO), likewise identified rotamer **65** as the preferred conformation in all cases, marginally so for dopamine (Table 4.18).

NMR methods of conformational analysis have the advantage of providing information of solute conformation under near-physiological conditions if D_2O (or H_2O with solvent suppression) is employed as solvent. The approach of Ison *et al*,[174] will be presented in some detail, as it illustrates the potential and limitations of the technique. The authors assumed that only the three staggered rotamers I–III (Table 4.20) of a phenylhydroxyethylamine contribute to the conformational equilibrium—all rapidly interconverting so that averaged NMR parameters are observed. If only two vinical (^3J) couplings operate, namely J_{trans} between protons separated by a 180° dihedral angle, J_{gauche} (60° dihedral angle), and the couplings between H_1 and H_3 of I and H_2 and H_3 of II can be identified by spectral analysis, the populations may be calculated from the following relationships:

$$p_{\mathrm{I}} = \frac{J_{1,3} - J_g}{J_t - J_g} \tag{1}$$

$$p_{\mathrm{II}} = \frac{J_{2,3} - J_g}{J_t - J_g} \tag{2}$$

$$p_{\mathrm{III}} = 1 - (p_{\mathrm{I}} + p_{\mathrm{II}}) \tag{3}$$

In the case of p_{I}, if $J_{1,3} = J_t$ (exactly) then $p_{\mathrm{I}} = 1$, and is the sole rotamer. If the populations of II and III are significant, overall $J_{1,3}$ will be made up of both J_t and J_g contributions and thus have a value below J_t. In the case of NA HCl, the ^3J

TABLE 4.18.
Rotamer Population Predicted by PCILO Calculations[173]

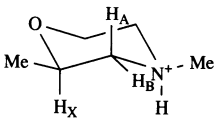
Compound	p_{I}	p_{II}	p_{III} (see Table 4.20)
NA	0	0.755	0.245
Adrenaline	0	0.955	0.045
β-Phenethylamine	0.290	0.420	0.290
Ephedrine	0	0.721	0.279
Norephedrine	0	0.959	0.041
Dopamine	0.345	0.352	0.303

values 9.05 and 3.35 Hz were derived by spectral analysis (see below). If the larger value applies to H_1, then I is preferred, but if to H_2 then II is preferred (if III were favored, both ^3J values would be small). A decision was made that the larger ^3J value applied to H_2 on steric grounds (non-bounded interactions between the bulky Ar and $H_2{}^+NR$ substituents are least when antiperiplanar is as in II) and consideration of the single ^3J value of the spectrum of ephedrine HCl. In 1*R*2*S*-(−)-ephedrine, H_2 of I is replaced by methyl. Hence the ^3J value must apply to the $H_1 - H_3$ coupling and its small value (4.4 Hz) is likewise evidence of a preferred antiperiplanar rotamer (II, H_2 replaced by Me).

4.13. Spectral Analysis

Spectra of all trisubstituted derivatives were of the ABX type ($CHCH_2$). They displayed 4-line X and multiplet AB signals from which approximations of J_{AB}, J_{AX}, J_{BX}, and ν_A–ν_B (chemical shift differences) could be obtained by inspection (spectra were recorded at 100 or 220 MHz—superior raw data may be derived from spectra recorded at higher frequencies; see the 400-MHz spectrum of NA salt, Fig. 4.2). These data were then refined by iterative fitting using appropriate computer programs. Some examples are shown in Table 4.19. Values of J_t and J_g in 1,2-disubstituted ethanes may be calculated on the basis of substituent electronegatives,[176] but since no comparable equations have been developed for trisubstituted ethanes data from substituted morpholines (taken as rigid-model compounds) were employed in the population calculations.

Values for *cis*-2,6-dimethylmorpholine HCl **66** (J_{AX} 11.2, J_{BX} 2.1 Hz) were chosen—these were derived from first-order treatment of the spectrum.[177]



66

Coupling data for *cis*- and *trans*-3-methyl-2-phenylmorpholine salts (^3J 10.52 and 2.78 Hz in D_2O; see ephedrine, Scheme 4.9) might have been more appropriate. Uncertainties in regard to J_t and J_g values together with the various assumptions

TABLE 4.19.
Coupling Constants in Catecholamines and Related Compounds (HCl Salts)[174]

	J_{AB}	J_{AX}	J_{BX}	$[\nu_A - \nu_B]$
NA	13.2	9.05	3.35	0.053
Adrenaline	12.8	9.15	3.65	0.01
Isoprenaline	12.8	9.65	3.15	0.014
Phenylephrine (3b) (*m*-synephrine)	12.8	9.45	3.55	0.023
β-Phenylethanolamine	13.2	9.70	3.00	0.075

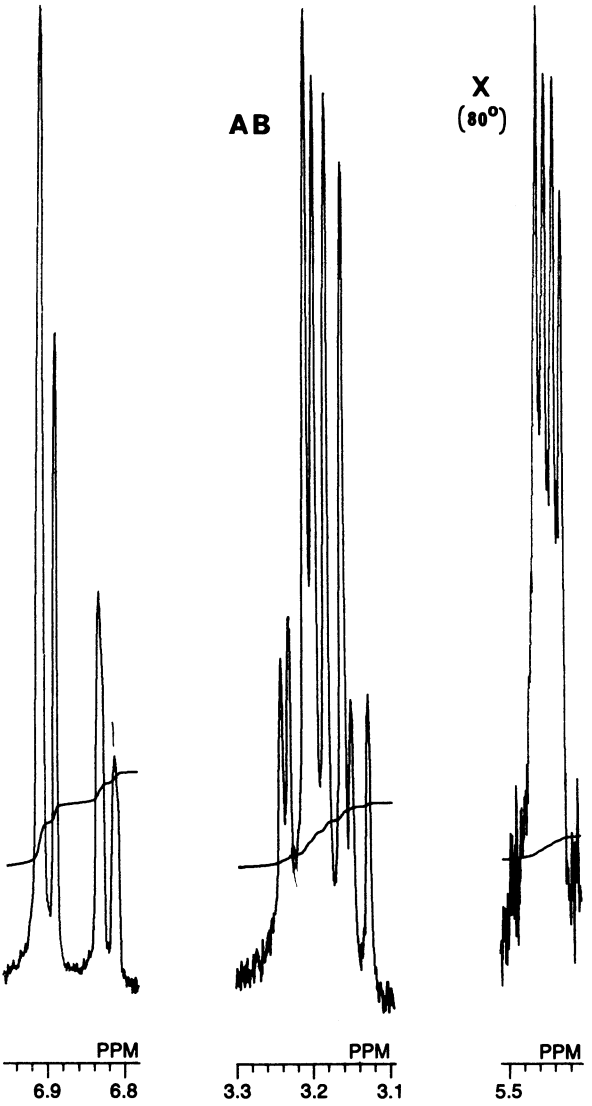
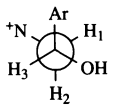


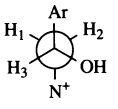
FIGURE 4.2. Part of the 400-MHz ^{1}H-NMR spectrum of noradrenaline (NA) hydrochloride in D_2O at ambient probe temperature (20° C) recorded at Bath. First-order analysis of the 8-line methylene proton signal AB(CHOHCH_2) gave δ_A 3.29, δ_B 3.125, ^2J 12.8 Hz, ^3J 4.3 and 8.5 Hz. Spectral parameters require computation because the $\Delta\delta$ value of 65 Hz still falls below 6 × ^2J (77 Hz) (Ref. 175). The methine signal (X) was obscured by the HDO resonance at 20° C; it was resolved as 4 lines centered near 5.46 ppm when the temperature was raised to 80° C.

made in the calculation procedure detract from the significance of reported population differences (Table 4.20), but the experiments do at least serve to identify the preferred rotamer and to establish an approximate idea of its population relative to other conformations. Conformational preferences are rather weak, in fact; a value of 0.8 for II represents an energy difference of only 0.83 kcal/mol for II compared to I and III. Electrostatic and/or hydrogen bonding interactions between OH and H_2N^+R substituents appear to be the chief factor for the conformational

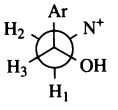
TABLE 4.20.
Rotamer Populations in Catecholamines and Related Compounds (HCl Salts in D_2O)[174]



I



II

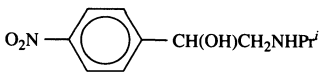


III

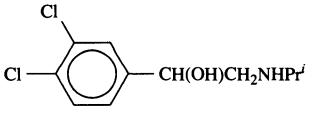
	p_I	p_{II}	p_{III}
Noradrenaline (NA)	0.14	0.76	0.16
Adrenaline	0.17	0.77	0.06
Isoprenaline	0.11	0.83	0.06
m-Synephrine (phenylephrine)	0.16	0.81	0.03
Amphetamine	0.45	0.50	0.05
Ephedrine	0.10	0.90 ($p_{II} + p_{III}$)	
Dopamine	0.43	0.57 ($p_{II} + p_{III}$)	

preference of II; cf. β-phenethylamine p_{II} 0.56, β-phenethanolamine p_{II} 0.84 (the low population of III is attributed to adverse steric interactions). Introduction of an α-methyl group raises the population of I in which α-methyl is *trans* to phenyl (cf. NA p_I 0.14, norephedrine p_I 0.21).

In a later study[178] conformational analyses for the β-blockers, INPEA **67** (and its NH_2 and NHMe analogues) and DCI **68**, were carried out by the same techniques. Corresponding 3-arylmorpholines **69** were employed as effectively rigid analogues from which model J_t and J_g values could be derived. To aid spectral analysis (carried out at 60–100 MHz) 5,5-dideutero derivatives of **69** were examined



67



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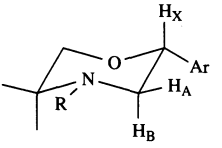


(a) R = Pr^i; (b) R = H; (c) R = Me

69

rather than the normal morpholines whose spectra displayed overlapping 3-CH_2 and 5-CH_2 resonances. Conformers of type II were identified as those of highest population for hydrochlorides of INPEA (88%) and DCI (77%). IR spectra of bases in CCl_4 at low concentrations (5×10^{-3} M or below to exclude intermolecular effects) showed a strong band near 3450 cm^{-1} and a weak band near 3620 cm^{-1}

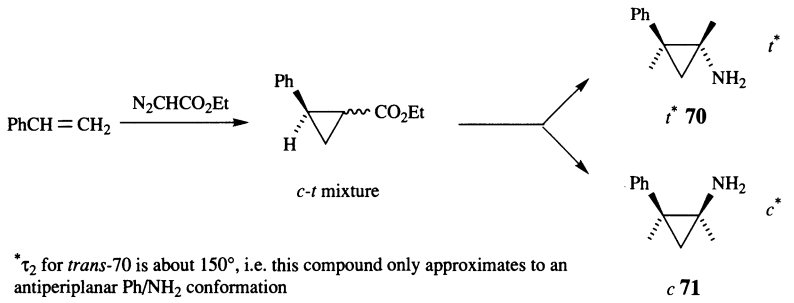
attributed to intramolecular H-bonded OH and free OH stretching, respectively, in support of conclusions based on NMR.

Gaggelli *et al.* reported an NMR ($^1H_1$ ^{13}C) study of isoprenaline HCl in DMSO-d_6—again the *t*-rotamer was deduced to be the favored form.[179]

4.14. Conformational Restraints

Despite much effort and ingenuity, little success has been achieved in the design of conformationally restricted analogues of sympathomimetic amines. Apart from the 1986 work of Macchia *et al.* (page 118),[180] no agent with significant direct action (agonist or antagonist) at adrenoceptors has been identified. Hence the data derived from analogues of this kind throw little light on the active conformation of adrenoceptor ligands. The object of conformational restraint is to design an analogue of a flexible ligand in which its pharmacodynamic functions are locked in a narrowly defined spatial relationship by use of a rigid framework of minimal additional bulk. Many of the restricted adrenergic agents reported provide molecules of well-defined geometry, but most include many extra atoms which clearly reduce the affinity of the ligand for its receptor. In only a few cases have catecholamine analogues (with 3,4-dihydroxyphenyl, *sec*-OH and NHR functions) been prepared—most products are related to ephedrine (Ar = Ph) and amphetamine (Ar = Ph, OH absent). A review covering work up to 1980 has been published[4] and only a selection of examples are presented here.

Small-ring systems which would be expected to result in the least interference with ligand affinity are cyclopropane (cf. work on acetylcholines, Chap. 8), cyclobutane, and azetidine, and all have been utilized in this work. The *trans*-isomer **70** (tranylcypromine) is a potent inhibitor of monoamine oxidase (MAO)[181] and of catecholamine uptake in cortex,[182] hypothalamus, and striatum[183] while the *cis*-isomer **71** is far less potent in these regards. Relative



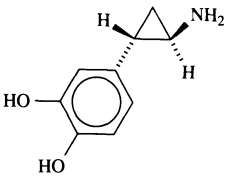
*τ_2 for *trans*-70 is about 150°, i.e. this compound only approximates to an antiperiplanar Ph/NH_2 conformation

potencies for inhibition of MAO were: *rac-trans* 1.0, (+)-*trans* 1.2, (−)-*trans* 0.3, *rac-cis* 0.4.[184] Configurational assignments were based on ozonolysis of precursor carboxylic acids to 1,2-cyclopropane dicarboxylic acids of known geometry (the absolute configuration of dextro **70** was later reported as 1*S*,2*R*).[185]

In antipodal comparisons of *trans*-**70**, the dextro isomer proved the more potent inhibitor of MAO (*in vitro* 10- to 16-fold),[186] but was somewhat less effective

in blocking the uptake of [^{3}H]NA into synaptosomes of rat hypothalamus [ID_{50}(+) 1.0–1.5 × 10^{-6}, (−) 4.5 × 10^{-7}].[183] The fact that (+)-**70** was more effective than its antipode in preventing reserpine-induced sedation in rats was therefore attributed to its influence on MAO.[186] In a human pharmacokinetic study[187] plasma and urine levels of (−)-**70** exceeded those of the (+)-isomer by severalfold. Levels of both isomers were elevated after equivalent oral dosage of the racemic mixture—evidence of mutal inhibition of hepatic biotransformation. Chiral analysis involved derivatization with (+)-benoxaprofen chloride and chromatography on silica gel.

The *trans*-dopamine analogue **72** was made by a stereospecific route

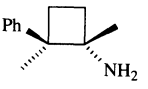


72

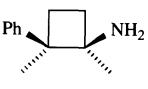
(Scheme 4.10) while the tranylcypromine procedure gave a *c*/*t* mixture (separation achieved at CO_2Et stage).[188] The ^{1}H-NMR features of *trans*-**72** were similar to those of tranylcypromine. According to Borne *et al*,[190] coupling constant data may be employed to assign stereochemistry ($^3J_{cis} > {}^3J_{trans}$ for substituted cyclopropanes, Ref. 189). However, the ^{1}H-NMR spectrum of *trans* (tranylcypromine) **70** is complex even at 400 MHz (HCl in D_2O) and difficult to analyze (Bath result).

Trans-72 and its isomer were devoid of dopaminergic activity (renal vasodilation) but had weak α-adrenergic activity (rabbit aorta, *trans* 4–5 × *cis* but 1/50 × phenylephrine). The compounds were also weak stimulants of cardiac muscle (*t* 15 × *c*) but with indirect action.

Amphetamine analogues **73** and **74** based on cyclobutane did not inhibit



73



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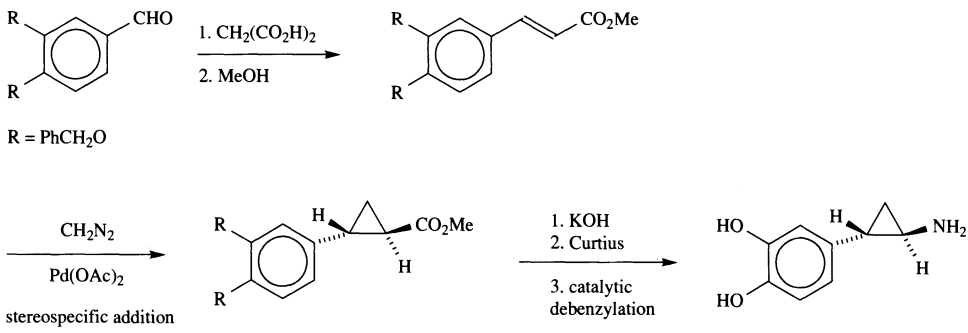
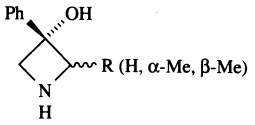
MAO; both inhibited uptake of NA into cortex, the *trans*-isomer being the more effective but much less so than either antipode of amphetamine itself.[191] The



Scheme 4.10. Synthesis of the *trans*-cyclopropane analogue of dopamine.[188]

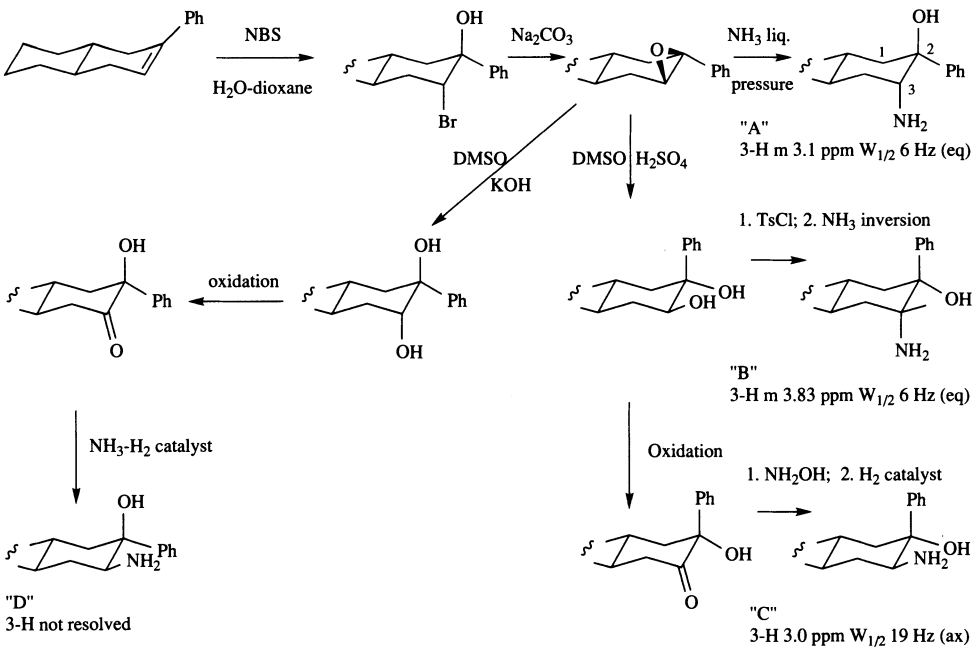
azetidines **75** (ephedrine analogues) all inhibited NA uptake into rat vas deferens, but were far less potent than tranylcypromine in this assay.[192]



75

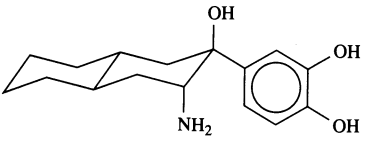
Smissman's group made much use of *trans*-decalin as a rigid framework on which to hang pharmacophoric groups (see also Chapter 8). The chemistry involved in the preparation of the four racemic norephedrine analogues is shown in Scheme 4.11.[193] Geometries were confirmed by the NMR characteristics of methine proton signals. In the rat vas deferens (α-sites) all racemic mixtures gave a response equivalent to that of NA but at 100-fold greater dose levels (the direct component of action was not defined). In the corresponding N-isopropyl set, the eq Ph, eq NHR member "D" of Scheme 4.11, NHPr^i, with no agonist response itself) potentiated NA in rat and GP vas deferens, i.e., inhibited NA uptake into storage sites. Activities of the others fell off in the order eq Ar, ax N ("A" of Scheme 4.11) > ax Ar, eq N ("C") > ax Ar, ax N ("B") (all N-isoPr derivatives).[194]

The NA analogues **76** were examined for their ability to inhibit uptake of dopamine into synaptosomes from striatum and hypothalamus, and rate of O-methylation by catechol-O-methyltransferase.[195] The geometry **76** (eg Ar, ax NH_2) best fitted the active site of COMT, since this isomer was methylated 11.3

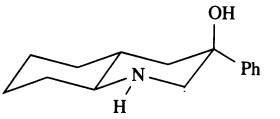


Scheme 4.11. Synthesis of the four diastereoisomers of 3-amino-2-hydroxy-2-phenyl-*t*-decalin.[193] $W_{1/2}$ = width at half maximum height.

times as fast as levo NA; rates for the other isomers were "D" 3.07, "B" 0.47, and "C" 0.45 (see Scheme 4.11, Ph replaced by 3,4-dihydroxyphenyl). Similar studies were made of dopamine analogues (2-OH of **76** replaced by H)—in this case only the ax Ar, ax NH_2 isomer exceeded the methylation rate of dopamine.[196] Analogues of ephedrine based on decahydroquinoline were inactive as α-agonists (rat VD); the antiperiplanar NH, Ph isomer **77** was more effective than its epimer in potentiating NA in the vas.[197]



76 (and diastereoisomers)



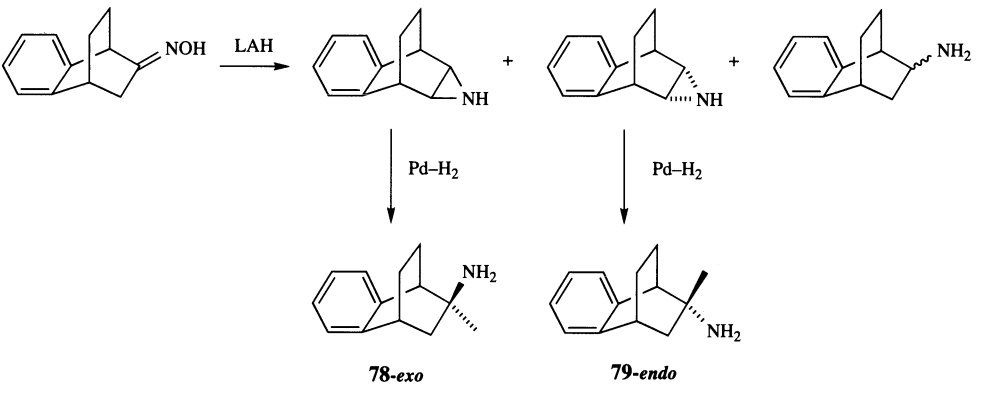
77

Bicyclic ring systems have also been employed in the design of restrained analogues of phenethylamines.

The 2-*endo*-derivative **78** (see Scheme 4.12) places the nitrogen atom synclinal ($\tau_2 = 60°$) to the aromatic ring while the 2-*exo*-isomer **79** mimics the antiperiplanar conformation. Both amphetamine analogues **78** and **79** showed indirect activity in the rat vas deferens with potencies 3×10^{-4}M for *endo*- and 3×10^{-5}M for *exo*-isomers,[198] while *exo*-compounds were also more potent in potentiating NA in reserpinized vas.[199] Regarding inhibition of NA uptake in cortex and dopamine in corpus striatum, *exo*-compounds **78** (NH_2, NHMe) were almost as potent as amphetamine, while *endo*-epimers were much less effective.[200] These results provide good evidence that the antiperiplanar rotamer of amphetamine is the active form at adrenergic sites concerned with catecholamine uptake and release.

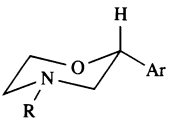
The compounds were made by the method of Kitahonoki[201] as shown in Scheme 4.12; the isomer with the lower-field NR (R = H or Me) resonance was assumed to be the *endo*-isomer.

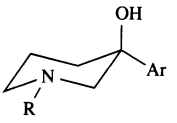
An Italian group has made much use of the cyclic systems morpholine and piperidine as frameworks for restrained adrenergic ligands and achieved notable

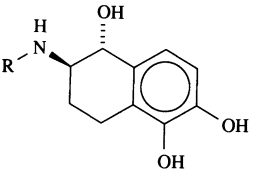


Scheme 4.12. Synthesis of amphetamine analogues based on benzobicyclo[2,2,2]octene.[201]

success in regard to agonists.[180] The data of Table 4.21 includes results for the morpholines **80**, 3-piperidinols **81**, and tetrahydronaphthalenes **82**. In β-receptor assays the 3-piperidinols and tetrahydronaphthalenes approached or exceeded pD_2 values of the acyclic parents (NA and isoprenaline). Results were rationalized in terms of a common antiperiplanar $^+$NHR/Ar–synclinal $^+$NHR/OH conformation







(a) R = H; (b) R = Pr^i; Ar = 3,4-diOHC$_6$H$_3$

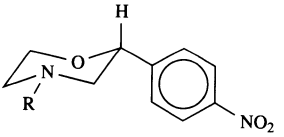
80 **81** **82**

important for uptake at the receptor (Fig. 4.3) (see Ref. 226 for recent revision). In α-assays, the morpholine **80**a approached the pD_2 of NA and was superior to the piperidinol **81**a, e.g., rat vas deferens pD_2NA was 4.96, **80**a 4.75, and **81**a 4.53.

The N-isopropyl derivatives **80**b and **81**b were inactive in this test as were also the tetrahydronaphthalenes (cf. Ref. 202).

If **82**a (NH_2), in addition to **82**b (NHPr^i), is regarded as an analogue of isoetharine (page 96) rather than NA (the 3,4-dimethylene chain constitutes a bulky feature close to nitrogen), then its failure as an α-agonist may be understood. Preferred conformations of the morpholines **80** were established from the ^3J values of the proton α to the aryl group, and those of the 4-piperidinols **81** from IR evidence of an intramolecular OH·····N hydrogen bond.[203]

The morpholines **83**a–b, analogues of the β-blocking agent INPEA (page 140)



(a) R=H; (b) R = Me; (c) R = Pr^i

83

TABLE 4.21.
β-Adrenergic Activities of Some Constrained Analogues of NA and Isoprenaline[180]

	Isolated guinea-pig atria		Isolated guinea-pig tracheal strip	
	pD_2	ia[a]	pD_2	ia
NA	6.50	1	6.04	1
Morpholine **80**a (NH)	5.31	0.91	4.76	0.70
Piperidine **81**a (NH)	6.75	1	4.20	0.77
Tetrahydronaphtalene **82**a (NH_2)[b]	6.74	1	7.49	1
Isoprenaline	8.35	1	8.47	1
Morpholine **80**b (NPr^i)	5.74	0.88	6.34	0.93
Piperidine **81**b (NPr^i)	8.14	1	8.26	1
Tetrahydronaphthalene **82**b (NHPr^i)	7.56	1	8.41	1

[a] Intrinsic activity.
[b] The *trans*-NHMe analogue (pD_2 8.01 trachea; 7.55 atrium) was about 10 times more active than the corresponding *cis*-diastereoisomer (pD_2 7.06 trachea; 6.34 atria).[202]

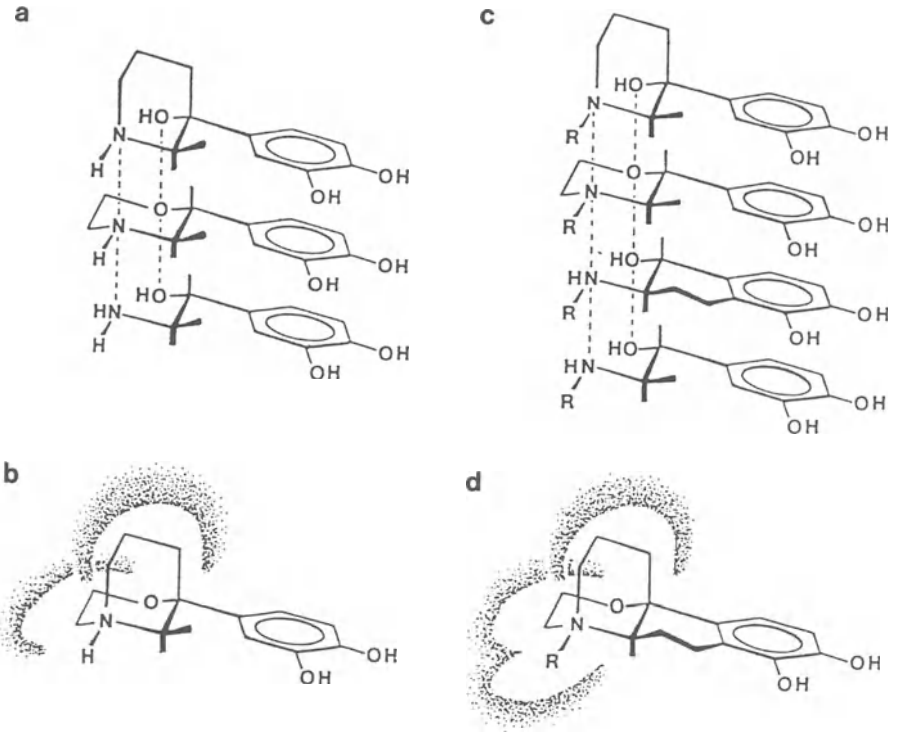


FIGURE 4.3. Molecular models (b and d) arising from the superimposition of the pharmacophoric groups (aryl moiety, amine nitrogen, and alcoholic or ethereal benzylic oxygen) of the eutomers **80** – **82**, NA, and isoprenaline depicted in the conformations in which they are presumed to bind to α- and β-receptors, respectively (after Macchia *et al.*).[180]

were ineffective as β-antagonists; they had weak α-adrenergic stimulant properties and potentiated NA.[204] These results point to the need for an $^{+}NH_2$ group in N-isopropyl antagonists.

4.15. Binding Experiments and Influence of Adrenoceptor Ligands on Adenylate Cyclase

During the 1970s several groups studied the binding of [^{3}H]-(−)-NA to microsomal preparations isolated from rat liver, heart, spleen, and fat cells and other tissues, and its displacement by various agents. Antipodal forms of NA and adrenaline were found to compete equally well; thus specifically bound [^{3}H]-(−)-NA was reduced from 17,700 to 9200 cpm by levo, and 9000 cpm by dextro NA both at a 0.5 μg/ml concentration.[205] Evidence was obtained that measurements related to ligand uptake at enzyme COMT sites rather than adrenoceptors themselves, which are probably sparsely populated in these preparations. Axelrod had previously noted that COMT does not distinguish between (−)- and (+)-adrenaline.[206] In functional tests (lipolytic activity and stimulation of adenylate cyclase in isolated fat cells or microsomes), (−)-NA proved far more effective than

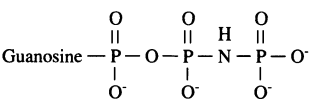
TABLE 4.22.
Binding of β-Adrenoceptor Antagonists

Compound			Reference
	Concentration (M) for 50% inhibition of [^{3}H]-(−)-alprenolol binding to frog erythrocyte membranes		209
(−)-Alprenolol	2×10^{-9}		
(+)-Alprenolol	3×10^{-7}		
(−)-Propranolol	3×10^{-9}		
(+)-Propranolol	3×10^{-7}		
	K_i(M) *vs* [^{127}I]HYP[a]		
(−)-Propanolol	2.1×10^{-9}		208
(+)-Propranolol	3.7×10^{-7}		
(turkey erythrocytes)			
	lung	heart	
(−)-Propranolol	5×10^{-10}	3×10^{-10}	210, 211
(+)-Propranolol	3×10^{-8}	3.9×10^{-8}	
rat vs. [^{3}H]-dihydro-alprenolol			

[a] HYP = hydroxylbenzylpindolol.

its antipode. Binding of NA and isoprenaline to turkey erythrocyte ghosts also lacked stereospecificity and only required the catechol moiety.[207] Brown[208] using [^{127}I]hydroxybenzylpindolol (HYP), a radioligand of high specific activity, was able to detect significant degrees of stereoselectivity in the binding of antipodal adrenalines (×30) and soterenols (×26) (for the formula see Table 4.4) to turkey erythrocytes. Binding experiments performed with β-adrenergic antagonists invariably reveal pronounced degrees of stereoselectivity (Table 4.22).

Miller *et al.*,[212] working with rat striatal homogenates, also found (−)-NA to be superior to its antipode in stimulating *c*-AMP production, while Kaumann[213] reported that both isomers of isoprenaline enhanced adenylate cyclase activity with (−)-40 to 100×(+) in cell-free membrane particles from kitten heart. However, Lefkowitz[214] observed no difference in the stimulant properties of (+)- and (−)-adrenaline, a result which correlated with their equal ability to compete with [^{3}H]-(−)-NA for binding sites on subcellular fractions of cardiac muscle (cf. Cuatrecasas[205]). Brown *et al*,[208] measured molar K_D values for adenylate cyclase stimulation in turkey erthrocytes of 9.6×10^{-6} for (−)- and 1.3×10^{-4} for (+)-adrenaline, obtained in the presence of 10^{-6}M GppNHp **84**, a hydrolysis-resistant analogue of GTP which activates G proteins (such proteins form part of the ATP

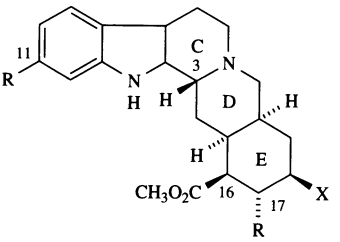


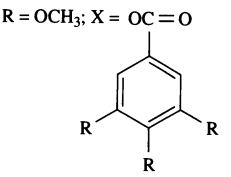
c-AMP reaction sequence).[215] At the higher GppNHp concentration of 10^{-5} M, (−)-adrenaline was 25 times more effective than its antipode while (−)- was only a fewfold times superior to (+)-soterenol. In kitten atria levo isomers of propranolol, oxprenolol, and KL 255 ($2MeC_6H_4OCH_2CHOHCH_2NH$-*i*-PR) blocked the adenylate cyclase and chronotropic effects of (−)-isoprenaline 50–300 times more effectively than corresponding (+)-antipodes.[213]

In view of the established role of *c*-AMP in the effector pathways of both α_2- and β-adrenoceptors, knowledge that the enzyme adenylyl cyclase has now been cloned and its primary amino acid sequence determined is of considerable significance in regard to the work described here.[216]

Appendix: Reserpine

This alkaloid, much employed as a pharmacological tool for depleting NA, DA, and 5-HT neurones of their transmitter amines and enjoying clinical use as an antihypertensive agent during the 1950–1960s (*Martindale* **29**, page 498), has the structure and absolute configuration **85**. Deserpine differs from reserpine only in





85

the absence of the 11-methoxy substituent, and has the same actions as the methoxy derivatives.[217] The stereochemistry of reserpine differs from the yohimbine group (Chapter 5, page 135) in having a β-H at C-3, i.e., rings C and D are *cis*-fused. It shares a *cis*-D/E ring junction with that of α- and allo-yohimbine (page 00). Geometry about C-16 and C-17 is the same as that of α-yohimbine.

Elucidation of the overall structure and relative stereochemistry of reserpine required an immense amount of work[218] culminating in the stereospecific total synthesis of Woodward.[219] An X-ray study[220] has confirmed the relative geometry of the alkaloid. The absolute configuration shown (**85**) is based on optical rotational evidence[221–223] and application of Prelog's asymmetric synthesis to methyl reserpate.[224]

According to Schlittler[225] stereoisomers of reserpine derived form methyl isoreserpate (3-epi), 16-epireserpate, 16-epi-17-epi-reserpate, 18-epireserpate, and 18-epi-isoreserpate all lacked the pharmacological properties of the parent alkaloid.

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5

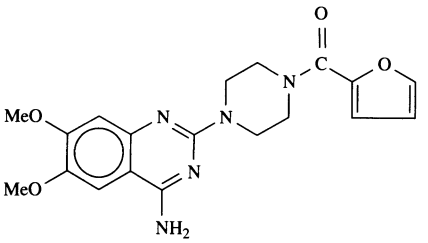
Adrenoceptor Antagonists

5.1. Introduction

This chapter is devoted to ligands which block adrenoceptors. Although some agents of this kind have already been mentioned in the preceding chapter as a result of the often quite close structural resemblances between agonists and antagonists, the amount of published material on antagonists warrants their separate discussion. Adrenoceptor antagonists fall into two classes, namely α- and β-blocking agents, a fact which in itself adds credence to the overall division of adrenoceptors into these two principal subtypes.

5.2. α-Antagonists[1]

Agents of this class counteract the actions of well-characterized α-adrenoceptor agents such as phenylephrine (metasynephrine) and clonidine, which are α_1- and α_2-selective agonists, respectively. Prazosin (**1**), the standard α-blocking agent, is achiral.

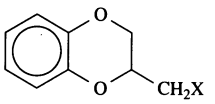


1

5.3. Benzodioxans

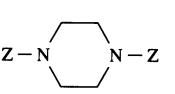
Discovery of the adrenergic blocking properties of 2-aminoalkyl-1,4-benzodioxans (**2**) goes back to the 1930s.[2] Agents such as prosympal **2**a, piperoxan **2**b, and dibozane **3** were subsequently established as valuable α-adrenoceptor

antagonists which acted competitively.[3] Nelson *et al.*,[4] established the absolute configurations of the resolved benzodioxans **2–3** and compared their pA_2 values in preventing methoxamine-induced contractions of rabbit aortic strips (methoxamine is an α-agonists, see page 109) (Table 5.1).



(a) X = NEt_2; (b) X = $N(CH_2)_5$

2



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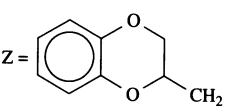
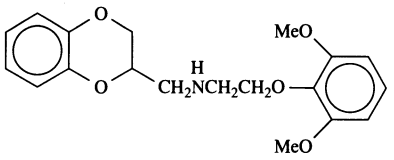


In all three sets an *S*-eutomer was identified, although eudismic ratios were modest (likewise in binding experiments). In a later paper[6] antipodes of WB-4101 (**4**) were examined in the same manner. The *rac*-material is a potent α-antagonist, more active at postsynaptic (pA_2 9.8) than presynaptic sites (pA_2 6.24).[7] An *S*/*R* potency ratio of 47 was obtained in the aortic strip assay (pA_2 *S*-**4** 9.02, *R*-**4** 7.35, *rac*-**4** 8.83). See Ref. 144 for a 1992 study.



4 WB-4101 (Benoxathian, O-4 replaced by S)

Chemistry. 2*R*-Tosyloxy-1,2-propanediol acetonide (**5**) (from mannitol, see page 150) was converted in several steps to the ditosylate **6** which sufferred intramolecular ring closure with inversion at C-2 when treated with NaOMe in ethanol to provide 2*R*-**7**. This dioxan was then converted to the desired 2-*S*-amino-

TABLE 5.1.
Competitive Antagonist Effects of Antipodal Pairs of Benzodioxans on Methoxamine-Induced Contractions of Rabbit Aortic Strips[4]

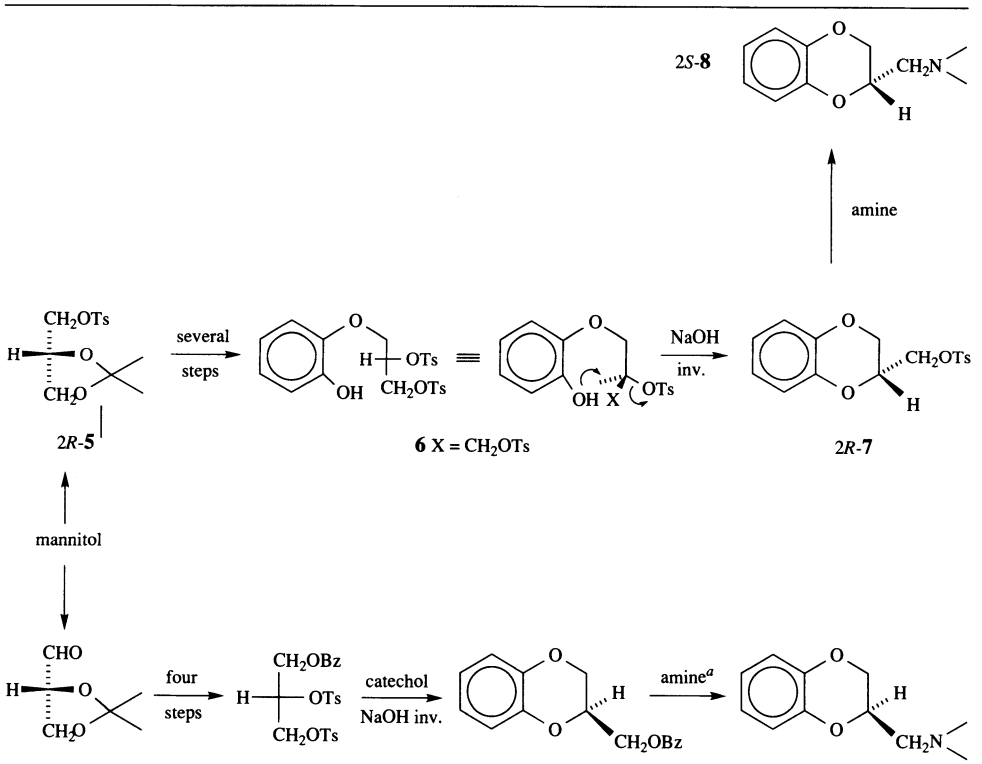
Compound	pA_2	*S*/*R* ratio	$-\log K_D$[a]
S-prosympal **2a**	5.60	2.2	6.21
R-prosympal **2a**	5.20		
S-piperoxan **2b**	7.12	18.2	6.51
R-piperoxan **2b**	5.86		5.99
SS-dibozane **3**	7.66	19.1	6.85
RR-dibozane **3**	6.38		6.25
meso-dibozane **3**	7.53		

[a] Affinity for [^{3}H]dihydroazepetine binding sites of α-adrenoceptor related protein from rat vas deferens.[5]

methyl derivatives **8** by displacement of OTs by the appropriate secondary amine (Scheme 5.1). 2-*R*-antipodes of **8** were obtained form *R*-glyceraldehyde-2,3-acetonide as shown in the Scheme. The dibozane enantiomers were obtained from 2*S*- and 2*R*-**8** [2-$CH_2N(CH_2)_4NH$] by reaction with the 2*S*- and 2*R*-tosylates **7**. Reaction between 2*R*-**7** (tosylate) and 2*R*-**8** (piperazine) provided the *meso*-isomer of **3**. Evidence of optical purity was provided by CD spectra and NMR spectra recorded in the presence of a chiral shift reagent (THFC-Eu; the NCH_2Me signal of *rac*-**2**a was resolved into two triplets at 60 MHz).

The *R*- and *S*-intermediates **7** (2-CH_2OTs) were also employed to prepare antipodes of WB-4101 (**4**). First the tosyloxy group was displaced by potassium phthalimide followed by hydrazinolysis of the products to give the corresponding primary amines (2-CH_2NH_2). Reaction of the amines with 2,6-dimethoxyphenoxyacetaldehyde followed by reduction of the intermediate imines with $NaBH_4$ gave the desired products.

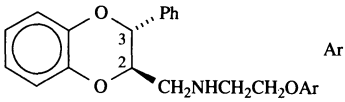
A direct comparison between the stereochemistries of *R*-adrenaline and the *S*-benzodioxans is not possible. Nevertheless, the authors have presented space-filling CPK models of 2*S*-**2**b (piperoxan) and *R*-adrenaline which share similar spacial relationships among amine, oxygen, and aromatic features, not observed between adrenaline and 2*R*-**2**b.



Scheme 5.1. Synthesis of antipodal benzodioxans.

Benoxathian, an analogue of WB-4101 (**4**) with O-4 replaced by sulfur, proved more potent that its parent and prazosin as an α_1-antagonist with a greater α_1/α_2 selectivity ratio.[8] Antipodal potency ratios in tests on the blockade of α_1-sites of rat vas deferens (*vs* NA) were 9.55 for those of benoxathian and 17.8 for those of WB-4101, with levo isomers the eutomers in both cases.[9] Optical purities of the benoxathian isomers were judged to be >90% by use of the chiral shift reagent $Eu(TFC)_3$; absolute geometries were not assigned.

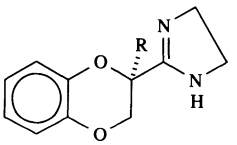
Insertion of phenyl *trans* to the C-2 substituent of WB-4101 (**4**), giving **8a**, reduced its α-blocking activity($\alpha_1 \times 5$, $\alpha_2 \times 150$) but raised the α_1/α_2 selectivity ratio from 794 to 23442[10]; *cis*-insertion depressed affinity and selectivity at all α-sites. The analogue of **8a** (Phendioxan) with O-1 replaced by C=O was also a potent



8a (one antipode shown)

α_1-blocker with an α_1/α_2 selectivity ratio (2630) likewise higher than that of the parent. Compounds were examined as racemic mixtures, and relative stereochemistry was based on ^3J coupling magnitudes between H-2 and H-3 (7.09 Hz for *t*, 2.51 Hz for *c*).

The benzodioxan idazoxan **9**a, which incorporates a 2-imidazolyl substituent



(a) R = H; (b) R = OMe; (c) R = Me; (d) R = MeC=CH_2

9

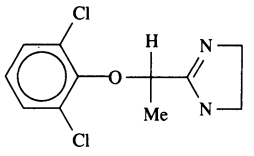
TABLE 5.2.
***In Vitro* Pharmacology and Binding Studies of Antipodal Idazoxan Derivatives[13]**

		Prejunct α_2 rat vas deferens		Postjunct α_1 rat anococcygeus		Receptor binding[c]	
R in **9**	Isomer	Antag pot[a]	Eudismic ratio	Antag pot[b]	Eudismic ratio	K_i(nM) [^{3}H]idazoxan	K_i(nM) [^{3}H]prazosin
OMe	(+)	15.5	419	16.0	> 160	0.36	27
OMe	(−)	0.037		< 0.1		256	67,863
Me	(+)	1.3	260	0.09	> 3.9	2.4	285
Me	(−)	0.005		< 0.023		487	13,329
MeC=CH_2	(+)	0.0003	2666	0.03	20	8734	46,439
MeC=CH_2	(−)	0.8		0.6		8.0	1,473

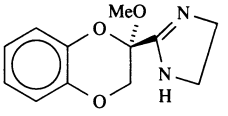
[a] vs. clonidine, relative to idazoxan.
[b] vs. phenylephrine, relative to idazoxan.
[c] Ability of test compound to displace radioligand from saturable binding sites of rat brain cerebral cortex.

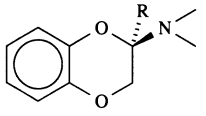
as present in tolazoline and related compounds (see page 101), is a selective α-2 antagonist of high potency.[11] In the rat vas deferens (−)-idazoxan was found to be only three times more potent than the (+)-isomer in antagonizing the clonidine-induced inhibitions of electrically induced contractions.[12] The discovery of a larger potency separation with idazoxan isomers against α_2-mediated sedation in chicks has led to the suggestion that central α_2-adrenoceptors may be more stereoselective than peripheral receptors. However, a Reckitt and Colman group (who found eudismic ratios of high magnitude for antipodes of **9** with R ≠ H, see below) considers that partial racemization of isomers of idazoxan may contribute to the low eudismic ratios observed, and has provided evidence for the lability of the proton at C-2 of **9**a.[13] Some data related to their own work on antipodal idazoxan derivatives are shown in Table 5.2. The results demonstrate a marked selectivity for α_2-adrenoceptors, and at these sites eudismic ratios range from 260 to 2666. The binding data concur, as do *in vivo* screening results (reversal of inhibitory effects of clonidine in the vas deferens of the pithed rat).

In central α_2-tests (hypotension and mydriasis) eudismic ratios for **9**b remained high (559 and 918, respectivity). In general, eudismic ratios reported for chiral imidazoline *agonists* are low and rarely exceed 10–20-fold (Chapter 4, page 101). Values up to 30 have been reported for lofexidine (**10**).[14] The absolute configuration of the eutomer (+)-**9**b was established as *S* by X-ray crystallography,[15] a result which correlates with the absolute geometry of WB-4101 and its analogues (see **11**). The antipodes (+)-**9**c and (+)-**9**d are probably related in configuration to (+)-**9**b.



10 Lofexidine



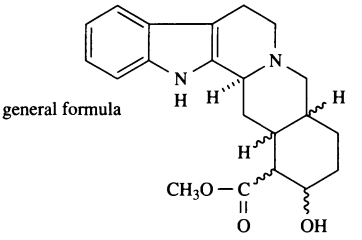


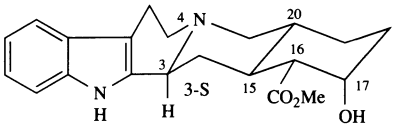
11

5.4. Yohimbine and Its Stereoisomers and Analogues

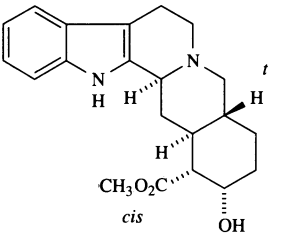
Indole alkaloids of the yohimbine group are of special interest in regard to the blockade of α_2-adrenoceptor sites and reviews are available.[16,17] Yohimbine (**12**, Scheme 5.2) was first recognized as more potent in antagonizing the prejunctional than the postjunctional α-adrenoceptor of the rabbit pulmonary artery in 1975[18] and has since been used as a pharmacological tool in the investigation of α_2-adrenoceptors. The selectivity of yohimbine for α_2-sites is shared by two of its stereoisomers (all of established stereochemistry, see below) and is most pronounced for rauwolscine (**13**, α-yohimbine) as judged from ligand binding studies. In the data of Table 5.3 [^{3}H]yohimbine was used to label α_2-adrenoceptors in human platelets and [^{3}H]prazosin to label α_1-receptors in rat liver membranes. The same order of α_2/α_1 ratios for the first three alkaloids was found in tests on calf cerebral cortex membranes.[20]

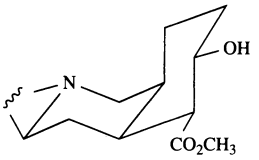
Functional assays confirm the relative selectivities of the first three members of Table 5.3, e.g., antagonism of the central hypotensive response to clonidine



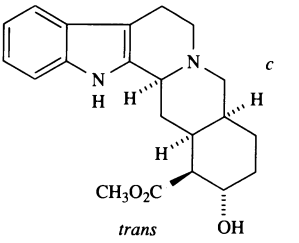


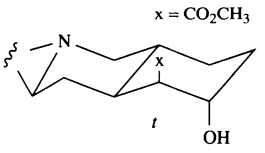
12 yohimbine



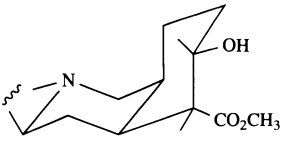


13 rauwolscine (α-yohimbine)





14 corynanthine



15 alloyohimbine

Scheme 5.2. Stereochemistry of yohimbine and some of its diastereomers.

TABLE 5.3. α-Adrenoceptor Binding for Yohimbine and Related Compounds[19]

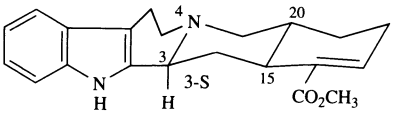
Compound	Binding K_i (nM)		
	α_1 [a]	α_2 [b]	α_2/α_1
Yohimbine	127	2	63.5
Rauwolscine	336	3	112
Corynanthine	20	557	0.036
Alloyohimbine	280	6	46.6

[a] vs. [^{3}H]prazosin (rat liver membranes).
[b] vs. [^{3}H]yohimbine (human platelets).

(α_2-agonist) in cats[21] and effects on NA transmission in the pulmonary artery of the rabbit.[22]

The four isomeric yohimbines differ in the stereochemistry of their terminal decahydroisoquinoline nuclei (see Scheme 5.2, and discussion below). It appears that both *trans*- and *cis*-ring fusion across position 15 and 20, as in yohimbine (**12**) and rauwolscine (**13**), respectively, provide ligands of high α_2-affinity. Affinity for α_2-sites is maintained in alloyohombine (**15**, *cis*-ring juncture) but not in corynanthine (**14**, *trans*-ring juncture) which differs from yohimbine in the β-orientation (axial and *trans* to α-OH at C-17) of its C-16 CO_2Me function—an arrangement which confers α_1-affinity instead. In the three α_2-selective ligands, the indole nucleus, N-4, and 16-CO_2Me all lie approximately in the same plane.

Apoyohimbine **16**, in which the methoxycarbonyl group is in a position intermediate between yohimbine and corynanthine, binds tightly to both types of α-adrenoceptor.[20]



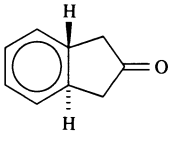
16 apoyohimbine

Binding K_i (nM)[20]	
α_1	20
α_2	14
α_2/α_1	1.4

The α_2-affinity of yohimbine is depressed when the C-16 CO_2Me is reduced or removed[20] but little changed when the α-C-17 hydroxyl is absent (in apoyohimbine it is enhanced in fact) or moved to a β-orientation as in β-yohimbine.[23] An α-hydroxyl at C-17 may impede binding to α_1-sites by repulsive steric interactions.

5.5. Stereochemistry of Yohimbine and Its Stereoisomers

Extensive investigations of the stereochemistry of the yohimbine group of alkaloids have been carried out.[24] Thus evidence of a *trans*-D/E ring junction for yohimbine was obtained by synthesis of the parent skeleton yohimbane **18** from *trans*-hydrindan-2-one **17** while use of *cis*-**17** led to the parent nucleus of alloyohimbane.[25,26] The fact of corynanthine being a C-16 epimer of yohimbine followed



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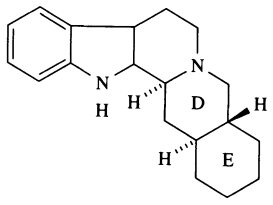
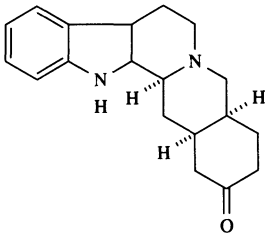


rac-**18**

from its conversion to the latter after saponification and re-esterification. The C-16 CO_2Me group was deduced to be equatorial in the more stable epimer, yohimbine.[27]

Alloyohimbine and rauwolscine are also related as epimers, since each yields alloyohimbone **19** after Oppenauer oxidation.[28,29] The fact of a configurational difference about C-16 rather than C-17 for this pair was not readily established. The 17-hydroxy group in rauwolscine must be equatorial, since treatment of this isomer with *p*-toluenesulfonyl chloride yields a quaternary tosylate **20** whose formation requires displacement of an α-OTs group.



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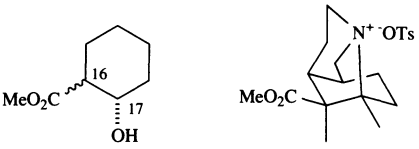
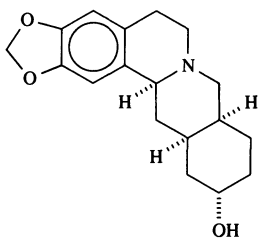


20 (partial structures)

The absolute configuration of natural (+)-yohimbine originally based on molecular rotational evidence and application of Prelog's method (C-17 geometry),[24,30] has now been confirmed as 3α, 15α, 20β with a 3*S* chiral center as shown in structure **12**, by X-ray crystallography.[31]

Several variants of yohimbine with α_2-blocking activity have been reported and are of stereochemical interest. All retain the quinolizidine fragment of the parent nucleus. The methylenedioxy derivative **21** (CH-38083) is a potent and selective α_2-antagonist in spite of lacking the carbomethoxy substituent of yohimbine.[32] Several stereoisomer of **21** (identical in relative configuration to that of



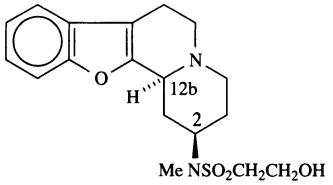
1657 x lower (rat vas deferens) and 933 x lower (rabbit pulmonary artery) affinities for postjuctional α_1-receptors than α_2-receptors (rat/mouse vd and GPI)[32]

21 CH-38083

rauwolscine) have been reported—all of lower potency. Antipodes of **21** (details of preparation unreported) were equipotent in increasing the electrically induced release of [^{3}H]NA.[142] However, the action of exogenous NA in decreasing this release was antagonized more effectively by (−)-**21** than by its antipode (K_B values levo 14.29, dextro 97.18 nmol/l). These results provide evidence that two stereochemically different α-2 receptors modulate NA release in rat cerebral cortex.

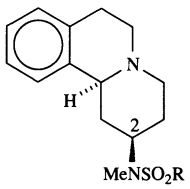
In the case of the hexohydrobenzofuroquinolizine **22**, the racemic mixture exhibited a 150-fold preference for [^{3}H]-clonidine over [^{3}H]prazosin sites in calf cerebral cortex and was effective in α_2-functional blockade tests.[33] The 2*R*, 12b*S* antipode was twice as potent as *rac*-**22** in these tests while the 2*S*, 12b*R* form dis-

played little or no activity.[33,34] Configurational relationships of the eutomer **22** with yohimbine and rauwolscine are again evident; these were established by X-ray methods.



22 L-654284 (2*R*, 12b*S* eutomer shown)

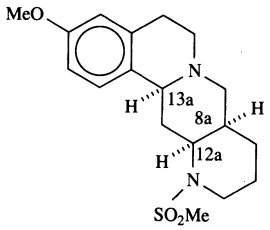
Benzoquinolizines of the same relative geometry to L-65484 have also been described[35–37] several of which had high α_2/α_1 activity ratios in isolated tissue and binding experiments, e.g., **23c** (WY26703) 93 form pA_2 values, 212 from binding in rat cortical membranes using [^{3}H]rauwolscine and [^{3}H]prazosin. Racemic mixtures were used in these tests; evidence of ring junction stereochemistry was provided by observation of strong Bohlman bands in IR spectra,[38] and 2-NMeSO_2R orientation by ^{1}H-NMR features of the 2-H proton resonance (higher field when axial as in **23**).



(a) R = Me; (b) R = Pr^n; (c) R = Bu^i (WY 26703)

23

A Syntex group have reported a hybrid of rauwolscine and WY26703 (**23c**), namely, the naphthyridine **24**.[39]

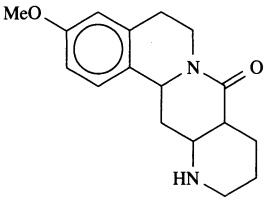


24

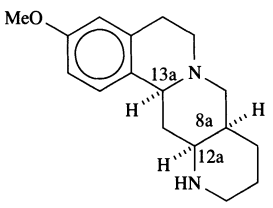
vs.	Ligand binding pK_i [^{3}H]prazosin	[^{3}H]yohimbine	Selectivity
rac	4.99	9.18	15,000
8a*R*, 12a*S*, 13a*S*	5.29	9.45	15,000
8a*S*, 12a*R*, 13a*R*	< 5	6.32	> 50

The compound is a potent α_2-antagonist and shows a remarkably high selectivity. Ligand binding data on *rac*-**24** and its two antipodes are shown alongside its formula. Similar results were found in functional tests using rabbit aorta (α_1, *vs* phenylephrine) and guinea-pig ileum (α_2, reversal of inhibitory effect of UK-14304 on contractile response to field stimulation). [A configurational relationship of the eutomer (8a*R*, 12a*S*, 13a*S*-**24**) with rauwolscine and related compounds is evident.] In a test for central α_2-activity, the eutomer effectively reversed clonidine-induced mydriasis in the rat while its antipode was without activity (IC_{50} μg/kg iv, eutomer 6.5, distomer $>10^4$).

Chemistry. Reduction of the naphthyridine **25** gave a mixture of diastereoisomers **26** from which one was separated in major field. This was converted in two steps to *rac*-**24**. Antipodes were obtained by resolving the *sec*-amine intemediate **26** via diastereoisomers formed by reaction with *R*-(+)-1-phenylethyl-isocyanate (separated by HPLC, isomeric purity >98% by ^{1}H NMR). The chiral



25
naphthyridine intermediate

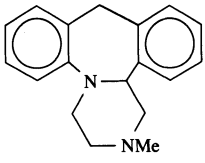


26
8aα, 12aα, 13aα major diastereoisomer (60% yield)
(8a*R*, 12*S*, 13a*S* shown)

auxilary urea was subsequently removed with sodium butoxide in 1-butanol at reflux, and the optical purity of the antipodes **26** determined to be >99% by chiral HPLC analysis using an Enantiopac α_1-acid glycoprotein column. Molecular geometry was established by X-ray crystallographic analysis of one of the diasteroisomeric ureas relative to the known *R* auxiliary group.

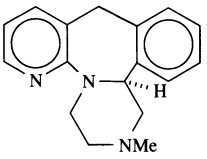
5.6. Miscellaneous Agents

The antidepressant agent mianserin **27** acts as a nonselective α-adrenoceptor antagonist in addition to its monoamine neuronal uptake inhibiting properties (it is also an antagonist of H-1 histamine receptors; see Chap. 11). Mianserin exhibits stereoselectivity in all the actions named. However, differences in the ability of antipodes to displace triated α-ligands from rat brain membranes were not great:



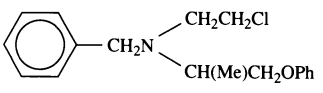
27

log IC_{50}, *vs* [^{3}H]prazosin *S*-(+) 6.58, *R*-(−) 5.65; *vs* [^{3}H]clonidine *S*-(+) 6.42, *R*-(−) 6.03.[40] In a test of potentiation of depolarization-induced release of NA from rat cerebral cortex slices, dextro isomers of both mianserin and its pyridyl analogue **28** proved to be the eutomeric forms.[41] While (+)-mianserin inhibits release of both [^{3}H]-NA and [^{3}H]-5HT, its *levo* antipode has an influence on NA release only.[42]

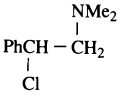


28

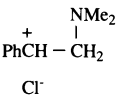
Phenoxybenzamine **29**, the now obsolete antihypertensive agent which irreversibly blocks α-adrenoceptors with a preference for the α-1 subtype,[44] has been resolved and its antipodes evaluated.[43] Dextro-**29** was found to alkylate α-1 receptor at a 15-fold higher rate than the levo isomer. The affinities of the intermediate aziridium species must therefore be influenced by the chirality of the phenoxyalkyl side chain. No difference was observed for the blocking potencies of *rac*-, (+)- and (−)-**30** in atropinized cats, evidence that it acts via the carbonium ion species (**31**) in which asymmetry is lost rather than the ethyleniminum ion.[45]



29



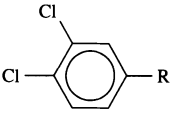
30



31

5.7. β-Antagonists

Some adrenoceptor ligands with β-blocking action have already been mentioned in Chapter 4. This section deals specifically with such agents of which there are two main classes. Those of the first generation are based on the structure of isoprenaline with dichloroisoprenaline (**32**, DCI) as prototype. Those of the

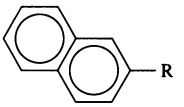


R = $CH(OH)CH_2NHPr^i$

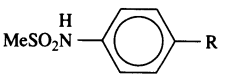
32 DCI

second generation are aryloxypropanolamines with propranolol (see later) as the prototype member. DCI and its analogues are derived by variation of the

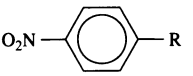
3,4-dihydroxyphenyl moiety of isoprenaline and all retain the chiral β-carbon chain of the natural catecholamines. Example which have been resolved are DCI (**32**), pronethalol (**33**, a 2-naphthyl derivative), sotalol (**34**), INPEA (**35**, nifenalol), and clenbuterol (**35a**). In all cases the levo isomer proved the more potent antipode in blocking a variety of β-adrenergic effects. Some details are provided in Table 5.4.



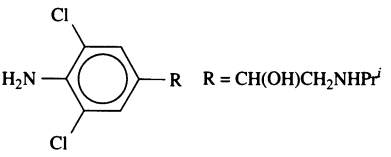
33 Pronethanol



34 Sotalol
(cf. β-agonist soterenol, page 87.)



35 INPEA

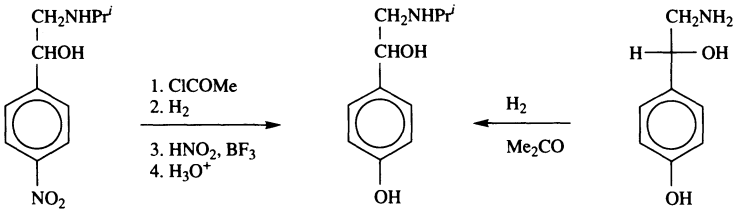


35a Clenbuterol

Optical rotatory evidence is cited as evidence for *R*-configurations in the cases of (−)-DCI and (−)-pronethalol (their ORD spectra show negative Cotton effects

TABLE 5.4.
Antipodal Comparisons of DCI and Related Compounds

Compound	Test	Ref.
DCI (**32**)	Blockade of tachycardia induced by isoprenaline in chloralosed cat	46
	(−) 2 × racemic mixture, at least 40 × (+)	
	Also true for antipodes of the —$NHCMe_2CH_2OH$ analogue	
Pronethalol (**33**)	GP trachea pA_2 vs. (−)-isoprenaline	47
	rac 7.3, (+) 5.2	
	(−) not tested (the more potent antipode)	
Sotalol (**34**)	pA_2 vs. (−)-isoprenaline	47
	(−) 6.77, (+) 5.09, GP atria (β_1)	
	(−) 7.73, (+) 6.13, GP trachea (β_2)	
	(−) 20 – 30 × more potent than (+), inhibition of hyperglycemia and elevation of plasma free fatty acids (FFA) induced by adrenaline and NA in dogs; vs. isoprenaline in GP trachea (−) 14 × (+)	48
INPEA (**35**)	pA_2 vs. (−)-isoprenaline	47
(see also Table 5.5)	(−) 6.81, (+) 5.44, GP atria (β_1)	
	(−) 7.12, (+) 4.72, GP trachea (β_2)	
	(−) more active than (+) in preventing adrenaline-induced tachycardia in rats	49
	pA_2 vs. NA (free fatty acid mobilization)	
	(−) 6.32, (+) 4.20	
Clenbuterol (**35a**)	pA_2 vs. (−)-isoprenaline	98
	(−) 7.61, (+) 5.39, GP trachea (β_2)	
	(−) 7.23, (+) 5.22, GP papillary muscle (β_1)	



(–)-antipode INPEA

R-(–)-antipode *p*-octopamine[a]

[a]Configuration assumed.[50]

Scheme 5.3. The configuration of INPEA antipodes.[49]

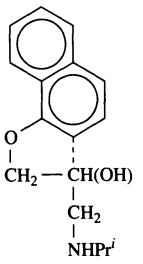
similar to those of levo adrenaline and NA)[46] while (−)-INPEA has been linked chemically to (−)-octopamine of assumed known configuration (see page 98),[49] as shown (Scheme 5.3).[49] All of these agents have an agonist component to their activity; in the example of DCI this has been shown to be a property of the levo isomer.[51]

The α-methyl (and ethyl) analogues of DCI of *erythro*-configuration lacked the intrinsic sympathomimetic activity of the parent and were feeble antagonists of isoprenaline at cardiac sites.[52]

Methoxamine and its analogues (Chapter 4, page 109) may also be regarded as members of first generation β-blockers. The compounds are α-methyl derivatives and carry a 2,5-dimethoxyaryl substituent, i.e., of a kind distinct from that of catecholamine agonists. Levo antipodes had pA_2 values in the range 6.3–7.2 vs. isoprenaline in GP trachea—dextro isomers were far less effective antagonists.

5.8. Second Generation β-Blockers

The parent member of this group is propanolol **36** developed in the 1960s by ICI. These aryloxypropanolamines differ from DCI and its analogues by the presence of oxymethylene inserted between the chiral $CH(OH)CH_2NHPr^i$ chain and aromatic ring, in addition to modification of the aryl unit itself. The drawing **36** presents an arrangement which mimics the structure of conventional catecholamines (see later). Antipodal activity comparisons versus isoprenaline in the cat (prevention of tachycardia: levo at least 40 × dextro),[46] GP trachea (pA_2:



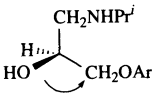
36

TABLE 5.5.
pA_2 Values *vs* Isoprenaline in Rat Tissues and Adipocytes[53]

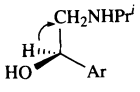
Compound	Atrium (β_1)	Diaphragm (β_2)	Adipocytes (β_2)
(−)-Propranolol	8.98	9.18	7.02
(+)-Propranolol	7.04	7.16	5.73
(−)-Practolol[a]	7.30	4.72	4.52
(+)-Practolol	5.63	3.58	3.51
(−)-Nifenalol (INPEA)	6.83	6.28	5.61
(+)-Nifenalol	4.87	4.33	4.25
(−)-Alprenolol	9.02	9.08	6.80
(+)-Alprenolol	7.03	7.71	5.72

[a] Eraldin

racemic mixture 8.5; dextro 6.5).[54] and rat (see Table 5.5)[53] have demonstrated the superior potency of the levo isomer. Results in rat atrium and diaphragm show an isomeric potency ratio near 100 in these tissues and a lack of specificity for β-subtypes, except in the case of practolol (see page 151). (+)-Propranolol has been related chemically to *S*-(+)-lactic acid and shown to have the *R*-configuration (Scheme 5.4).[55] Thus *S*-(−)-propranolol **37** and *R*-(−)-isoprenaline **38** have the same arrangement of substituents about their chiral centers, i.e., $CH_2OAr \equiv Ar$.

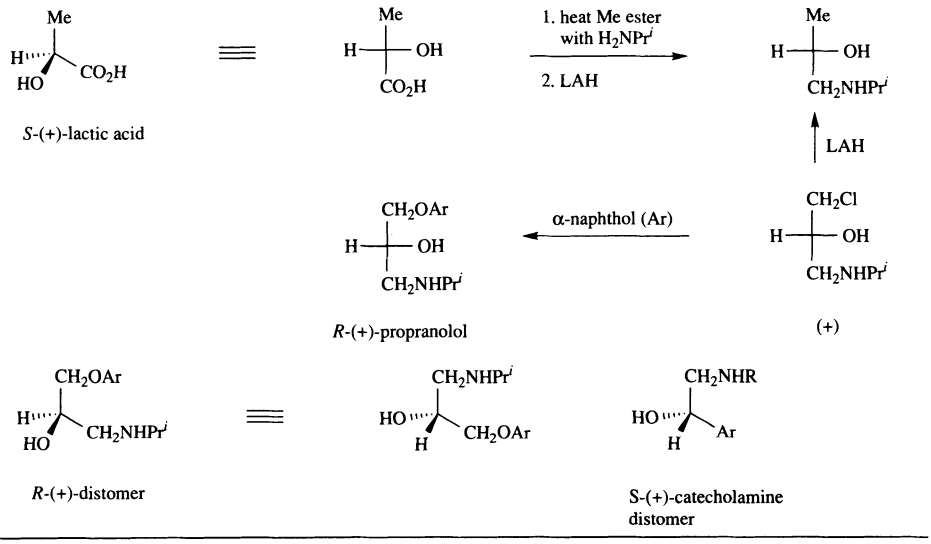


S-(−)-**37**



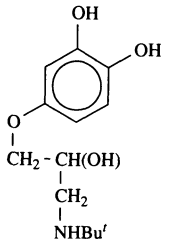
R-(−)- **38**

The fact that aryloxypropanolamines appear to be tailored to fit the same receptor as catecholamines is emphasized by the fact that the former type yield



Scheme 5.4. The configuration of (+)-propranolol.[50]

agonists as well as antagonists provided a suitable aromatic function is chosen. The derivative **39** with a catechol substituent and *t*-butylamino function is a highly potent β-agonist of direct action.[56]



39

310 × isoprenaline (GP atrium) for racemic mixture)
22 × isoprenaline (GP trachea)

5.9. Pharmacokinetics of Propranolol and Its Analogues

The marked stereoselectivity of action of antipodal β-blockers of the propranolol type extends to their clinical use. Thus in hypertensive patients, in which daily doses of 120 to 320 mg of *rac*-propranolol decreased supine systolic and diastolic as well as standing systolic blood pressure, Rahn *et al.*,[57] demonstrated that the same doses of (+)-propranolol had no effect on blood pressure (see also Refs. 58 and 59). For these reasons many investigations have been conducted on the stereoselectivity delivery of β-receptor antagonists, particularly propranolol.[60] Quite early in the use of this agent it was discovered that *rac*-propranolol was 2 to 3 times more effective after oral than iv routes of administration given in doses which produced equal plasma concentrations measured by nonchiral procedures.[61] This result was correlated with the greater oral clearance of the (+)-distomer as compared with that of the (−)-eutomer (see below), although a contributing factor could be the higher concentrations of the active metabolite 4-hydroxypropranolol detected after oral than after iv doses.[62] Following the development of chiral assay procedures, several reports of plasma enantiomer levels in normal subjects receiving oral doses of racemic drug were

TABLE 5.6.
Plasma Propranolol Enantiomer Levels in Normal Subjects Receiving Oral Doses of Racemic Drug (modified from Walle *et al.*)[60]

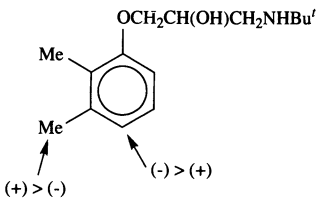
(−)/(+)-Ratio of plasma levels[a]			
Mean	Range	Dosage	Number studied
1.48[b]	0.99 – 2.04	Single 40 mg	15
1.87[c]	1.07 – 3.71	Repeated 40 – 80 mg	4 × 3 doses
1.55[c]	1.39 – 1.74	Single 80 mg	6 × 2 doses
1.39[c]	1.08 – 1.78	Single 80 mg	17

[a] Data relate to four studies; work of Walle *et al.* involved use of pseudoracemates with one antipode deuterated in the Ar ring (2D) or the NPr^i group (6D).
[b] Plasma enantiomer levels at 2 hr after dose.
[c] Area under the plasma concentration–time curves, i.e. complete kinetics.

made (Table 5.6). An earlier study giving evidence of higher plasma levels after *rac* than after the dextro antipodal form was flawed by the use of a nonselective fluorometric assay.[63]

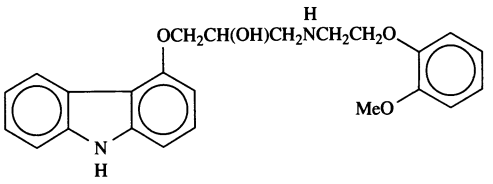
Results for most of the 42 subjects of the tests of Table 5.6 showed (−)/(+) plasma ratios above one—the highest value seen was 3.7. The preferential removal of the (+)-isomer was traced to hepatic metabolism, and specifically to the greater rate of 4-hydroxylation (and subsequent conjugation with glucuronic acid) of the (+)-isomer [partial metabolic clearance l/min (+) 0.88, (−) 0.35].[64] Two of three individuals with (−)/(+) plasma levels near unity were shown to be poor oxidizers of debrisoquine—a genetic trait associated with suppression of enzymes capable of the ring oxidation of propranolol. Clearance of antipodes by other routes, such as glucuronidation of side-chain OH and side-chain oxidation (to naphthoxyacetic acid), showed little stereoselectivity. (See Ref. 145 for effects of aging.)

Other β-blockers which undergo stereoselective oral clearance are metoprolol (1.37),[65,66] bufuralol (1.97),[67,68] penbutolol (2.68),[69] and xibenolol **40** (0.43)[70] [(−)/(+) plasma ratios in parentheses, other formulas in Table 5.7].



40 Xibenolol
(+) > (−) and (−) > (+) denote hydroxylation rates

A recent example is that of carvedilol **41**, a β-blocker with vasodilating activity.[71] After oral administration of *rac*-**41** to humans, the AUC for the *R*-(+)-distomer was 2–8 times that of the *S*-(−)-eutomer [*S*-(−) ∽ 160 × *R*-(+) as a β-blocker, with no difference in vasodilating properties]. This difference was ascribed to the greater liver clearance of the *S*-(−)-isomer and its lower degree of plasma protein binding compared with the *R*-isomer. Chiral analysis was by chromatography after derivatization with GITC (see page 34).



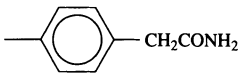
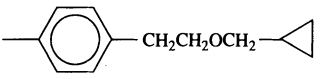
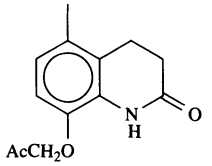
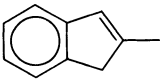
41 Carvedilol

Dextro metoprolol is O-demethylated at a faster rate than the levo (eutomer) form.[72] Opposite stereoselectivity was shown by xibenolol in this context—this drug produces two metabolites (4-OH and 3-CH_2OH) which are 2–3 times more potent than the parent as β-blockers.[70]

The levo antipode is 4-hydroxylated far faster than the dextro form—itself the preferred substrate in regard to production of the 3-CH_2OH metabolite. The net

TABLE 5.7.
Potency Comparisons Among Antipodal Pairs of Miscellaneous Blockers Related to Propranolol

$ArOCH_2CH(OH)CH_2NHR$

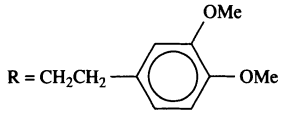
Compound	Eutomer[a]	Eudismic ratio from binding or functional tests	Ref.
Atenolol Ar =  R = Pr^i	(−)	6 (β_2, binding pA_2 vs. *S*-propranolol) 1.2 (β_1, binding pA_2 vs. *S*-propranolol)	93
Betaxolol Ar =  R = Pr^i	*S*	98 (β_1, pA_2, ventricle vs. ICYP)[d]	94
Bometolol Ar =  R[e]	*S*	257 (β_1, pA_2, atrium vs. isoprenaline) 93 (β_2, pA_2, trachea vs. isoprenaline)	95
Bufuralol[f] Ar =  R = Bu^t	(−)	12 (β_1, ED_{50} inhibition of tachycardia) 154 (β_1, ED_{50} inhibition of vasodepressor response to isoprenaline) 19 (partial agonist ED_{50}, increase in heart rate)	96, 143

[a] Where *R*- and *S*-configurations are specified, isomers were obtained by stereospecific syntheses.

[b] (+)-Isomers of pindolol and bupranolol are significantly more β_2-selective than the (−)-forms, resulting in lower antipodal affinity ratios for β_2- than β_1-sites.

[c] pK_B vs. binding of ^3H propranolol to kitten ventricle membranes (−) 8.77, (+) 6.95; vs. (−)-isoprenaline in rat atrium (−) 9.43, (+) 7.72; kitten papillary muscle (−) 9.28, (+) 7.20. Optical purities established by differential scanning calorimetry.[109]

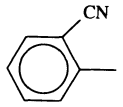
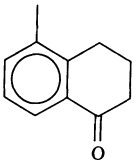
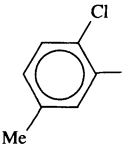
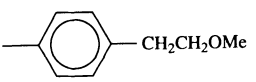
[d] ICYP denotes iodocyanopindolol.

[e] 

[f] See Ref. 143 for a report of stereoselective metabolism.

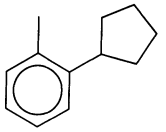
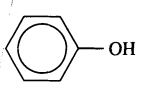
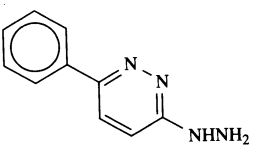
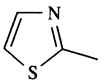
(table continued)

TABLE 5.7. ***(Continued)***

Compound	Eutomer[a]	Eudismic ratio from binding or functional tests	Ref.
Bunitrolol Ar =  R = Bu^t	*S*	26 (β_1, binding K_i, ventricle vs. ICYP)[d]	94
Bunolol Ar =  R = Bu^t	(−)	5.4 × *rac* (β_1, pA_2 tachycardia vs. iv isoprenaline)	97
Bupranolol[b, c] Ar =  R = Bu^t	(−)	27.5 (β_2, binding pA_2 vs. *S*-propranolol) 65 (β_1, binding pA_2 vs. *S*-propranolol)	93
Carvedilol (see **41**)	*S*	113 (β_1, K_B atrium vs. isoprenaline) 1.1 (α_1, K_B aorta vs. NA)	99
Falintolol (see **49**a)	*R* *S*	3.2 (β_1, pA_2 atrium) 2.0 (β_2, pA_2 trachea)	101
IPS-339 (see **48**)	*R* *S*	2.2 (β_1, pA_2 atrium vs. isoprenaline) 1.1 (β_2, pA_2 trachea vs. isoprenaline)	100
Metoprolol Ar =  R = Pr^i	*S*	98 (β_1, pA_2 atrium) 60 (β_2, pA_2 trachea)	100

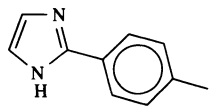
(table continued)

TABLE 5.7. ***(Continued)***

Compound	Eutomer[a]	Eudismic ratio from binding or functional tests	Ref.
MK-761 (see **50**)	*S*	19 (ED_{50} blockade of isoprenaline-induced hypotension) 67 (ED_{50} blockade of isoprenaline-induced tachycardia)	102
Penbutolol Ar =  R = Bu^t	*S*	50 (β-sympatholysis)	103
Pindolol[b] (see **44**)	(−)	200 (β_2, binding pA_2, lung vs. *S*-propranolol) 1260 (β_1, binding pA_2, heart vs. *S*-propranolol)	93
Prenalterol Ar =  R = Pr^i	(−)	83 (β_1, pA_2, soleus vs. terbutaline) 83 (β_2, pA_2, trachea vs. terbutaline)	104
Prizidilol Ar =  R = Bu^t	*S*	21 (β_1, pA_2 atrium) *R* & *S* equiactive as vasodilators (partial agonists)	105
Tazolol Ar =  R = Pr^i	*S*	1.2 × *rac*, myocardial β-blocking 2 × *rac*, myocardial β-stimulant	106

(table continued)

TABLE 5.7. ***(Continued)***

Compound	Eutomer[a]	Eudismic ratio from binding or functional tests	Ref.
Timolol (see **45**)	*S*	74 (β_1, pA_2 atrium) 58 (β_2, pA_2 trachea)	100
R = Bu^t	*S*	54 (β_1, pA_2 rat atrium) 30 (β_1:β_2 K_i ratio, ICYP binding assay)	107
Unnamed Ar =  R = Bu^t	*S*	26 (ED_{50}, block of isoprenaline-induced hypotension) 13 (ED_{50}, block of isoprenaline-induced tachycardia)	108

result is a higher concentration of the dextro isomer in plasma. This study was carried out by a pseudoracemate procedure.

In the examples of bufuralol and metopropol, evidence of enzyme deficiency (polymorphism, page 57) has been revealed by metabolism studies. Poor metabolizers excrete less of the 1′-hydroxy metabolite of bufuralol (the formula is given in Table 5.7) than do members of the EM group; furthermore, the transformation is stereoselective for the latter (the *R*-distomer is the preferred substrate), but not for the PM group. This difference in metabolic handling was traced to the absence of the enzyme isocytochrome P450 buf II from microsomes of the PM group.[73,74] The polymorphic oxidation of β-adrenoceptor antagonists has been reviewed by Lennard.[75]

Differential binding of propranolol antipodes to proteins of blood also plays a role in the observed plasma (−)/(+) ratio—this is small in degree but judged significant.[60] This phenomenon, which is rare for basic drugs, was traced to interaction with α_1-acid glycoprotein (α_1-AGP) [fraction α_1-AGP unbound: (+) 0.162, (−) 0.127] rather than with HSA [fraction unbound: (+) 0.607, (−) 0.649][76] and was felt of possible clinical importance in the case of patients with elevated α_1-AGP levels as in myocardial infarction.

Finally, the possibility of stereoselective storage and release of β-receptor antagonists by adrenergic neurons has been investigated. Tests were conducted using the PC12 clonal line of rat pheochromocytoma cells as a model neurosecretory system.[77] The cells were loaded by incubation overnight with *rac*-[^{3}H]atenolol and then subjected to a washout procedure. Samples of radiolabeled atenolol released from these cells under varying experimental conditions were analyzed by chiral derivatization and subsequent separation by reverse-phase HPLC. Preferential secretion of (−)-atenolol was observed in response to membrane depolarization by high potassium [50 mM K^+; 2 mM Ca^{2+} (−)/(+) ratio 3.6].[78]

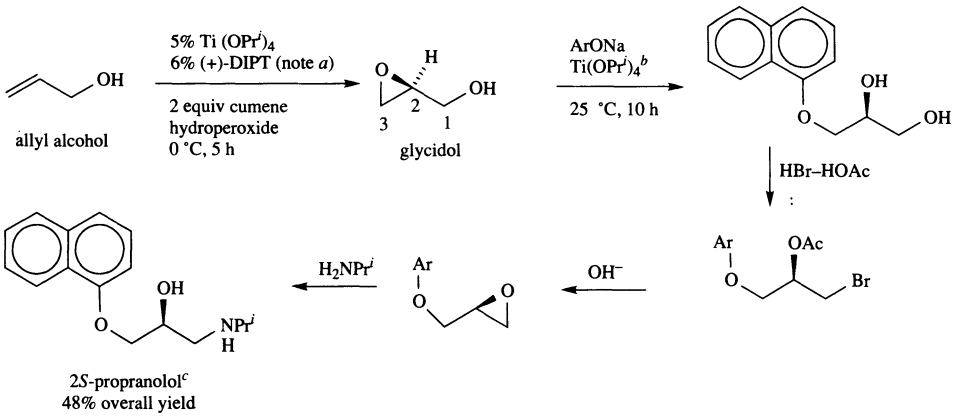
Takahasi *et al.*,[79] employed chiral stationary phase LC[80] to determine the distribution of propranolol antipodes to the major organs of the rat. Higher

concentrations of *levo-* than *dextro*-antipode were found in all tissues studies (lung, brain, kidney, GI tract, muscle, and heart) at 60 min after administration of the *rac*-drug. However, in blood the (+)-concentration exceeded that of the (−)-antipode. All results were traced to a difference in the plasma binding of the antipodes. Binding capacities of plasma protein, measured by ultrafiltration and employing [^{3}H]-*rac*-drug, were 2.089 μM for *dextro-* and 1.678 μM for (−)-propranolol. The opposite results for rats and man in regard to protein binding of antipodes of propranolol are just one example of the species variation seen in pharmacokinetic studies.[81] The stereochemistry of tissue distribution of *rac*-propranolol has also been studied in the dog.[82]

Another clinical use of propranolol is in the treatment of hyperthyroidism, and its action in inhibiting peripheral T4/T3 conversion has been traced to the *R*-antipode.[139,140] A study in man has shown that the chief 4-hydroxy-metabolite of the *R*-eutomer (prepared synthetically via chromatographic resolution of D-tartaric acid monoester diastereoisomers of RS-4-methoxypropranolol) was without influence on thyroid metabolism but possessed negative chronotropic effects (pulse rate decreases were noted).[141]

5.10. Stereoselective Synthesis

Stereoselective synthetic procedures are now available for the production of *R*- and *S*-propranolol and its congeners and access to antipodal pairs of known chirality is now a routine matter. The most recent method exploits the titanium-catalyzed epoxidation of allylic alcohols (Ref. 83 and Chapter 2, page 30) and is outlined in Scheme 5.5.[84] Use of (+)-diisopropyl tartrate at the epoxidation stage leads to 2*S*-propranolol while use of the (−)-ester produces the 2*R*-antipode.



Notes

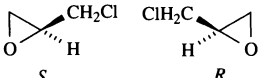
[a] Diisopropyl tartrate.

[b] Required to mediate the opening of the glycidol by the aryloxide.

[c] Also obtained by an alternative route involving use of (–)-DIPT at the epoxidation stage, followed by a different sequence of reactions.

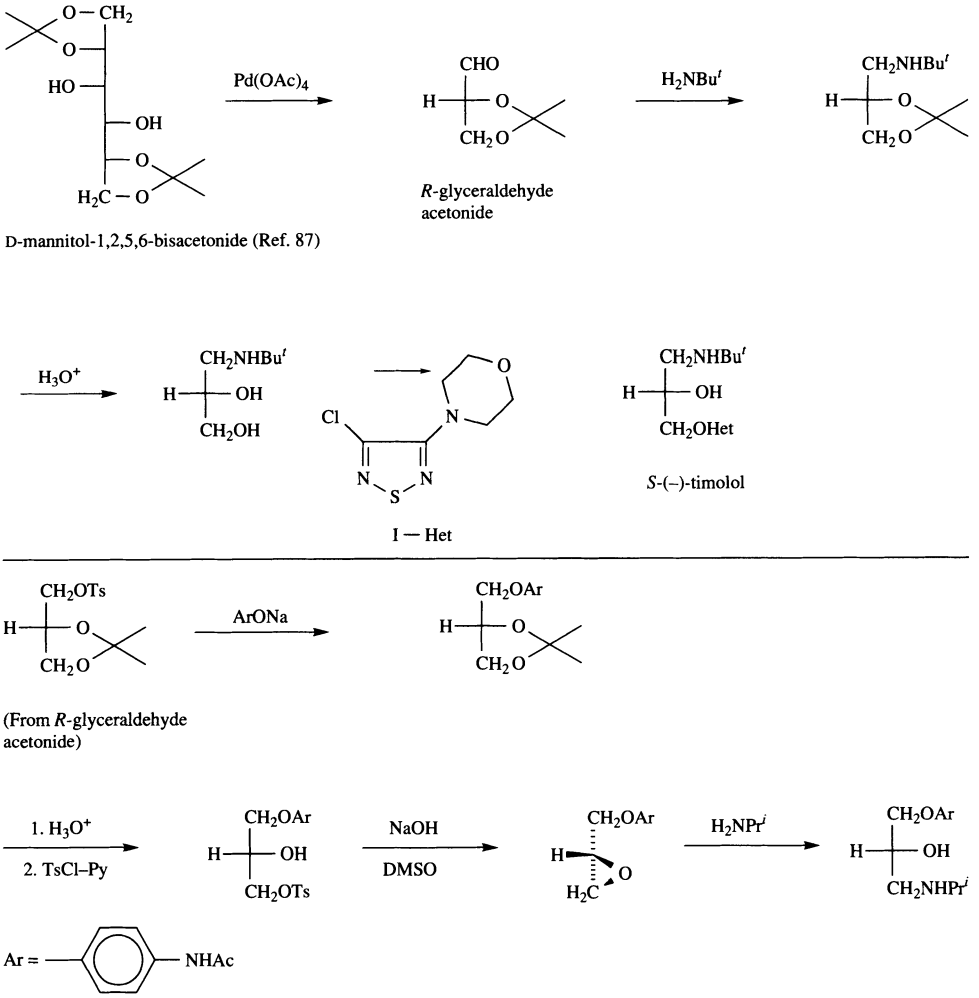
Scheme 5.5. Stereospecific synthesis of *S*-propranolol by the Sharpless procedure.[84]

Earlier methods were based on synthesis of *R*- and *S*-epichlorhydrin **42** from the bisacetonide of D-mannitol by Baldwin and his colleagues[85] and their stereoselective reactions with aryloxy and other nucleophiles.[86] Chiral building blocks of these kind are now commercially available, such as *R*-(+)- and *S*-(−)-glycidol **42**. Scheme 5.6 illustrates application of the *R*-glyceraldehyde acetonide route of the synthesis of *S*-(−)-timolol and *R*-(+)-practolol. Note that, dependent on the sequence followed, the D-mannitol intermediate can lead to either the *R*- or *S*-antipode.



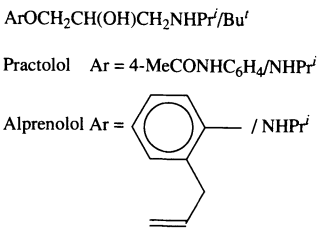
42

Following the development of propranolol, a host of analogues of general structure **43** have been marketed as β-blocking agents for use in cardiovascular dis-



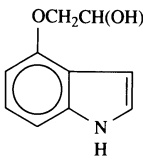
Scheme 5.6. Stereospecific syntheses of *S*-(–)-timolol [88] and *R*-(+)-practolol.[89]

eases, some of which (unlike propranolol) are selective for β_1-sites. Practolol (Eraldin), introduced in the early 1970s, was the first β_1-selective agent of this kind (it is recommended that its use now be restricted to life-threatening arrhythmias,



43

because of its serious adverse side effects; *Martindale* **29**, page 798, 1989). Many of these novel β-blockers have been resolved or secured in homochiral states by stereoselective synthesis and their antipodes compared; with few exceptions (see later) the levo isomer is the more potent antipode. It is generally assumed that levo isomers have the *S*-configuration, i.e., identical with that of (−)-propranolol, although this has only been established in cases of stereospecific synthesis as detailed above. pA_2 values for antipodal forms of practolol and alprenolol (Table 5.5, see **43**) illustrate the superior activity of the levo isomers and the β_1-specificity of practolol. The levo antipode of the 4-indole analogue **44** (pindolol)



44 Pindolol

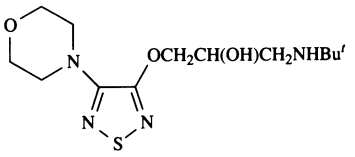
pA_2 (GP muscle vs. isoprenaline)[90]

	tracheal (β_2)	soleus (β_2)	papillary (β_1)
(−)	9.59	9.17	9.68
(+)	7.42	6.97	7.20

likewise is much more effective than its dextro counterpart in blocking β-sites of guinea-pig muscle (see legend alongside **44**).[90] By contrast agonist properties in equal degree were found for both isomers (relaxation of tracheal or vascular smooth muscle[91]). Practolol and alprenolol are also partial agonists. Further data on antipodal β-blockers are shown in Table 5.7 (see Main and Tucker for a general review).[92]

5.11. Timolol

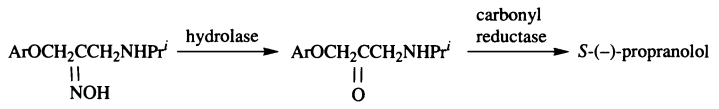
The example of timolol **45** deserves special attention. This compound is one of the few β-blockers which is used clinically in a homochiral form (levo timolol, *Blocadren*, *Betin*); another example is levo bunolol.[110] Its configuration is known to be *S* from its stereospecific synthesis from natural mannitol (Scheme 5.6).[89]



45 Timolol

One application of *S*-timolol is the treatment of glaucoma through its blockade of ocular β-adrenoceptors of the β_2 type which control the formation of aqueous humor.[111] Since timolol lacks specificity for β-subsites,[112] unwanted cardiac and pulmonary effects are seen in certain patients as a result of concurrent stimulation of β_1-receptors.[113,114] In animals, *R*-(+)-timolol is a more potent receptor antagonist in the eye than in pulmonary and cardiac tissues, and only moderately weaker (3-fold) than the *S*-antipode in lowering intraocular pressure.[115] Hence *R*-timolol may be a safer compound for the treatment of glaucoma than the marketed *S*-enantiomer.

A novel process of site- and stereo-specific ocular drug delivery by sequential enzymatic bioactivation has been reported.[116] When the oxime of the keto-precursor of propranolol **46** was instilled into the iris-ciliary body and cornea of rabbits, the extract residue was shown to contain the eutomeric form of propranolol. This transformation depends on the presence of an oxime hydrolase (producing the ketone **47**) and a carbonyl reductase—action of the latter is stereospecific. No cardiovascular effects followed iv administration of the oximes **46** and no active β-blocker could be detected. Chiral HPLC analysis was applied to the ocular extracts after derivatization with 2,3,4,6-tetra-*O*-acetyl-D-glycopyranosyl isocyanate (GITC).



46
syn/anti oxime mixture
Ar = 1-naphthyl

47
inactive ketone

Morris and Kaumann[93] determined the binding parameters of ten *RS* pairs of adrenoceptor ligands (chiefly antagonists) in an attempt to uncover differences in the stereoselectivity of β_1 and β_2-adrenoceptors. Since higher antipodal affinity ratios were found for heart tissue (assumed β_1) than for lung tissue (β_2), β_1-sites were taken to have the more stringe steric requirements. Kaumann and Testa believe, however, that the lipophilicity factor should be taken into account in the analysis of such data,[117] particularly because β_2-selective adrenoceptor antagonists are known to be more lipophilic than β_1-selective agents.[118,119] The authors carried out eudismic and classical regression analysis of the binding data of Morris and Kaumann[93] and lipophilicity data (apparent *n*-octanol/water partition coefficient at pH 7.4) of Woods and Robinson[120] (Table 5.8) to seek quantitative evidence. They point out that the consistently *lower* eudismic index (EI) of

TABLE 5.8.
Receptor Affinity and Selectivity of 10 Enantiomeric Pairs of β-Adrenoceptor Ligands[117]

Ligand	α_1-adrenoceptors[a]			β_1-Adrenoceptors[a]			β_1-Selectivity[d]	Lipophilicity[b]
	pK_{Eu}	pK_{DIS}	EI[c]	pK_{EU}	pK_{DIS}	EI[c]		log $P_{7.4}$
Isoprenaline	6.82	4.90	1.92	7.07	4.46	2.61	0.25	− 1.93
Adrenaline	5.91	4.80	1.11	5.47	4.33	1.14	− 0.44	− 2.50
Noradrenaline	5.04	3.80	1.24	5.55	3.97	1.58	0.58	− 2.86
Prenalterol	6.49	5.29	1.20	6.62	4.70	1.92	0.13	− 0.28
Practolol	5.38	4.81	0.57	5.58	4.52	1.06	0.20	− 1.31
Alprenolol	8.90	7.56	1.34	8.53	5.90	2.63	− 0.37	0.36
Pindolol	9.45	7.15	2.30	9.26	6.10	3.16	− 0.19	− 0.09
Atenolol	5.46	4.68	0.78	5.96	4.89	1.07	0.50	− 1.88
Bupranolol	9.47	8.03	1.44	9.20	7.39	1.81	0.27	1.08
Propranolol	9.16	7.20	1.96	8.71	6.51	2.20	− 0.45	1.31

[a] Values from Morris and Kaumann.[93]
[b] Apparent *n*-octanol/water partition coefficient measured at pH 7.4; data from Woods and Robinson.[120]
[c] EI correspond to the affinity ratio of the stereoisomers (eudismic index).
[d] Values calculated as $pK_{Eu}(\beta_1)$ minus $pK_{Eu}(\beta_2)$.

β_2-agents has its origin in significantly *higher* pK_{Dis} (β_2) versus pK_{Dis} (β_1) values of distomers.

In equations developed to fit the data, hydrophobic interactions were found to play an important role in the affinity of stereoisomers at both β_1- and β_2-adrenoceptors with that of β_2-ligands being slightly more dependent, at least for distomers. The plot of Fig. 5.1 further illustrates this conclusion—β_1-selectivity falls with a rise in lipophilicity (log $P_{7.4}$). The conclusions are subject to the assumption of the high optical purity of the homochiral ligands employed in the binding experiments.

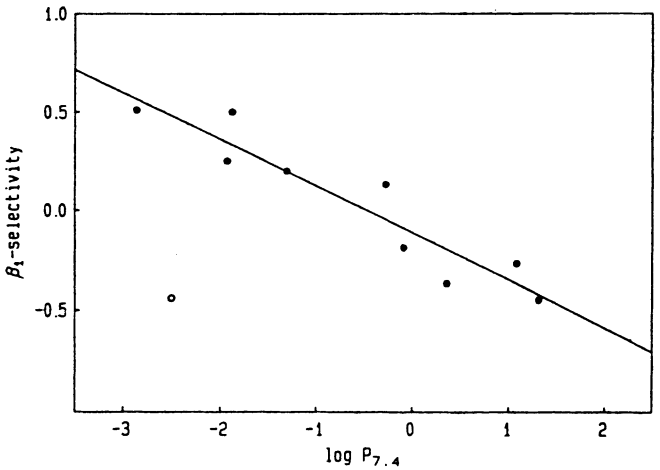
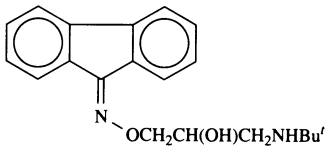


FIGURE 5.1. Relationship between lipophilicity (log $P_{7.4}$) and β_1-selectivity for the eutomers of Table 5.8. Adrenaline is an outlier in this plot (after Kaumann and Testa).[117]

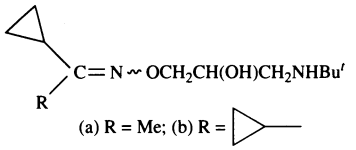
5.12. Oxime Class

The oxime **48** (IPS-339, low chiral preference) is unusual in that its antipodes show little difference in their powers to block β-adrenoceptors (orders of potency are modest—see data of Table 5.7).[100] This may be a result of the additional



48

geometrical factor of the oxime link which allows antipodal superimposition of all molecular features except the OCH_2 link (Fig. 5.2). Falintolol **49**a is also an



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oxime.[101] The data of Table 5.7 relate to mixtures of *anti*- and *syn*-forms; eudismic ratios are small and unusual in that the *R*-antipode is the eutomer at β_1-sites ($3 \times S$)

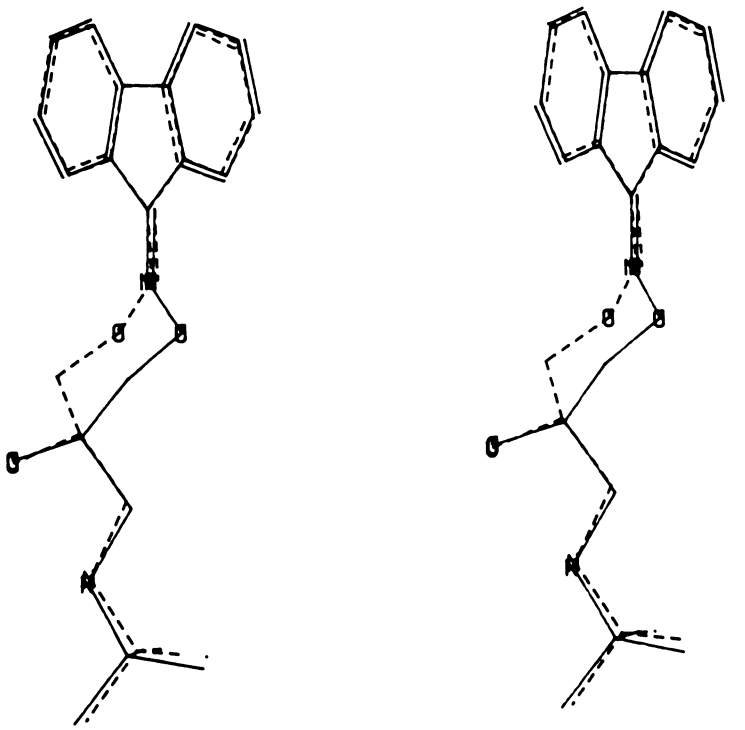
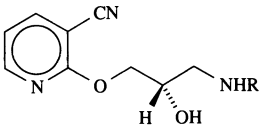


FIGURE 5.2. Superimposed stereoscopic view of *R*-**48** (solid lines) and *S*-**48** (dotted lines) illustrating the spacial relationship of the aryl – hydroxy – amine triad (after Baldwin *et al.*).[100]

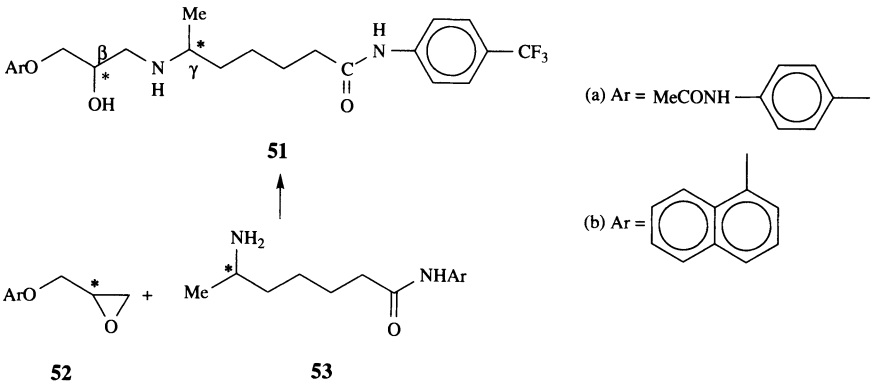
while the *S*-form is the eutomer at β_2-sites ($2 \times R$) (results for IPS-339 are similar in all these respects). When the separate geometrical forms of *rac*-material were tested, the *anti* exceeded the potency of the *syn*-form at both β_1 ($\times 4$) and β_2 ($\times 7$) sites (oxime stereochemistry followed from ^{1}H-NMR *Me* C=N shifts—lower field for *anti*-isomer when Me close to N—*O*). *R*- and *S*-forms of the symmetrical oxime **49**b differed little in potency (*S*, $3 \times R$ on atria; *R*, $2 \times S$ on trachea—note inversion of stereoselectivity again) and the authors' account for their findings in terms of pseudosymmetry as proposed by Baldwin.

Some diastereoisomeric analogues of the 3-cyanopyridine **50** (see MK-761 of Table 5.7) with *N*-aralkylamino substituents showed α_1-blocking properties in addition to β-blockade.[121] β-Affinity required *S*-chirality at the *sec*-OH carbon, while α-affinity was greatest when the N-substituent chirality was *R* (γ-type; see Chapter 4, page 98)



50

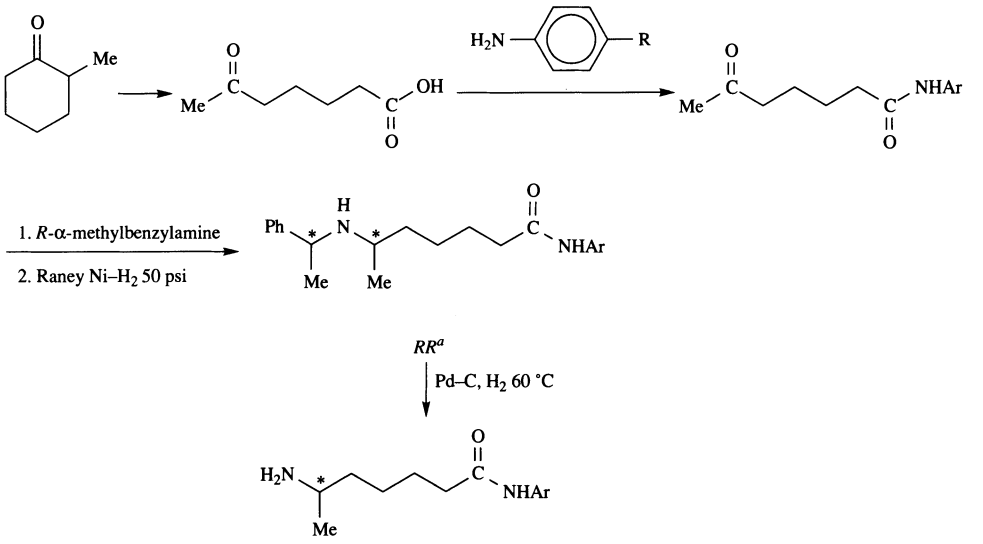
Goodman's work on the attachment of an anilide of heptanoic acid to β-adrenoceptor ligands (Chapter 4) has been extended to β-blocking agents.[122] Isomeric sets of the practolol **51**a and propranolol **51**b analogues were made by linking the homochiral intermediate **52** (obtained from the Sharpless epoxide, p. 149) with the amino amide **53**, also of known configuration (Scheme 5.7 for *R*-product).



51 **52** **53**

Biological activity was determined by utilizing both *S*-**49** mouse lymphoma cells (β_2) and rat fat cells (β_1) and was measured by the *in vitro* accumulation of *c*-AMP.[126] Data for the practolol and propranolol series are shown in Table 5.9.

The alkyl-anilide side chain enhanced the potency of practolol in all but the *RS*-isomer. The most potent form [$93 \times (\beta_2)$, $22.6 \times (\beta_1) \times$ *rac*-practolol] had the anticipated *S*-configuration at the β-chiral center (cf. page 73 for use of α, β, γ to denote chiral centers). *R*-Geometry at the γ-center was preferred for β-receptor blockade—*RR*- and *SS*-isomers had similar activities, probably the consequence of a balance between optimal β-geometry in one case, and optimal γ-geometry in the



R- **53** amino-amide component[b]

Notes
[a] Assignment of absolute configurate based on literature precedent for hydrogenation of imines formed with α-methylbenzylamine.[123,124]
[b] Optical purity verified from ^{1}H-NMR analysis of the Mosher amides.[125]

Scheme 5.7. Stereospecific synthesis of *R*-**53**.[122]

TABLE 5.9.
Inhibition of Isoprenaline-Induced Accumulation of *c*-AMP by Analogues of Practolol and Propranolol[122]

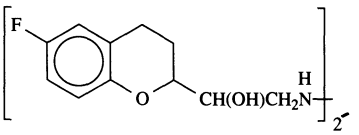
Compound		K_i(μM)[a] S-49 mouse lymphoma cells (β_2)	Rat fat cells (β_1)
rac-practolol (**51a**)		13.5	0.7
analogue	*RR*[b]	2.17	0.24
	RS	62.4	6.7
	SR	0.146	0.031
	SS	2.85	0.35
rac-Propranolol (**51b**)		0.78	
analogue	*RR*	130	N/D
	RS	179	
	SR	45.9	
	SS	39.0	

[a] *c*-AMP determined by radioimmunoassay.[126]
[b] *R*(β) *R*(γ) etc. see **51**.

other; cf. compounds of Table 4.12 (page 99) where γ(*R*) chirality is also conducive to β-blockade.

All derivatized propranolol isomers fell well below the potency of the *rac*-parent at β_2-sites. The configuration of the γ-center appears of less import in this series since the *SR*- and *SS*-isomers had similar potencies, severalfold superior to *RR*- and *SS*-forms.

The stereoselectivity of the blockade of *presynaptic* β-adrenoreceptors has been investigated by several workers. NA released at low frequency of nerve stimulation stimulates these receptors and triggers a positive feedback mechanism leading to increases in transmitter release.[127] The receptors are characterized as β, since they are activated by (−)-isoprenaline (the dextro antipode is ineffective) and blocked by propranolol at concentrations devoid of neuron blocking action. The experiments involve the use of rat isolated atria and other tissue preloaded with [^{3}H]-NA, and measurement of released radioactivity. In work on nebivolol **54** and its isomers the order of potency vs. adrenaline in these test was *SRRR*> nebivolol > *RSSS*.[128]

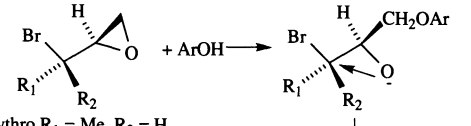


R 67138 *SRRR* } nebivolol
R 67145 *RSSS* } 1 : 1 mixture

54

5.13. Further Analogues Including α-Methyl Derivatives

α-Methyl analogues (**55**) of propranolol, practolol, and oxprenolol have been described in the form of their *threo*-racemic mixtures.[129] All were of reduced cardiac β-blocking potency in comparison with the parent compounds (cf. result for DCI and its α-Me analogue, (page 141), e.g., dose (μg/kg) giving 50% inhibition of tachycardia produced by isoprenaline: propranolol 62, *threo*-α-methyl 744. Preliminary reports on the *erythro*-racemic mixture at β_1-sites showed it to be twice as potent as the *threo*-isomer and to resemble the parent in its β_2-activity. Treatment of the *erthro*- and *threo*-l-bromoethyloxirane **56** with a phenol followed by isopropylamine provides a stereospecific route to *erthro*- and *threo*-**55**, respectively[130]; the oxiranes were obtained from the geometrical isomers of crotyl alcohol.



erythro R_1 = Me, R_2 = H
threo R_1 = H, R_2 = Me

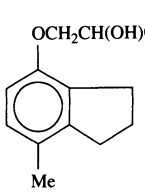
56

$ArOCH_2CH(OH)CHMeNHPr^i$
γ α

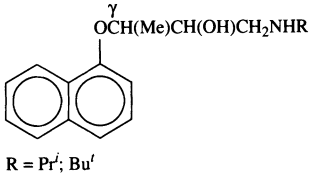
55

Later, α-methylpropranolol (*erythro*) was found to be selective for β_2-sites[131] and is listed as a selective β_2-antagonist in the *TiPS* receptor supplement of January 1990 along with the related α-methyl derivative ICI 118551 (**57**). A β_2-selectivity ratio of 123: 1 has been reported for **57** from *in vitro* studies and greater than 250: 1 *in vivo*.[132] It is more β_2-selective than its *threo*-isomer.[92]

All α-methyl derivatives reported by Lemke *et al*,[133] were feeble β-antagonists—however, the γ-methyl analogues of propranolol **57a** were more effective than propranolol at both atrial and tracheal sites. Stereochemistries were not established.

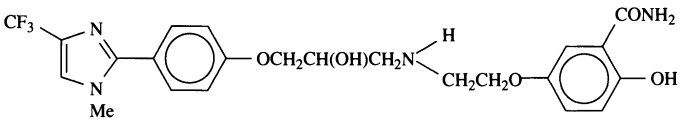


57 ICI (118551)
rac-erythro



57a

A further addition to selective pharmacological tools is CGP 20712A (**58**), a specific β_1-adrenoceptor antagonist.[134] This compound is yet another variant of propranolol; it has a 4-(1-methyl-4-trifluoromethyl-2-imidazoy)phenyl Ar group and carries a phenoxylethyl N-substituent. Binding of **58** to rat neocortical membranes (rich in β_1-receptors), as judged by displacement of [^{3}H]dihydroalprenolol (DHA), was biphasic, indicative of uptake at β_1 followed by β_2-sites. The IC_{50}- β_2 to IC_{50}-β_1 ratio was ~10,000 in evidence of the high degree of β_1-selectivity of CGP 20712A. When antipodes of **58** were examined, the levo isomer proved to be the eutomer (1.6 times more potent than *rac*-**58**) with IC_{50} values 0.45 nM (β_1) and 4200 nM (β_2). The (+)-enantiomer gave only a slightly biphasic curve and its IC_{50} value of 650 nM showed it had far less β-affinity than its antipode.



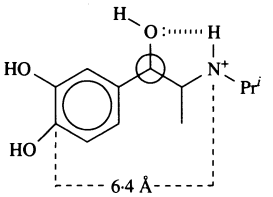
58 CGP 20712A

Beer *et al*.,[135] have developed a binding assay to establish the selectivity of β-adrenoceptor ligands in rat brain, by use of the selective antagonists CGP 20712A and ICI 118551 (**57**) to block β_1- or β_2-subpopulations, respectively, in rat cerebral cortex membranes. This permitted the selective labeling of β-adrenoceptors with the antagonist (−)-[^{125}I]pindolol, a ligand of notably high radioactivity.

IC_{50} (μM) values for clenbuterol (**35a**) were levo: 0.14 (β_1), 0.12 (β_2); dextro: 9.13 (β_1), 11.40 (β_2), results which reveal this agent to be nonselective for β-sites.

5.14. Conformational Aspects

Jen and Kaiser[136] have proposed that aryloxypropanolamines adopt a conformation at β-adrenoceptors that mimics that of isoprenaline and related catecholamines. Conformation studies of isoprenaline provide evidence for a preferred conformation in which the nitrogen function is *trans* to the aromatic ring and *gauche* to benzylic hydroxyl (**59**) (see separate discussion on page 109).



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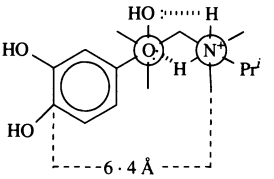

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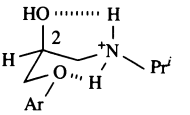
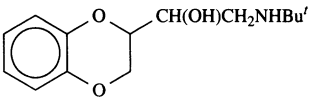


61

A propanolamine conformation which may be superimposed on **59** is the arrangement **60**, shown for the agonist example (**39**). Conformation **60** is presumed to be stabilized by a pair of intramolecular hydrogen bonds, one comparable with that operating in the isoprenaline structure and the other formed between aryloxy oxygen and the second $^{+}N-H$ atom, whereby accounting for the need for a secondary amino function (see **61**). Such a conformation is not found in the solid state where *trans*-$R^{+}NH_2/CH_2OAr$ geometry is found,[137] but there is NMR evidence for conformational preference of type **60** for propranolol HCl as solute in $CDCl_3$. The evidence (from 60–90 MHz spectra) relates in particular to the β-proton (C*H*OH) whose abnormally low chemical shift in the salt (4.8 ppm HCl, 4.10 ppm base) is attributed to various deshielding influences operating in the bidentate H-bonded structure **60**. In H-bonding solvents such as water, intermolecular H-bonding is considered to reduce the population of the intramolecularly bonded kind as so prevent their detection. It would be of interest to apply state-of-the-art NMR, which would yields details of proton coupling interactions and proximities, to this conformational problem (a 400-MHz spectrum of propranolol HCl in $CDCl_3$ recorded at Bath showed a C*H*OH multiplet at 4.82 ppm; its base width of ~35 Hz was greater than that anticipated for four small couplings required if conformation **61** is preferred). Absolute configurational relationships of catecholamines and aryloxypropanolamines provide strong support for these ideas.

Activity data on the antipodal forms of the benzodioxan **62** would be of special interest in the present regard. The two racemates of **62** were both 5 to 10 times more potent than propranolol as β-antagonists in tests on the heart; only NMR evidence of relative configuration is available.[138]

Deshydroxy analogues of **62** are α-blocking agents, and are discussed separately (page 129). Agents with mixed α/β blocking actions are described in Chapter 4 (page 88).



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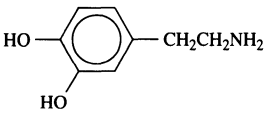
6

Dopamine and Dopaminergic Ligands

6.1. Introduction

Dopamine (DA) **1** is an achiral molecule and thus presents a case akin to those of acetylcholine and histamine discussed in later chapters in which a pair of pharmacophores are separated by a bimethylene unit. Its receptors, nevertheless, display marked degrees of stereoselectivity toward chiral dopaminergic ligands of both agonist and antagonist character as reviewed by Cannon[1] and a Danish group,[2] respectively, and briefly by Baindur and Neumeyer.[3] The dopamine system has much in common with that of adrenergic agents, including features of *in vivo* synthesis, metabolism, uptake and release, and interaction with adenylate cyclase.



1

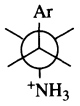
Indeed dopamine and N-methyldopamine (epinine) are the deoxy analogues of noradrenaline and adrenaline respectively and, at high dose levels, exhibit both direct and indirect action at adrenergic receptors (see Chapter 4). DA induces both inotropic and chronotropic responses at the heart (β_1-sites)[4,5] and contractions of α_1-preparations such as rat vas deferens (direct action potency $\sim$0.1 X NA).[6] Receptors specific to dopamine are, however, the special interest of this chapter and recent reports of the cloning of the D_1 (linked to adenylate cyclase activation),[7,8] D_2,[9] and D_3 (represents both an autoreceptor and postsynaptic receptor)[10] dopamine receptors are of major significance in this regard (see also Ref. 155). DA is a central and perhaps also a peripheral neurotransmitter in its own right and several dopaminergic pathways have been identified in the brain associated with motor function and functions related to mental state and emotion. The development of specific ligands has led to the definition of subspecies of

dopamineric receptors. Two subtypes, namely, D-1 and D-2, have been discovered by binding experiments and by anatomical location, and these may be differentiated by their association with adenylate cyclase (D-1), leading to a rise in *c*AMP, or lack of (sometimes negative) any influence (D-2). Pharmacological tests have led to the delineation of the DA_1 variety which is linked to the relaxation of smooth muscle (including vasodilation) and release of parathyroid hormone. Stimulation of DA_2 receptors is associated with emesis, various behavioral syndromes, and inhibition of prolactin release; prejunctional DA_2 receptors control the synthesis and release of DA by a feedback mechanism (autoreceptors: agonists promote a fall and antagonists a rise in DA and DOPA levels). Other tests of subtype specificity are described within the text. Ligands selective for either D-1/DA_1 or D-2/DA_2 receptors are known; most, but not all, behave pharmacologically as antagonists, and are described in Chapter 7. It is probable that the D-1/DA_1 and D-2/DA_2 varieties are identical or similar in nature, although discrepancies have been found in this regard (thus *R*-sulpiride antagonizes renal vasodilation induced by DA_1 stimulation but fails to block activation of adenylate cyclase by DA—a D-1 effect).[11] The classification of DA receptors therefore remains controversial.[12] Present nomenclature is included in a January 1991 *TiPS* supplement, and DA classification has most recently been discussed by Andersen *et al.*[13] Ligands in which the DA pharmacophore (ArCCN) is constrained withing a molecular framework of varying complexity form the principal groups of chiral DA agonists and antagonists. This situation stands in sharp contrast to adrenergic systems where conformationally restrained analogues of high potency are rare, and is well illustrated by the example of the polycyclic DA agonist apomorphine.

A useful account of assay and binding procedures employed in DA studies is given by Horn in Pergamon's *Comprehensive Medicinal Chemistry*.[14]

6.2. Conformational Studies[15]

In the solid state DA, hydrochloride exists as the *trans*-conformer **2** (X-ray analysis).[16] However, computational and ^{1}H-NMR methods predict only a small preference for this conformer in the solute condition. Thus Pullman *et al.*[17] calculated (PCILO) a population 0.352 for *t*-**2** and 0.345/0.303 for the two *gauche*-conformers (see Chapter 4, page 110) while Bustard and Egan (extended-Huckel and NMR) deduced a *t*-population of 0.43 over a 60° temperature range.[18]



2
Ar = 3,4-di-OHC_6H_4

Similar conclusions were reached by Ison *et al.*, by NMR methods.[19] The ^{1}H-NMR spectrum of DA HCl in D_2O recorded at 400 MHz is, indeed, close to that of an A_2B_2 system, and indicative of a lack of conformational preference (Fig. 6.1; cf. 100-MHz spectrum of Ref. 18).

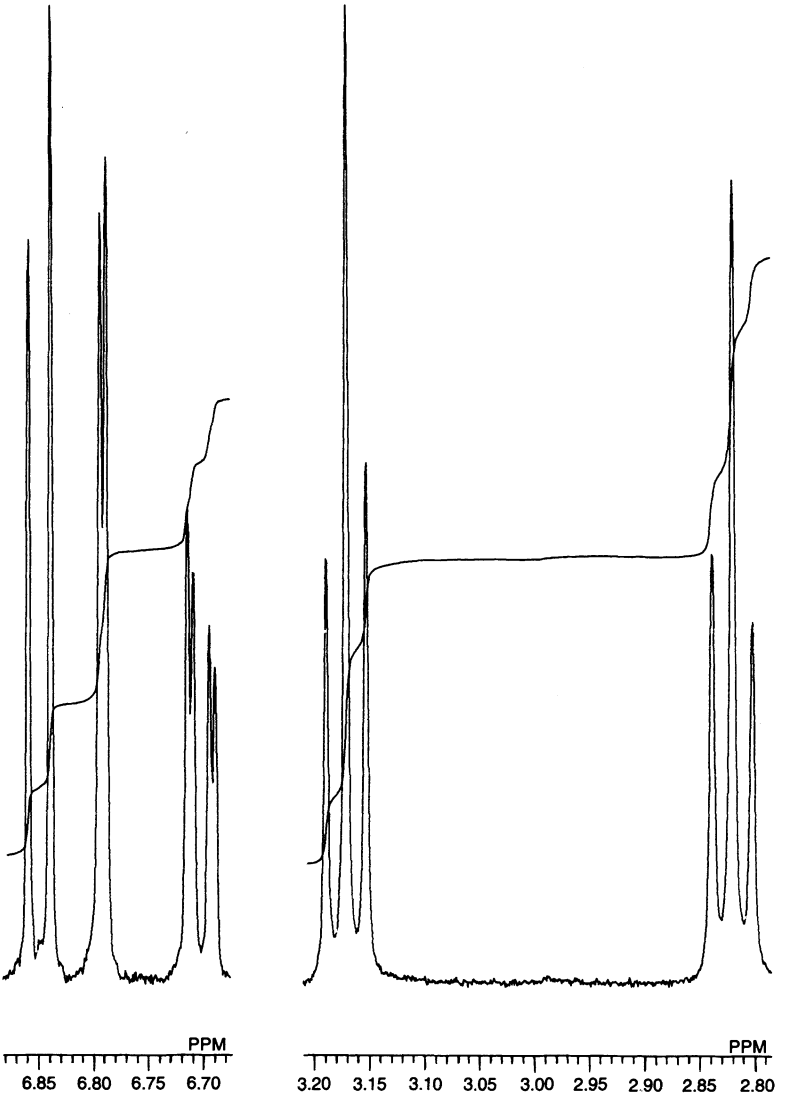
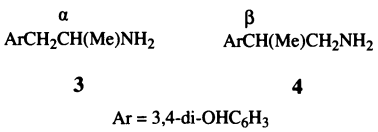


FIGURE 6.1. The 400-MHz ^{1}H-NMR spectrum of dopamine hydrochloride in D_2O. The appearance of the NCH_2 and $ArCH_2$ resonances (near 3.17 and 2.84, respectively) are typical of an ACH_2CH_2B system of low conformational preference. First-order ^3J value 7.33 Hz; cf. 7.7 and 6.35 Hz calculated by Ison *et al.*[19]

6.3. Methylated and Small-Ring Analogues of Dopamine

The stereochemistry and adrenergic properties of α-methyldopamine (**3**) have already been presented in Chapter 4. Its lack of dopaminergic properties is seen directly in its failure to act at renal DA receptors (see below), and indirectly in the inability of *S*-(−)-α-methyl DOPA to be effective in treating Parkinsonism (α-Me DA is a metabolic product). In rat caudate nuclei which contain DA-sensitive adenylate cyclase[20] *rac*-**3** was 100-fold less effective than DA in raising the *c*AMP

level.[21] When antipodes were evaluated in this test the *R*-(−)-isomer proved a weak stimulant while the *S*-(+)-form was inactive. However, in a β-adrenergic system (erythrocyte ghosts) α-methyl DA was more effective than DA with *S*-(+)-**3** the more active antipode.



Goldberg and Kohli reported that no DA analogue carrying an α- or β-substituent in the phenethylamine chain had activity in the canine renal artery assay (DA_1 model) and stated that α-methyl DA was only a very weak agonist.[22] Riggs *et al.*[23] resolved β-methyl DA **4** and tested the ability of the antipodes (absolute configuration established by X-ray crystallography) to stimulate rat retinal adenylate cyclase (D-1 preparation).[12] At a concentration of 10 μM, the *R*-(+)- and *S*-(−)-enantiomers were equieffective and had about one-fifth of the potency of DA. The finding that the racemic mixture **4** was only 25% as effective as either antipode was puzzling, but confirmed.

It is therefore evident that, unlike the situation found for methylated acetylcholines (Chapter 8), replacement or any of the four hydrogens of the bimethylene of DA by methyl, reduces ligand affinity for DA receptors. At equal dose levels (10μM), racemic β-phenyl DA had half the stimulant activity of the parent molecule—evidence for receptor subsites capable of binding to an additional aromatic subsituent (cf. the 1-phenylbenzazepine **64** and dihydroxynomifensine **68**, pages 193 and 194).

6.3.1. β-Hydroxy Analogues

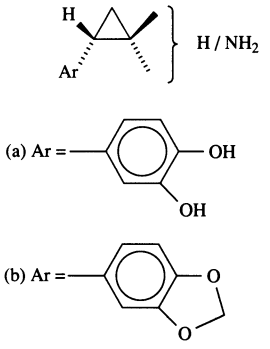
In binding experiments carried out on calf caudate homogenates, dopamine and epinine competed more effectively with [^{3}H]-haloperidol and [^{3}H]-dopamine than their respective β-hydroxyl analogues NA and adrenaline.[24] In these tests, *R*-(−)-antipodes were more potent than corresponding *S*-(+)-isomers (Table 6.1).

TABLE 6.1.
Inhibition of [^{3}H]-Haloperidol and [^{3}H]-Dopamine Binding to Calf Caudate Homogenates[24]

Compound	vs. [^{3}H]-haloperidol (nM) (antagonist)	vs. [^{3}H]-dopamine (nM) (agonist)
Dopamine	670	17.5
Epinine	530	23
R-(−)-NA	5,600	200
S-(+)-NA	21,000	800
R-(−)-Adrenaline	2,600	280
S-(+)-Adrenaline	37,000	1200

6.3.2. Small-Ring Analogues

References has already been made to the adrenergic properties of the cyclopropane derivatives **5**a (see Chapter 4).[25,26] Racemic *c*- and *t*-**5**a were inactive in several DA assays while the binding capacity of the *trans*-isomer was about one-fifth that of DA (displacement of [^{3}H]-spiperone from calf caudate tissue). Both isomers strengthened the heart beat of dog (*trans* ≫ *cis*, much weaker than isoprenaline). In the case of the methylene ethers **5**b, a clear selectivity of DA-like activity was established for the *rac-trans*-isomer although its overall potency was low.[27]



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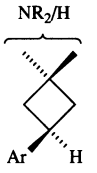
Uptake properties of the cyclobutanes **6**a and **6**b have been reported.[28] *Trans*-**6**a had a greater affinity for postsynaptic receptors and the presynaptic pump than the *cis*-isomer **6**a. In binding experiments, *trans*-**6**a and **6**b displaced [^{3}H]DA 20–30 times more effectively than corresponding *cis*-isomers, but the binding capacity of DA was 20-fold greater than that of the most effectively cyclobutane, *trans*-**6**b.[29]

The feeble activities of these small-ring DA analogues again reveals the low tolerance of DA receptors to the presence of additional saturated hydrocarbon features in their ligands and the results stand in contrast to the high potencies of DA agents of considerably greater complexity.



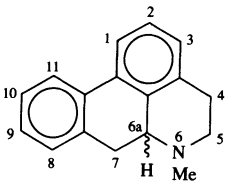
(a) R = H; (b) R = Me; Ar = 3,4-di-OHC_6H_3

6

6.4. Aporphines

Several groups of polyclic molecules include the DA pharmacophore locked within their molecular framework. The first group to receive attention in this

regard was the aporphines **7**, of which apomorphine **8** is the best-known member. Apomorphine is produced by acid-catalyzed rearrangement of morphine, a reaction that results in a molecule with a single chiral center (6a).[30] The chief action of apomorphine is emesis by direct stimulation of the vomiting center of the brain, an effect recognized as dopaminergic during the 1960s. Since that time apomorphine



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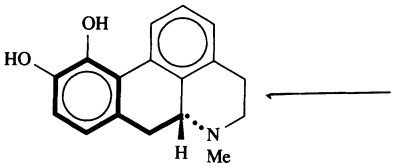

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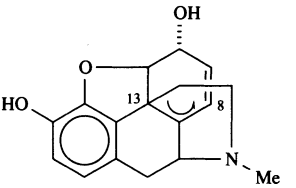
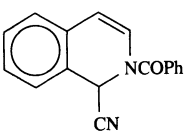
6a -*R*-(–)-apomorphine
Bold lines denote DA (N-MeDA, epinine) pharmacophore



(–)-morphine

has been shown to have DA actions in virtually all biochemical and pharmacological test systems.[31] However, its potency at peripheral sites is well below that of DA (unlike DA it can penetrate the brain), e.g., canine renal vascular bed assay potencies: DA 1.0, epinine 1.0, apomorphine 0.01 (partial agonist),[32] see also references cited by Saari *et al.*[38] The absolute configuration of (−)-apomorphine has been established as 6a-*R* by chemical,[33,34] ORD,[35] and X-ray diffraction[36] methods.

Neumeyer[37] prepared racemic apomorphine by total synthesis from the Reissert anion **9**. Saari *et al.*[38] resolved the racemic material and found the

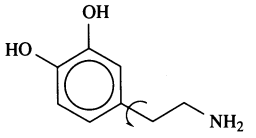


9

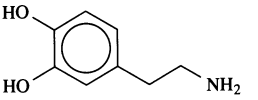
(+)-6a-*S*-isomer to be devoid to activity in three DA test systems (production of postural asymmetries in caudate-lesioned mice, emesis in dogs, renal blood flow increase). In line with these results *RS*-apomorphine proved only half as effective as the levo isomer in producing stereotyped behaviour in rats. *R*-(−)-*N*-*n*-Propylnorapomorphine proved 3 times more potent than its racemate in the same test [there was evidence that the *S*-(+)-antipode behaved as an antagonist, see later].[39] The binding IC_{50} (nM) values (vs.[^{3}H]apomorphine) were 2.5 for the *R*-(−)-*N*-*n*-propyl derivative and 5.0 for the racemic mixture.[40]

R-(−)-Apomorphine binds to both D_1 and D_2 receptors of rat striatal tissue but with more than 20-fold preference for D_2 sites; the *S*-antipode also binds at both sites but with reduced affinities. Reported inhibition constants (K_i nM) vs [^{3}H] SCH-23390 (D_1 antagonist) and [^{3}H] spiperone (D_2 antagonist) were: *R*-(−) 236 (D_1), 11.1 (D_2); *S*-(+) 2,980 (D_1), 58.3 (D_2).[41]

Various hydroxylated aporphines have been examined which, together with data on 2-aminotetrahydronaphthalenes (see below), provide evidence in support of the *trans*-α-rotomer of DA **10** being the receptor bound species rather than the β-form **11**.

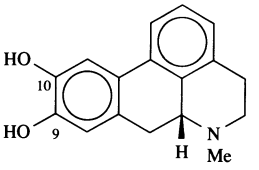


α
10
trans-α-rotamer

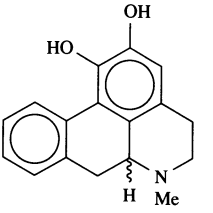


β
11
trans-β-rotamer

Thus the variants 9,10-dihydroxyapomorphine **12** and 1,2-dihydroxyaporphine **13**, both of which include a constrained β-rotamer of DA, were inactive in various DA systems such as the dog emesis assay (test of central postsynaptic DA

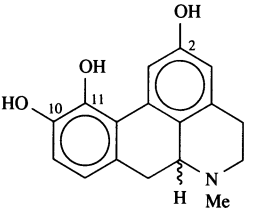


12
9,10-di-OH (isoapomorphine)
trans-β-rotamer



13
1,2-di-OH
gauche-β-rotamer

activity).[42] In binding assays the critical role of configuration at C-6a upon ligand affinity was demonstrated for antipodal forms of the 2,10,11-trihydroxyaporphine **14**: IC_{50} nM vs.[^{3}H]apomorphine, 6a-*R* 11, 6a-*S* 4000, 6a-*R*-apomorphine 1.0.[43] In this work the *R*-antipode was obtained from thebaine, and the *S*-form from bulbocapnine **15**, and naturally occurring aporphine of *S*-configuration at position 6a (X-ray evidence of absolute geometry).[44]



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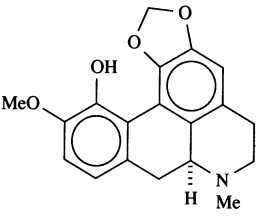
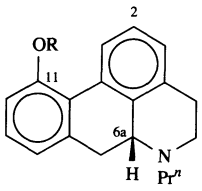


15 bulbocapnine

Stereotypic cage climbing induced by (−)-apomorphine in mice is antagonized by 6a-*S*-(+)-apomorphine (doses of 20–40 mg/kg of the dextro antipode caused a 50% depression in the stimulant action of 10 mg/kg of levo apomorphine.[45] The observation, that *RS*-*N*-*n*-propylnorapomorphine was one-third (not one-half) as potent as the *R*-antipode in another stereotype test (see above), correlates with an antagonist role for less active 6a-*S*-isomers (no antagonism effects were reported in tests employed by Saari *et al.*).[38] Likewise, *S*-(+)-11-hydroxy-*N*-*n*-propylaporphine produced a dose-dependent inhibition of arousal in rats induced by apomorphine. The 6a-*S*-aporphine bulbocapnine **15** is also reported to be a D-1 antagonist[46]; a 10^{-5} M concentration inhibited responses to 100 μM DA. Affinities for the *R*-(−)-antipode of the 11-hydroxy derivative were similar to those of the corresponding apomorphines and ranked as follows: DA agonist sites D-2 > D-1 (Table 6.2).[47] Absolute configurations were established by comparison of resolved materials with the authentic (−)-6a-*R*-antipode derived from morphine.[48]

Agents of this class were also given to rats by the i.p. route and stereotype behavior evaluated for 60 min. A strong dose-dependent induction of stereotyping was seen for apomorphine (ED_{50} 0.25 mg/kg) and *R*-(−)-11-OH **16a** (ED_{50} 0.8 mg/kg); responses to the *S*-(+)-antipode of **16a** and its methoxy analogue **16b**



(a) R = H; (b) R = Me

16

were much weaker ($ED_{50} \gg 10$ mg/kg).[50] Results confirm that hydroxy-substitution of aporphines at the 11-position, homologous to the 3-OH of DA, is critical for affinity and activity at the DA receptor. In contrast, a 10-hydroxyl group (≡4-OH

TABLE 6.2.
Affinity of Enantiomers of Hydroxylated *N*-*n*-Propylnoraporphines for Dopamine Receptor Sites[47]

Compound	Computed values of K_i(nM)[a]			
	[^{3}H]-SCH-233390 (D-1)	[^{3}H] spiperone (D-2)	[^{3}H]ADTN[d] (agonist)	D_2/D_1 potency ratio
R-**8** (NMe replaced by NPr^n)[b]	340	2.4	1.1	142
S	1345	115	278	11.7
(*R*:*S* potency)	(4.0)	(48)	(253)	
R-**16a**[c]	434	2.7	4.0	161
S	1413	105	229	13.5
(*R*:*S* potency)	(3.3)	(39)	(57)	

[a] Using corpus striatum membranes from rat brain.
[b] *N*-*n*-Propylnorapomorphine.
[c] 11-Hydroxy-*N*-*n*-propylaporphine.
[d] *rac*-6,7-Dihydroxy-2-aminotetralin (see later).

of DA) is not essential for D-2 site activation (see below for D-1 however). While *R*-(−)-**16**a potentiated locomotion stimulated by apomorphine, the corresponding *S*-(+)-antipode inhibited it. Aporphines with a catecholamine unit are usually inactive orally—however, both *R*-**16**a and its antipode were similarly potent after oral or parenteral administration.

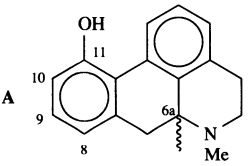
Some 11-hydroxyaporphines have also been examined in binding experiments and test of antagonism of adenylate cyclase activity induced by dopamine.[51] The results of Table 6.3 confirm the preference of *R*- and *S*-apomorphine for D-2 sites and the antagonism of D-1 receptors by the *S*-antipode (see, in addition, results for bulbocapnine). All analogues which lacked a 10-hydroxyl function behaved as D-1 antagonists with *R*-antipodes more effective than their *S*-partners (the difference was minor for the 8-Br pair in regard to effects on adenylate cyclase). A model was proposed for the binding of aporphines to D-1 receptors (Fig. 6.2), and the authors drew attention to evidence for a funcional interaction between D-1 and D-2 receptors in the CNS.[52]

All compounds were obtained from the *R*- and *S*-*N*-methyl analogues of **16**a (the latter antipode was made from the morphine-derived *R*-form by a racemization/resolution sequence) and use of a variety of bromination procedures.

Recently, the D_2-selectivity of the 2-fluoro analogue of *R*-(−)-*N*-*n*-propylapomorphine has been evaluated.[49] It proved remarkably high (D_2/D_1 18 300 compared with 133 and 6 for the parent compound and apomorphine, respectively).

Octahydro-benzo[*g*]quinolines (page 191), close analogues of apomorphine, are discussed later.

TABLE 6.3.
Binding and D-1 Antagonism Data for Some Aporphine Derivatives[51]



Compound (see structure **A**)	C-6a configuration	Binding IC_{50}(nM) vs. [^{3}H]SCH-23390 (D-1)	Binding IC_{50}(nM) vs. [^{3}H]spiperidone (D-2)	Antagonism of adenylate cyclase (% inhibition)[a]
Apomorphine	*R*	432	21	(91.5)[b]
(10-OH)	*S*	3620	680	42.3
(no other	*R*	107	58	87.8
substituent)	*S*	5061	2,242	38.0
(8-Br)	*R*	181	449	83.3
	S	8611	10,000	79.4
(9-Br)	*R*	818	2,579	73.4
	S	1000	1,000	3.2
(10-Br)	*R*	171	664	87.8
	S	3340	2,930	54.2
Bulbocapnine	*S*	739	14,047	85.1
SCH-23390		1.3	10,000	89.7

[a] Inhibition of 10 μM dopamine control in the presence of 10 μM of test compound.
[b] Agonist activity.

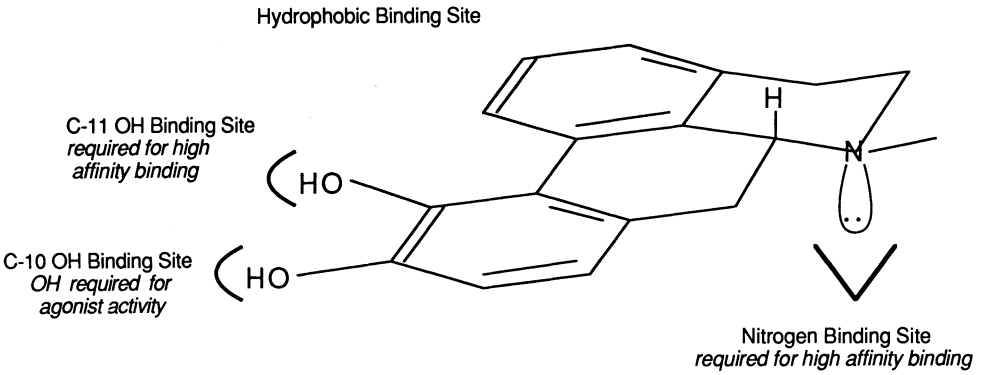
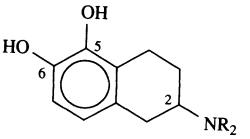


FIGURE 6.2. Proposed mode of binding of aporphines to the D-1 receptor (after Schaus *et al.*).[51]

6.5. 2-Aminotetralins

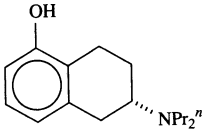
Of the various fragments of apomorphine investigated, 2-aminotetralin derivatives are of special interest in regard to the question of receptor preference for α- or β-rotamers of DA, since they represent another type of restrained DA ligand. Although only limited DA-like activity was observed for 5,6-dihydroxy-2-aminotetralin (**17**a) in dogs (renal vasculature relaxation), the dipropylamino analogue (**17**b) was very effective both in the renal test and as a stimulant of DA-sensitive adenylate cyclase.[53–55]



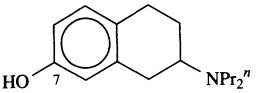
(a) $R_2 = H_2$; (b) $R_2 = Pr_2^n$

17

Several 2-aminotetralins have been resolved and the antipodal pairs compared for DA-like activities.[56] These include the 5-hydroxy **18**, 7-hydroxy **19**, 5,6-dihydroxy **20** dipropylamino derivatives, and 6,7-dihydroxy-2-aminotetralin **21**.*



18
S shown



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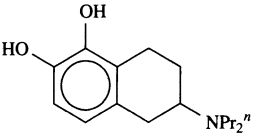

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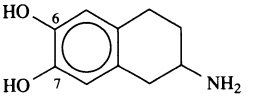


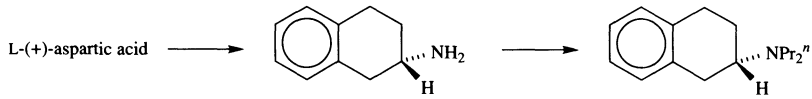
21 (6,7-dihydroxy ADTN)

* 8-Hydroxy-2-dipropylaminotetralin is a potent 5-HT agonist with the 2*R*-levo antipode twice as active as the dextro form.[57]

TABLE 6.4.
Dopaminergic Tests on Antipodal Aminotetralins[60]

	Emesis (μg/kg)	Stereotyped behavior
5-OH (**18**) *RS*	4	0.09
5-OH (**18**) *S*-(−)	2	0.05
5-OH (**18**) *R*-(+)	> 80	> 1.8
Apomorphine	26	0.05

Absolute configurations were established by conversion of the phenolic derivatives to *2S*-(−)-2-dipropylaminotetralin **23** or its antipode by standard deoxygenation methods. The 2*S*-(−)-tetralin **23** was derived from *S*-(−)-2-aminotetralin (**22**) obtained by stereospecific synthesis from *S*-(+)-aspartic acid.[58] All levo isomers have the 2*S*-configuration, equivalent sterically to the 6a-*R*-chiral center of apomorphine. X-ray evidence is also available.[59]



2*S*-(−)-**22** 2*S*-(−)-**23**

Assay data on the 5-hydroxy racemate **18** and antipodes are shown in Table 6.4.[60]

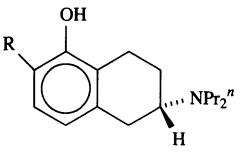
5-OH *S*-(−)-**18** also proved a more effective stimulant of adenylate cyclase in rabbit retina than its antipode.[54] Binding experiments confirmed the greater affinity of *S*-(−)-**18**, and also *S*-(−)-**20** (5,6-dihydroxy), over *R*-antipodes for both DA agonist and antagonist sites on calf striatal membranes (Table 6.5). *S*-Levo antipodes of the 5-hydroxy and 5,6-dihydroxy derivatives are able to present the α-rotamer of the DA pharmacophore to the receptor in the same stereochemical

TABLE 6.5.
Displacement of [^{3}H]-DA Ligands from Calf Striatal Membranes[56, 61]

		Binding IC_{50} values ($\times 10^{-8}$ M)		
Amino substituent		vs. [^{3}H]-spiperone	vs. [^{3}H]-apomorphine	vs.[a] [^{3}H] DA
NPr^n_2	5-OH **18** *S*-(−)	12	8	7.8
	5-OH **18** *R*-(+)	300	220	210
NPr^n_2	5,6-di-OH **20** *S*-(−)	1.0	3.8	
	5,6-di-OH **20** *R*-(+)	16	33	
NPr^n_2	7-OH **19** *S*-(−)	3200	> 10^4	
	7-OH **19** *R*-(+)	3.1	56	
NH_2	6,7-di-OH **21** *S*-(−)	190	24	29
	6,7-di-OH **21** *R*-(+)	8.7	1.4	1.8

[a] McDermed *et al.*[61]

sense as (−)-apomorphine (see **24**). The special interest of the binding data of Table 6.5 lies, however, in the *reversal* of receptor stereoselectivity seen in 7-hydroxy (**19**) and 6,7-dihydroxy (**21**) antipodes.



R = H or OH
24

Seiler and Markstein[55] reported similar differences between antipodal 5-OH and 7-OH amino tetralins (2-NH_2 and 2-NPr^n_2) in their abilities to activate adenylate cyclase and to displace [^{3}H]DA and [^{3}H]spiperone from binding sites.

The *S*-(−)-antipodes of the 7-hydroxy derivatives present the β-rotamer of DA (**11**). The importance of hydroxy positioned at C-5 to binding is clear from the affinity rankings 5-OH > 7-OH > 6 OH for monophenolic derivatives[60,62,63]—the same order is seen in the emesis assays.[60] A positioning of 7-OH that is more close to that of the *S*-(−)-5-hydroxy derivative **18** may be achieved with their *R*-(+)-7-hydroxy analogues by simple rotation of the molecule through 180° (**25a** → **25b**). Superimposition of molecules *S*-(−)-**18** and *R*-(+)-**25b** then allows a near (but not exact) coincidence of the phenolic hydroxyls and basic centers. In both molecules the NPr^n_2 group is directed below the plane of the near-planar structures.

25a **25b**

Figure 6.3 shows Freeman and McDermed's representation of the interactions of *S*-(−)-5-OH, *R*-(+)-7-OH, and *R*-(−)-apomorphine at a schematic DA receptor.[56] The figure also includes inactive isoapomorphine and shows why its *S*-(+)-antipode cannot adopt the same strategy as the *R*-(+)-7-OH tetralins because of its additional molecular bulk (cf. the receptor boundary proposal of Grol and Rollema[64] to account for the inactivity of isoapomorphine). To challenge this idea 5- and 8-propyl analogues of 6,7-dihydroxy-2-aminotetralin were examined as competitors of [^{3}H]-DA. The 8-propyl derivative (with its bulky propyl substituent directed away from the receptor surface—see Fig. 6.3) proved to be a fairly potent ligand, while the 5-propyl analogue (substituent directed toward the surface) had a much lower affinity.

An Uppsala group[65] examined antipodal 5-hydroxy **18** and 7-hydroxy tetralins **19** in tests to assess activity at pre- and postsynaptic DA receptors (see page 183). Both *S*-**18**-(5-OH) and *R*-**19**-(7-OH) were effective in DOPA accumulation (presynaptic) and motor activity (postsynaptic) tests and far exceeded the potencies of their respective antipodes in these respects—results in accord with the binding data of Table 6.5.

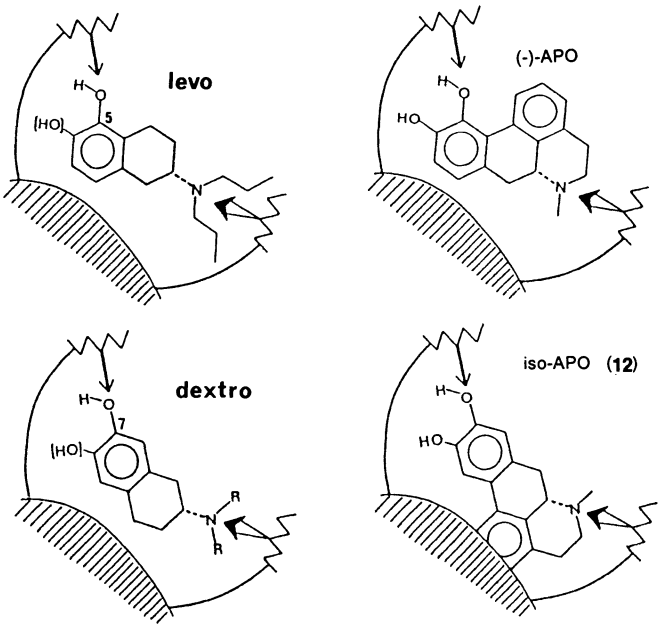
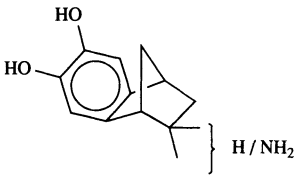


FIGURE 6.3. Binding of some aminotetralins and aporphines to a schematic receptor (after Freeman and McDermed).[56]

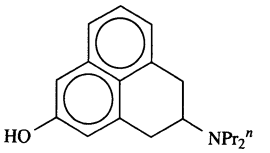
In a more recent report,[66] in which antipodes of high optical purity ($R >$ 99.7 ee) were used, the *S*-antipode (0.05 μmol/kg sc) was confirmed as a potent D_2 agonist in the DOPA accumulation test while the *R*-isomer acted as an antagonist (raising DOPA levels over those of a control) when given at high dose levels (5 and 50 μmol/kg); cf. results for 1-methyl analogues, page 178). Optical purities of the antipodes, derived from resolved 2-benzylamino-5-methoxytetralin intermediates, were established by chiral ion-pair chromatography using *N*-benzyloxycarbonyl-glycine-L-proline as the counterion and a porous graphite column. The authors present a table of previous 5-OH-DPAT DA activity comparisons in which the eudismic ratio varies from 10 to 176—the width this range is probably due to variations in the optical purity of materials used, especially those of the *R*-distomer.

Rigid analogues of 2-amino-6,7-dihydroxytetralin **21** based on benzonorbornene (**26**) were ineffective in dopamineric binding and behavioral tests.[67,68] However, the DA-agonist properties of 2-dipropylamino-7-hydroxytetralin were retained following cyclization across the 4,5-positions to give the phenalene derivative **27** (U-66444B).[69]



endo and *exo*

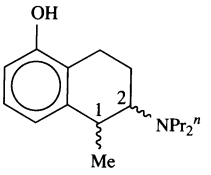
26



27

Interest in this compound has been directed at its selectivity for presynaptic receptors, which reduce DA synthesis when activated by a feedback mechanism (see page 183).[70,71] The phenalene **27** possessed such properties, as judged by its ability to decrease striatal 3-methoxytyramine (the proximate metabolite of DA) and to inhibit locomotor activity in rats. In these tests the dextro antipode was the more potent isomer (Table 6.6)—it would be of interest to compare its absolute configuration with that of *R*-(+)-dipropylamino-7-hydroxytetralin.

Several methylated 2-aminotetralins have been examined. Both racemic *cis*- and *trans*-1-methyl-2-dipropylamino derivatives **28** had similar low potencies at



28

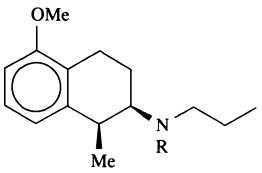
central DA receptors (DOPA accumulation and motor activity test).[72] The *cis*-diastereoisomer has been resolved and configurations established by X-ray crystallography.[73] The 1*R*,2*S*-levo isomer reduced DOPA formation in both reserpinized and nontreated animals with a potency approaching that of apomorphine, while the 1*S*,2*R*-isomer increased DOPA level in nontreated animals revealing its

TABLE 6.6.
Effect of Enantiomers of U-66444B (27) on Striatal 3-Methoxytyramine in Mice, and Antagonism of (+)-Amphetamine-Stimulated Motor Activity[70]

Antipode	Dose (mg/kg sc)	Striatal 3-MT (ng/mg)
(−)	0.3	10
	0.03	25
	0.003	28
(+)	0.3	11
U-66553	0.03	11
	0.003	21
(saline)		24
		Inhibition of motor activity (%)
(−)	0.1	63[a]
	0.1	32
	0.001	27
(+)	0.1	41[a]
	0.01	79[a]
	0.001	39[a]
	0.0001	32

[a] $p < 0.05$.

antagonistic action at presynaptic sites (see page 183 for methodology). A large group of antipodal pairs of *cis*-1-methyl-2-aminotetralins were examined for central DA agonist and antagonist activity and binding to DA receptors (*in vivo* interactions with *N*-diPr^n-5,6-ADTN).[74] Both 2*R*- and 2*S*-enantiomers were able to bind to DA receptors but only 2*S*-antipodes produced pharmacological results. O-Methylation of 1*S*,2*R*-2-amino-1-methyltetralins enhanced their DA receptor antagonistic activity. Both **28a** (R = H, AJ76) and **28a** (R = Pr^n, UH232) markedly elevated DA



28a 1*S*, 2*R*

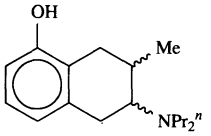
synthesis and turnover in brain, and antagonized locomotor hyperactivity induced by apomorphine (UH232 was the more potent in this respect).[75] In tests on the recently cloned D_3 receptor, AJ76 and UH232 proved 3- to 4-fold more potent at the D_3 than the D_2 receptor.[10] Some data are shown in Table 6.7. In further binding experiments 1*R*,2*S*-**28** was found to displace [^{3}H]-spiroperidol (spiperone) twice as effectively as its 1*S*,2*R*-antipode (antagonist); a 9-fold difference was seen vs. [^{3}H]-*n*-propylnorapomorphine (NPA). By contrast 2*S*-5-OH DPAT and 2*R*-5-OH DPAT differed far more in their affinities ($\times$ 120 vs. spiroperidol, $\times$ 27 vs. NPA), due presumably to the influence of 1-methyl which has an adverse influence on the binding of 2*S*-5-OH DPAT with little effect (or enhancement) on that of the 2*R*-isomer (e.g., pIC_{50} 6.24 $\rightarrow$ 5.42 for 2*S*; 4.15 $\rightarrow$ 5.15 for 2*R* vs. spiroperidol).[76] All four diastereoisomers of the 3-methyl analogues **29** were later examined in the same

TABLE 6.7.
Pharmacological and Binding Data on Antipodes of *cis*-1-Methyl-2-di-*n*-propylaminotetralin (5-OH and 5-OMe)[74]

	Dose (μmol/kg sc)	Di-Pr-5,6-ADTN (**17b**) binding[a]	DOPA accumulation[b] ED_{50} (μmol/kg sc)	
			Limbic	Striatum
1*S*,2*R*-**28** (OH)	10	61	10 (240%)	9.4 (340%)
(antagonist)	40	21		
1*R*,2*S*-**28** (OH)	10	90	0.84 (51%)	0.84%
(agonist)	40	37		
1*S*,2*R*-**28** (OMe)	3.2	84	12.8 (285%)	9.6 (380%)
(antagonist)	13	61		
	52	34		
	204	31		
1*R*,2*S*-**28** (OMe)	22	106	I	I
	45	94		

[a] Striatal level of Di-Pr-5,6-ADTN (after subtraction of cerebellum blank) expressed as percent of Di-Pr-5,6-ADTN controls (see references cited).[74]
[b] No pretreatment with reserpine.

tests.[77] The 2*R*,3*S*-levo antipode of *cis*-**29** proved a potent DA agonist in central tests (DOPA results shown alongside **29**)—all other isomers were of low activity. It is to be noted that 2*R* of the 3-methyl derivative = 2*S* of the 1-methyl molecule, hence there is a configurational correlation between the more potent antipodes of the two series. The compound approached the potency of its 3-desmethyl steric analogue (2*S*-5-hydroxy DPAT) and was about ten times as effective as apomorphine in the same test. X-ray crystallography was used to solve the stereochemistry.

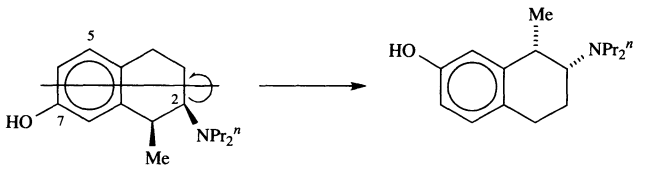


29

DOPA accumulation μmol/kg limbic[a]	
(+)-trans	2.5 (35%)
(−)-*trans*	3.5 (35%)
(+)-*cis*	inactive
2*R*,3*S*-(−)-*cis*	0.005 (35%)

[a] Doses giving half-maximal decrease of DOPA formation; minimal levels are shown in parentheses, control = 100%

In the case of 1- and 3-methyl derivatives of 7-OH-DPAT, the most active DA agonist proved to be the 1*S*,2*R*,-1-methyl derivative **30a**, itself with one-seventh the potency of 2*R*-7-OH-DPAT in the DOPA accumulation and locomotor activity tests.[78] All other compounds of this series had lower activities (Table 6.8). Again, absolute configurations were based on X-ray crystallography evidence. Rotation of **30a** about the axis shown produces a structure **30b** which may reasonably be superimposed on that of 2*R*,3*S*-**29**, the most potent *5-OH*-3-methyl isomer (cf. correlation of *S*-5-OH and *R*-7-OH aminotetralins, page 176).



30a **30b**

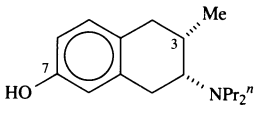
The reverse chirality relationships at the C-2 center of 5-OH- and 7-OH-DPAT eutomers appears to extend to derivatives which behave as antagonists. Thus 2*R*,3*S*-**31** proved to be the more effective antipode in blocking presynaptic DA_2

TABLE 6.8.
DA Activities of Some 7-Hydroxytetralins (No Resperine Pretreatment)[78]

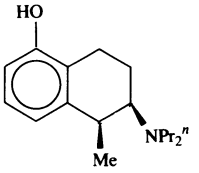
	DOPA accumulation ED_{50} μmol/kg sc		Locomotor activity total counts after 30 min (dose: μmol/kg sc)
	Limbic	Striatal	
1*S*,2*R*-**30** (1-methyl)	0.07 (35%)	0.06 (20%)	50 ± 4 (4.0)[a]
1*R*,2*S*-**30**	3.5 (35%)	3.0 (20%)	20 ± 13 (16.0)
2*R* 7-OH DPAT (**19**)	0.01 (35%)	0.01 (20%)	46 ± 18 (0.31)[b]
2*S* 7-OH DPAT (**19**)	1.9 (35%)	2.4 (20%)	33 ± 6 (39.0)[b]

[a] $p < 0.001$.
[b] $p < 0.05$ *vs* saline controls.

receptors as judged by the DOPA accummulation test—its C-2 chirality is the opposite of that of 1*S*,2*R*-**32**, also an antagonist.[79] The stereochemistry of **31** was established by X-ray crystallography of its HCl salt.

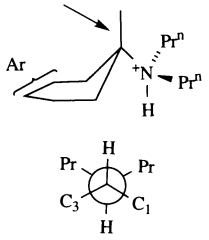


2*R*,3*S*-**31**



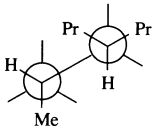
1*S*, 2*R*- **32**

Conformational studies of 2-aminotetralins carried out by a combination of X-ray crystallography, NMR spectroscopy, and molecular mechanics (MMP2) calculations have led to the conclusion that the optimal arrangement for DA-agonism is a half-chair tetralin nucleus with a pseudoequatorial amino substituent (**33**). Further, a C_1-C_2-N-H (τ_N) dihedral angle close to 60° also appears to favor uptake at DA receptor sites.[76] The last requirement accounts for the differing affinities of the 1*R*,2*S*- and 1*S*,2*S*-1-methyl-5-hydroxy derivatives.



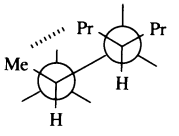
33

In the strongly bound 1*R*,2*S*-ligand a τ_N value of 60° is possible, since 1-methyl does not interact with the NPr^n substituents in this conformation **34**. The same conformation is unfavored, however, in the poorly bound 1*S*,2*S*-diastereoisomer where serious nonbonding interactions between 1-Me and an *n*-Pr group must arise (**35**). The same arguments explain the superior affinity of the corresponding 2*R*,3*S*-3-methyl congenor (*cis*) over the 2*R*,3*R* (*trans*) isomer.[77]



1*R*

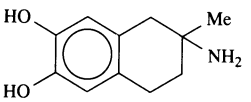
34



1*S*

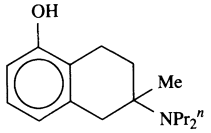
35

C-2-Methylation of 6,7-ADTN giving **36** abolished the DA_1 agonist activity of the parent (no vasodilation in dog renal artery)[80]—a result consistent with the lack of activity of α-methyl DA at this receptor.[81]



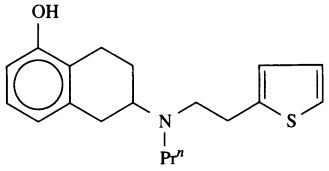
36

The 2-methyl derivative **37** similarly proved to lack dopaminergic acitivity,[82] as confirmed by a recent study of antipodes which also gave evidence that *S*-**37** affects 5-HT turnover.[157]



37

A Dutch group has investigated analogues of 2-dipropylamino-5-hydroxytetralin in which one of the N-propyl groups is replaced by a 2-arylethyl substituent.[83] Among several potent agonists, the *N*-2-thienylethylamino derivative **38**

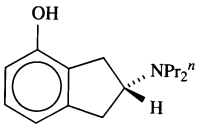


38

(N-0437) has received special attention. The [^{3}H]-analogue (obtained by reducing the N-propargyl precursor with tritium) binds with a high affinity (K_D=0.17 nM) to calf caudate membranes and is recommended as a useful tool in studies of central DA receptors.[84] The levo antipode of **38** displaced this radioligand 142 times more effectively than the dextro form. However, in functional tests antipodal differences were less clear-cut.[85] In presynaptic models (antagonism of γ-butyrolactone -induced DOPA elevations and the induction of hypomotility) both enantiomers exhibited a similar degree of potency. In postsynaptic tests (induction of stereotypy in rats and rotation in 6-hydroxydopamine-lesioned rats), the levo isomer behaved as an agonsit while its (+)-antipode was inactive. The postsynaptic agonist properties of (−)-**38** were confirmed by its inhibition of release of [^{3}H]acetylcholine from rabbit striatal slices (see page 192 and Ref. 112); (+)-**38** behaved as an antagonist in this test. Thus the profiles of (−)- and (+)-**38** differ at pre- and postsynaptic D-2 receptors. No absolute configurations have been reported. The enantiomeric purity of resolved forms of **38** was established by an HPLC method which involved precolumn glucuronidation catalyzed by uridine 5′-diphosphoglucuronyltransferase (UD PGT).[86] Data concerning the influence of the antipodes **38** on dopamine release have also been obtained in conscious rats by use of intracerebral dialysis.[87] Levo N-0437 (i.p.) induced a 60% decrease in DA release (DA, DOPAC, and HVA quantitated by HPLC) which was still seen after destruction of postsynaptic sites by kainic acid. Stereotyped behavior was also apparent, hence the levo isomer is both a pre- and a postsynaptic agonist. Dextro N-0437 did not affect DA release under these conditions and failed to induce stereotypy.

Indans related to the 2-aminotetralins also display dopaminergic properties with receptor selectivity for the *R*-antipode. Thus in a test on cat atrium (inhibition of tachycardia, effects reversed by haloperidol) ID_{50} values were 0.007 μM for

R-**39**, 0.72 μM for *S*-**39**, and 0.007 μM for apomorphine.[88] Configurations were established by X-ray analysis of the D-tartrate of a 4-methoxy precursor which had a solid state conformation with a pseudoaxial amino substituent. A configurational relationship with *S*-**18**, 2-aminotetralin eutomers, is apparent. A molecular mechanics (MM2) conformational analysis of *R*-**39** and its 4-methoxy analogue has shown the phenol and the methyl ether to be more stable in the N-equatorial and N-axial conformations, respectively.[89] The authors argue that an equatorial NPr^n_2 conformation places the basic group close to the aromatic ring plane which is one of several prerequisites for potent DA receptor agonism.



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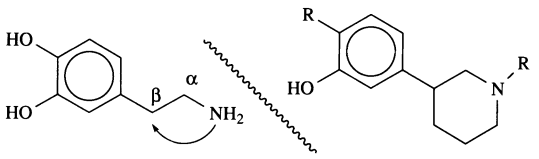
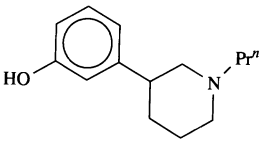
6.6. 3-Phenylpiperidines

This class **41** represents a constrained form of DA in which basic nitrogen is linked to the β-carbon of the phenethylamine chain (see **40**). The compound of principal interest is the mono-phenol, *N*-*n*-propyl derivative (**42**), well known as



40 **41**



42

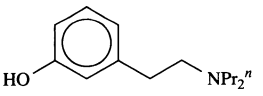
3-PPP. It lacks D-1 agonism effects; e.g., it fails to stimulate the D-1 receptor of carp retina,[90] but is active at DA (D-2) autoreceptors at modest levels of potency.[91] Autoreceptor activation was investigated by use of the phenomenon of receptor-mediated feedback inhibition of the presynaptic neurone. Thus, the synthesis rate of DA and NA is inhibited by agonists (and activated by antagonists) at DA and α-adrenergic receptors, respectively. The DOPA accumulation following decarboxylase inhibition by means of 3-hydroxybenzylhydrazine (NSD 1015) is used as an indicator of the DA-synthesis rate in DA-rich areas (limbic system, corpus striatum) and the NA-synthesis rate in NA-dominant areas (cortex). HPLC procedures with electrochemical detection provide a sensitive assay for DOPA.[92] Tissue obtained from rats pretreated with resperpine is often used in this assay in order to render the D-2 receptors supersensitive (Wikström, private communication, 1989). A test compound which behaves as an antagonist in tissue where endogenous agonist is present may perform as an agonist in reserpinized tissue. This was the case for (−)-*S*-3-PPP, classified as a partial agonist, but not for its dextro antipode which was an agonist in both models (Table 6.9, footnotes *c* and *d*). A second biochemical test measures the effect of the test compound on striatal

TABLE 6.9.
Biological Data for 3-PPP and Standard DA Agonists in Reserpinized Tissue[95, 96]

	Binding[a] IC_{50} (nM)	DOPA accumulation[b] ED_{50} (μmol/kg) limbic	Dose (μmol/kg)	Motor activity total counts after 30 min
R-(+)-3-PPP	1710	1.0[c]	13	78 + 14[e]
S-(−)-3-PPP	420	0.8[d]	213	12 + 2 ns
Apomorphine		0.19	2.3	361 + 42
S-5-OH-2-NH_2 tetralin		3.7×10^{-3}	0.31	155 + 27
R-7-OH-2-NH_2 tetralin		9.5×10^{-3}	0.31	46 + 18

[a] Displacement of [^{3}H]-5,6-di-OH DPAT and [^{3}H]-SCH 23390 (D-2 ligands).
[b] Dose giving a half-maximal decrease of DOPA formation estimated from a dose-response curve.
[c] 0.8 (86% control)[e] in nonreserpinized tissue.
[d] 213 (95% control, ns)[e] in nonreserpinized tissue.
[e] Significant; ns, nonsignificant.

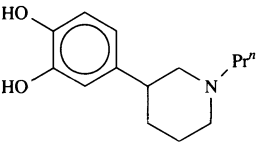
levels of the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Postsynaptic effects were assessed by behavioral tests—*increase* in locomotor activity and the induction of stereotyped behavior. Racemic 3-PPP was about one-quarter as active as the acyclic analogue (**43**) in producing a half-maximal decrease of the DOPA level in rat brain.[91] Several studies of antipodal forms of 3-PPP have been reported; the configurations *S*-(−)[93] and *R*-(+) (methoxy derivative)[94] were established by X-ray crystallography. An efficient and inexpensive resolution of 3-PPP has been described.[154]



43

In binding tests (displacement of the D-2 ligand [^{3}H]-N-0437, **38**) the following IC_{50} (nM) values have been reported: (−)-3-PPP 90.0, (+)-3-PPP 650, dopamine 8.1.[84] In tests on seven *RS* pairs, all *R*-(+)-isomers behaved as classical DA agonists with affinity and intrinsic activity for both pre- and postsynaptic receptors of the type discussed in this section (Table 6.9).[95,96] The same bifunctional profile was valid for *S*-(−)-isomers with *N*-substituents larger and bulkier than *n*-propyl. However, the *S*-(−)-*N*-ethyl and *N*-propyl derivatives had affinity for both sites but behaved as *agonists* at pre- and *antagonists* at postsynaptic sites. The antagonist actions of *S*-(−)-isomer were first revealed by its ability at an sc dose of 27 μmol/kg to counteract locomotor hyperactivity in rats induced by apomorphine (1 mg/kg sc.)[97] Activity induced by (+)-amphetamine was also attenuated by this isomer. The racemate (*RS*-3-PPP) decreased DA-synthesis in rat brain, an effect blocked by haloperidol, and failed to produce signs of postsynaptic DA-receptor activation.[98] Thus *S*-(−)-3-PPP has the ability to attenuate DA function in two ways.[92] Supporting pharmacological data on (−)-3-PPP is included in a paper reporting a fused-ring analogue (**27**) of the 2-aminotetralin group (page 177).[70]

Metabolic effects (hydroxylation to the catechol **44**, 1–5%) and O-methylation (both isomers are good substrates for COMT) were not considered to influence pharmacological profiles of *R*-(+)- and *S*-(−)-3PPP significantly.[99]

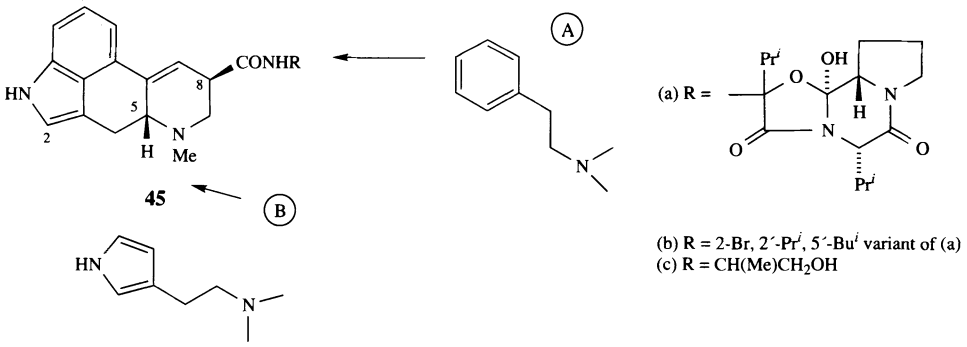


44

In studies of the binding of tritiated [^{3}H]-*R*-(+)-3PPP, its characteristics were found to resemble those of ligands specific for σ-opioid receptors such as SKF 10,047 (see Chap. 13, page 461) rather than DA ligands (although it can displace radiolabeled forms of the latter).[100] In an extensive study of the σ-affinities of a variety of ligands, judged by their ability to displace *R*-(+)-[^{3}H]-3-PPP, haloperidol proved to have the highest affinity (IC_{50}3nM) while *R*-(+)-3PPP (IC_{50} 32 nM) bound about five times more effectively than its *S*-(−)-antipode (IC_{50}165 nM).[101]

6.7. Ergot Derivatives

The ergolines **45** present a further polycyclic system on which DA agonists are based, the phenylethylamine pharmacophore being represented either by **45**,A or pyrrole CH_2CHN (**45**,B). There are many examples of ergolines with dopaminergic properties, such as ergocornine **45**a, bromocriptine **45**b (the latter is in clinical use, *Martindale* **29**, page 1011), and ergometrine **45**c. In general, ergots



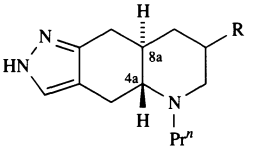
(b) R = 2-Br, 2′-Pr^i, 5′-Bu^i variant of (a)
(c) R = $CH(Me)CH_2OH$

produce a great number of pharmacological actions. Their DA effects include inhibition of prolactin secretion, decreased turnover of DA in the CNS, and displacement of DA ligands from dopaminergic binding sites.[102] They also have direct DA-like actions in the lesioned rat model, and produce stereotypy and emesis.[103] The absolute configuration of naturally occurring derivatives is 5*R*,8*R*.[104,105] Access to antipodal pairs is rare in this group and few isomeric com-

parisons of activity have been made. Burt and others[24] found that 5*R*,8*R*-(+)-lysergic acid diethylamide, LSD, **45** (8-CONHR replaced by $CONEt_2$) effectively displaced [^{3}H]-haloperidol and [^{3}H]-DA from DA binding sites in calf caudate tissue with the levo isomer several orders less active. The C-8 configuration also appears important, since C-8 isobromocryptine is inert as a DA agonist[106] (see also Bach *et al.*).[107] Ergolines with 8-amido substituents have a tendency to epimerize to the corresponding 8-*iso*-diastereoisomer which is generally stated as being pharmacologically inactive.[108]

The configuration of bromocriptine (5*R*,8*R*) has also been established by X-ray crystallography.[109]

During the course of the synthesis of some partial structures of ergoline, a Lilly group discovered two pyrazoles, **46**a and **46**b, of high dopaminergic activity in lowering prolactin levels in reserpinized rats, and the 6-hydroxydopamine-lesioned rat turning assay.[107] The *trans*-4a/8a ring junction was established by X-ray crystallography. In a later paper[110] the pyrazole **46**a was resolved and the levo antipode (termed *quinpirole*, 4aR configuration by X-ray analysis) found to be the more potent antipode by a large margin in a variety of test for DA ligands, including binding assays (IC_{50} nM *vs* [^{3}H]apomorphine: (−)-**46**a 71, (+)-**46**a 3900; pergolide 1.6).

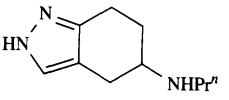


(a) R = H (LY-141865) levo isomer is quinopirole

(b) R = CH_2SMe (as in pergolide, see Table 6.10)

46

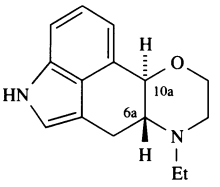
The pyrazole **46**a is reported to be selective for D-2 receptor,[111] a claim supported by its failure to stimulate DA-sensitive adenylate cyclase (a D-1 assay) and ability to activate striatal D-2 receptor modulation of ACh release (agonists inhibit release).[112] The significant DA activity of **47**, a bicyclic analogue of *rac*-quinpirole, supports the idea that both pyrrole and pyrazole ring systems can function as a catechol bioisostere.[113]



47

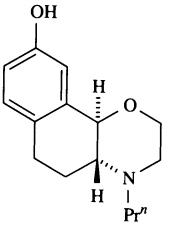
Further evidence of configurational requirements at C-5 of ergolines (≡C-4a of the pyrazole **46**) comes from work on 9-oxaergolines.[114] The most active N-ethyl derivative **48** was resolved and DA-like activity shown to reside chiefly in the levo isomer of 6a*R*,10a*R* configuration—see legend.[117] X-ray analysis of its salt with di-*p*-toluoyl-(+)-tartartic acid[115] established the stereochemistry. Related 2*H*-naphth[1,2-*b*]-1,4-oxazines **49** were active in the same DA assays with receptor selectivity for the (+)-1a*R*,4a*R*-antipode of the N-Pr^n derivative (see legend); the

compounds were specific for D-2 sites, since they failed to stimulate *c*AMP synthesis in the carp retina assay. These compounds relate more closely to 3-PPP (**42**) than to the ergolines—the DA-receptor affinity of *rac*-**49** fell 10-fold when the 9-OH substituent was absent.



48

Isomer	DA binding vs. apomorphine IC_{50}(nM)	Contralateral turning in 6-OHDA lesioned rats ED_{50}(mg/kg)
6a*R*,10a*R*-(−)	2.0	0.027
6a*S*,10a*S*-(+)	219	7.5



49

	DA receptor binding vs. apomorphine IC_{50}(nM)	Contralateral turning in 6-OHDA lesioned rats ED_{50}(mg/kg), ip
1a*R*,4a*R*-(+)	23	0.005
1a*S*,4a*S*-(−)	25,300	30.0
trans-rac (1a,4a)	19.6	0.006
cis-rac	19,600	15.0

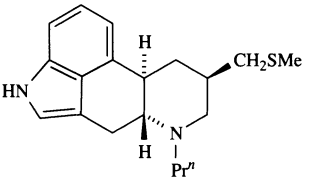
Antipodal 9-oxaergolines **48** and oxazines **49** also bind to α_2-adrenoceptors (*vs* clonidine) with the same stereoselectivites. Another report, however, claims that (−)-**48** is mainly responsible for the dopaminergic actions while α_2-activity is due to the *dextro* antipode.[116] Correlation of data for a series of the more potent antipodes (eutomers) of ergolines and their structural analogues is shown in Table 6.10.

Relationships to eutomers of 2-aminotetralins are likewise evident. It is noteworthy that the stereochemical correlations of Table 6.10 require that the rigid pyrrole-ethylamine fragment of ergolines and their relatives be regarded as the DA pharmacophore (see **45**,B; also Wikström *et al.*).[118] If phenethylamine be deemed

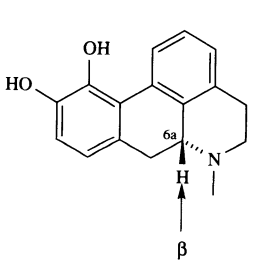
TABLE 6.10.
Dopaminergic Activities of the Eutomers of Some Ergolines and Related Compounds[117]

Compound	Structure	DA receptor binding IC_{50} (nM) *vs* apomorphine	Contralateral turning in rat ED_{50} (mg/kg)
9-Oxaergoline	**48**	2	0.027
Pergolide	*b*	3.1	0.10
LY-141865	**46a**	—	0.05[a]
(+)-1,4-Oxazine	**49**	23	0.005
Bromocriptine	**45b**	27.9	2.8
6a*R*-Apomorphine	**8**	1.1	0.14

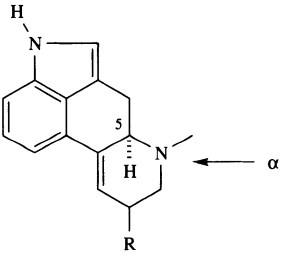
[a] Ref. 107.
[b] Structure:



the pharmacophore, then structures of type **51** would be required for correlation with apomorphine **50**—structures in which orientations at the secondary carbon α to nitrogen do not correspond (see also page 203 of Ref. 4).



6a*R*-**50**

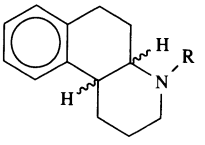


5*R*-**51**

Camerman *et al.*,[109] provide a stereoscopic view of the ergoline system of bromocriptine superimposed on *R*-(−)- and *S*-(+)-apomorphine.

6.8. Octahydrobenzo[*f*]quinolines

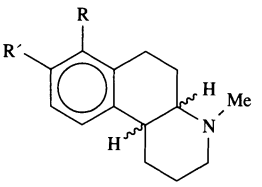
Cannon investigated the reduced benzoquinolines (**52**) in an attempt to attain conformationally discrete DA-like molecules.[1] The compounds may also be



52

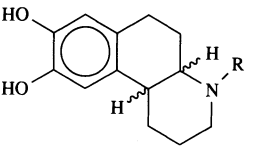
regarded as fragments of apomorphine bridged across N-methyl and C-7 carbons (also bridged 3-PPP and 2-aminotetralins). The work of his group covered racemic *cis*- and *trans*-fused derivatives. Ring junction stereochemistry was established by IR (observation of Bohlmann bands in the range 2700–2900 cm^{-1} for *trans*-derivatives) and NMR (PhCH_2 resonance of N-benzyl derivatives was a 4-line AB system in a *trans*- compared to two closely placed lines in the *cis*-derivative). X-ray analysis later confirmed these assignments.[119]

In a variety of derivatives *trans*-fused isomers were found to be more potent than corresponding *cis*-isomers. Thus relative emetic activity in dogs (apomorphine = 1) were: *trans*-**53**a 0.03, *cis*-**53**a inactive; *trans*-**53**b 6.61, *cis*-**53**b 0.02.[120] Both *cis*- and *trans*-**53**c were inactive. Doses inducing stereotypy (mg/kg sc) were 0.5–2.0 for apomorphine and 0.025–0.05 for *trans*-**53**b (N-Pr^n analogue); the *cis*-isomer was inactive in this test.[121]



(a) R = OH, R′ = H; (b) R = R′=OH; (c) R = H, R′ = OH

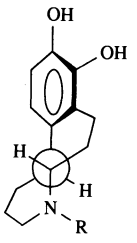
53



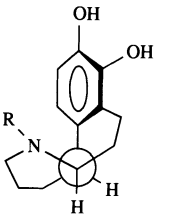
54

Dose to inhibit right cardioaccelerator nerves (μmol/kg)
trans 0.009 (R=Pr^n)
cis 0.45 (R=Pr^n)
antagonist by haloperidol

The positional isomer (**54**) displayed peripheral rather than central DA actions, again with *trans*-isomers the more active (see legend). The secondary amines **54** (R = H) were sympathomimetics with effects on cardiac nerves (*trans* 56 × *cis*) antagonized by phentolamine. Cannon rationalized the results in terms of *trans*-isomers (**55**) being essentially planar molecules (like apomorphine), which could attain an antiplanar Ar/N conformation. The more flexible *cis*-derivatives (**56**), in contrast, can exist in two interconvertible conformations neither of which are planar or provide the antiperiplanar disposition. It is noteworthy, however, that isomers of type **54**, include the β-rotamer of DA rather than the more effective α-variety.



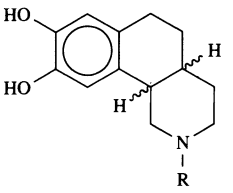
55 *t*



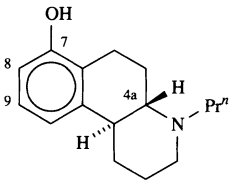
56 *c*

The series **57** had only weak DA-like actions in cardiovascular assays.[122] Wikström and others examined the *cis*- and *trans*-7-hydroxy-4-*n*-propyl derivatives (**58**) and found only the *trans*-isomers to be active in dopaminergic tests (DOPA accumulation and motor activity in rats)[123]; the 4a*S*-antipode **58** was the eutomer.

The *trans*-9-hydroxy analogue of **58** was particularly active in these tests (*cis* feeble or inactive) while the *trans*-8-hydroxy derivative showed the lowest potency of the three *trans*-compounds.



57



58

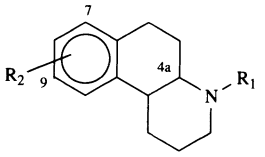
In further work[119] *cis*- and *trans*-**58** were resolved and antipodes examined in biochemical and behavioral tests (Table 6.11). Configurations were established by X-ray crystallography.[124] The results confirm the feeble activity of *cis*-stereoisomer and show that the 4a*S*,10b*S*-isomer is the more potent of the *trans*-7-OH-antipodes; eutomers are nonselective in stimulating both DA autoreceptors and postsynaptic receptors.

TABLE 6.11.
Pharmacological Data for Some Antipodal Octahydrobenzo[*f*]quinolines[119,125]

Compound[a]	DOPA accumulation ED_{50} (nmol/kg) limbic	Motor activity		Binding (D-2)[b] IC_{50} (nM)
		Dose (nmol/kg)	Accumulated counts (30 min)	
cis (**59**a) 4a*R*,10b*S* (7-OH, N–Pr^n)	*c*	50,000	31 + 23 ns	—
trans (**59**a) 4a*R*,10b*R*	320	3,100	59 + 11	160
trans (**59**a) 4a*S*,10b*S*	14	3,100	78 + 11	34
trans (**59**b) 4a*R*,10b*R* (7-OH, NH)	50,000	not tested		1550
trans (**59**b) 4a*S*,10b*S*	620	not tested		92
trans (**59**c) 4a*R*,10b*R* (9-OH, N–Pr^n)	4	1.06	155	25
trans (**59**c) 4a*S*,10b*S*	250	10.6	28	520
Apomorphine	190	not tested		not tested

[a] Optical purities, established by HPLC of diastereoisomeric amides formed by reaction with the acid chloride of *R*-(−)-*O*-methylmandelic acid, were 96% or greater.
[b] *vs* [^{3}H]spiperone.
[c] Inactive.

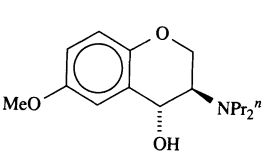
Further biological data were reported in 1987[125] on antipodes of the secondary amine and 9-hydroxy analogues of **59**—these are included in Table 6.11.



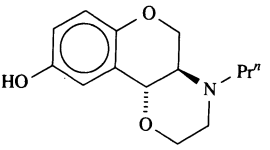
(a) R_1 = H, R_2 = 7-OH; (b) R_1 = Pr^n, R_2 = 7-OH;
(c) R_1 = Pr^n, R_2 = 9-OH

59

The configurational inversion in regard to eutomer geometry which follows replacement of 7-hydroxyl by 9-hydroxyl is striking and corresponds exactly with results found for related derivatives of the 2-aminotetralins (page 176).



60



61

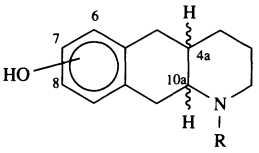
Isomer (**61**)	IC_{50} (nM)[a]	DOPA accumulation[b]	**61** (4a*R*,10b*R* shown) Inhib of LMA[c] ED_{50} (mg/kg) ip
4a*R*,10b*R*	490	88.4	5.4
4a*S*,10b*S*	$>10^5$	0	>30
apomorphine	27	100	0.3

[a] Binding *vs* [^{3}H] haloperidol.
[b] % age reversal of the γ-butyrolactone-stimulated increases in DOPA, at dosage of 30 mg/kg ip.
[c] LMA denotes locomotor activity in mice.

Findings with analogues of 9-hydroxy (**59**) with two oxygen-containing rings add further confirmation to stereochemical relationships established for DA agonists.[126] The racemic mixture **61** proved to have moderate affinity for D-2 and a profile consistent with that of a DA autoreceptor agonist. The eutomer was the 4a*R*,10b*R*-isomer—in accord with the eutomer stereochemistry of DA agonists of the same phenolic OH/basic center positioning. The absolute configuration was established by X-ray crystallography of the intermediate **60** as a salt with (+)-mandelic acid.

6.9. Octahydrobenzo[*g*]quinolines

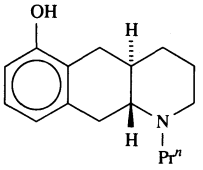
This group, closely related to aporphines, provides another means of restraining the 2-amino substituent of phenolic 2-aminotetralins. A series of derivatives **62**



62

have been examined in regard to their ability to stimulate DA-sensitive adenylate cyclase (a D-1 receptor assay) and striatal D-2 receptors that modulate ACh release.[112] Some of the data are shown in Table 6.12.

Results show that the racemic 6-hydroxy derivative **63** is active at both D-1 and D-2 sites, and that the more potent antipode is the 4a*R*,10a*R*-isomer [X-ray crystallography performed on the *R*-(−)mandelate salt]. Potencies at the D-2 sites are about 1000-fold greater than at D-1 sites. When N-Pr^n of **63** was replaced by *n*-butyl, activity was zero in both assays. The 6-hydroxy compound **63** is of the



4a*R*,10a*R*-antipode depicted
63

α-rotamer type of DA as are 5-OH-AT and 5-OH-DPAT (also active in the D-1 and D-2 assays). However, of the β-rotameric types, the tetralins 7-OH-AT and 7-OH-DPAT retained D-1 and D-2 activity while the 8-hydroxy analogue of the benzo[*g*]quinoline **63** was inactive. These results form the basis of a rotamer-based

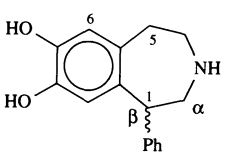
TABLE 6.12.
Effects of Octahydrobenzo[*g*]quinolines on DA-Sensitive Adenylate Cyclase and Electrically Evoked ACh Release[112]

	Adenylate cyclase (D-1 assay)		ACh release (D-2 assay)[127]	
	pD_2	Maximal stimulation (%)	pD_2	Maximal inhibition (%)
rac-**63** (6-OH)	5.68	43	8.55	− 91
(−)-**63** (4a*R*,10a*R*)	5.90	50	9.15	− 86
(+)-**63** (4a*S*,10a*S*)	4.75	24.5	7.63	− 82.5
rac-cis Analogue of **63**	0	0	0	0
rac-8-OH Analogue of **63**	0	0	0	0
Reference compounds (all *rac*)				
5-OH-2-aminotetralin (AT)	4.45	23	0	0
5-OH DPAT (**18**)	5.90	34.5	8.40	− 90
7-OH-AT	5.05	48.5	6.45	− 66
7-OH-DPAT	5.53	29.5	8.31	− 83

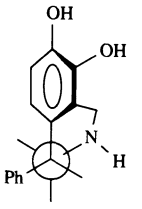
DA receptor model as outlined later (page 197). The absolute configuration of the more active antipode **63** correlates nicely with 6a*R*-(−)-apomorphines, 5*R*-ergolines, and the pyrazole **46**.

6.10. Miscellaneous Agonists Including Benzazepines

In the late 1970s the tetrahydro-1*H*-3-benzazepine **64** was characterized as an agonist of both central and peripheral DA receptors with D-1 selectivity.[4] Antipodal forms of **64** were later examined and the *R*-isomer (configuration by X-ray crystallography of MeI) found to be far more potent than *S*-**64** in several dopaminergic systems (Table 6.13).[128] Thus receptor uptake of the molecule **64**, which includes a DA pharmacophore of the α-*gauche* type, is extremely sensitive to the orientation of the phenyl substituent at C-1. *R*-**64** is highly active, notably in stimulating DA-sensitive adenylate cyclase in spite of being unable to attain the extended ArCCN conformation of dopamine; **65** shows a probable *gauche*-arrangement. It would be of interest to examine the analogue of **64** in which the phenolic



64



65

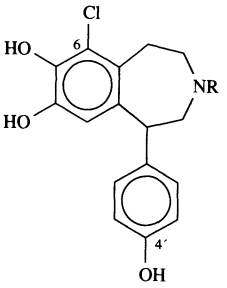
hydroxyls were present in the 1-phenyl substituent. The 6-chloro-4′-hydroxy analogues of **64** also showed DA-agonist properties.[129] Data for **66**a (fenoldopam) is included in Table 6.13; corresponding results for the 1*R*-N-allyl derivative **66**b

TABLE 6.13.
DA Activities of *R*- and *S*-2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine (64)[128, 129]

Test	*R*-64	*S*-64	DA
DA-sensitive adenylate cyclase stimulation EC_{50} (μM)	3.2×10^{-8} 66% at 10^{-5} (3.71×10^{-9})[a]	— 27% at 10^{-4} (1.47×10^{-6})	3.5×10^{-6} 100% at 5×10^{-5}
Inhibition of spiroperidol binding to rat caudate tissue IC_{50} (μM)	33.86 (0.54)	197.4 (18.2)	5.34
Contralateral rotation in lesioned rat RD_{500} (mg/kg) ip	0.50	2.0 = 50 ± 8 rotations	—
Renal vasodilator activity ED_{15} (μg/kg) renal vascular resistance reduction (RVR)	25 (0.31)	550 gave 9% reduction in RVR (0.94)	2.7

[a] Values in parentheses refer to the 6-chloro analogue **66**a.

were 8.76×10^{-9} (adenylate cyclase stimulation), 10.6 (spiroperidol binding), and 0.75 (RVR activity, ED_{20}). Antipodes of **66**a and **66**b were related chemically or by CD to *R*- and *S*-**64** (parent). It is evident that the 6-chloro substituent significantly enhances activity. Functional tests confirmed the D-1 activities of fenoldopam.[130] Thus the renal and vasodilator activities of *rac*-**66**a in dogs were shown to be properties of the *R*-antipode; the *S*-isomer was almost inactive.[131] These actions



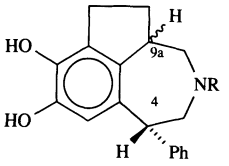
(a) R = H; (B) R = $CH_2CH{=}CH_2$

66

were antagonized by the related D-1 antagonist SKF R-83566, but not by the D-2 agent domperidone. When chlorine (and other halogen atoms) replace one of the hydroxyls of **64**, DA antagonists result (see Chapter 7, page 223).

5,6-Ethano bridged analogues of the benzazepine **64** have been prepared and *cis*- (H-4/H-9a) and *trans*-isomers **67** isolated.[132] The *cis*-racemate effectively displaced [^{3}H]fenoldopam (**66**a) from binding sites[133] and stimulated DA-sensitive adenylate cyclase, i.e., it was specific for D-1 sites, but was 5–7 times less potent than *R*-**64** in these respects. The *trans*-racemate **67** had feeble activities in these tests. Conformations of the two racemates differ in that the 4-phenyl group is fixed in a near-axial orientation in the *trans*-form but can alternate between axial and equatorial positions in the *cis*-derivative (X-ray, NMR, and molecular mechanism evidence). In the case of analogues of **64** which behave as antagonists, conformational restraint has been imposed by bridging positions 2 and 2′ of the phenyl substituents (page 224).

3′,4′-Dihydroxynomifensine **68**, like the benzazepine **64**, is a β-substituted analogue of DA with selectivity for D-I (DA_1) receptors. The presence of the



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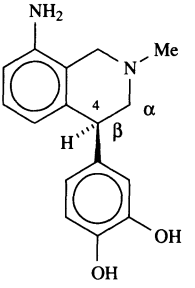
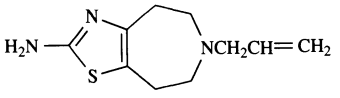


68 (4*S* shown)

amino substituent is not essential to its DA actions.[134] The compound was postulated as a metabolite of the antidepressant agent nomifensine to account for

the dopaminergic properties of this compound. While attempts to identify such a metabolite have failed, the DA-like actions of synthetic material are well established (see references quoted by Dandridge *et al.*).[134] Tests on antipodal forms showed the *S*-isomer to be the more active in stimulating DA-sensitive adenylate cyclase (2 × DA) and displacing clonidine from rat cortical membranes (affinity for α_2-adrenoceptors 0.1 × that of DA). Both *R*- and *S*-**68** were feeble competitors of [^{3}H] spiroperidol in binding experiments and hence lacked action at D-2 sites. Configurations were based on X-ray analysis of a methiodide and CD spectra. The authors consider that the enantioselectivities found for **68** are consistent with McDermed's model based on aminotetralins. In the *S*-antipode the bulk of the tetrahydroisoquinoline ring system is directed away from the receptor surface (Fig. 6.4) and does not impede binding at the key *m*-phenolic and amino sites. A correlation with *R*-**64** (benzazepine) was also proposed. Antipodes of nomifensive (**68**, OH groups replaced by hydrogen) itself have been compared in a variety of pharmacological tests.[135] One test involved inhition of [^{3}H]DA uptake into rat brain synaptosomes and observed IC_{50}(M) values were: *S*-(+) 5.3×10^{-8}, *R*-(−) ~ 10^{-5} (a similar order of difference was found in regard to [^{3}H]-NA uptake). It is significant that the eutomer of nomifensine and its dihydroxy analogue share the *S*-configuration at C-4. Note that eutomers of **68** and **64** have opposite configurations at equivalent chiral centers, a result which may relate to the fact that they also differ in the positions of their phenolic groups.

Evidence that the aminothiazoloazepine **69** (B-HT 920) inhibits synthesis of DA in the brains of rodents prompted Schneider and Mierau[136] to examine aminothiazole analogues of apomorphine and 2-aminohydroxytetralin as potential



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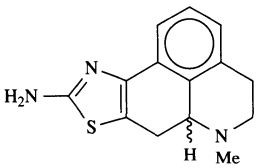


70

DA agonists with presynaptic selectivity. Some of their data are shown in Table 6.14. Of the two antipodes of the apomorphine analogue **70**, the levo isomer

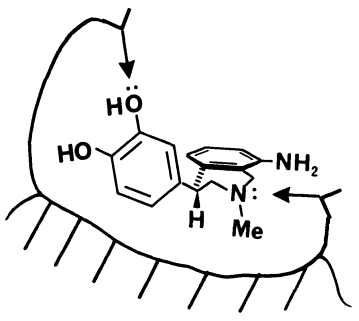


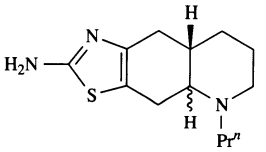
FIGURE 6.4. Proposed binding mode for *S*-3,4-dihydroxynomifensine.[134]

TABLE 6.14.
Biological Data of Some Aminothiolaze Analogues of DA Agonists[136]

Compound	Dose (mg/kg)	L-DOPA accumulation[a] nmol L-DOPA/g tissue weight corpus striatum	L-DOPA accumulation[a] % difference compared to saline	[^{3}H]spiperone receptor binding IC_{50} (nM)
Saline		24.1		
(+)-**70** (apo)[b]	10	24.4	+ 1	> 100,000
(−)-**70**	10	20.1	− 16	2900
71	10	9.1	− 62	310
R-(+)-**72** (tetralin analogue)	1	19	− 21	43,000
S-(−)-**72**		11.3	− 53	4700
Apomorphine	1	8.9	− 63	110

[a] L-DOPA accumulation elicited by injections of γ-butyrolactone (GBL) (blocks impulse flow in nigro-neostriatal pathways and thus eliminates postsynatically induced changes in DA synthesis) and NSD (DOPA decarboxylase inhibitor).
[b] Apomorphine analogue.

proved the more potent in DOPA accumulation and binding assays, but much less effective than (−)-apomorphine or a congener **71** which lacks the fused benzene ring of the latter compound. Both enantiomers of **72** were effective in tests of Table 6.14 with *S*-(−)-**72** the more active [configuration by X-ray analysis of



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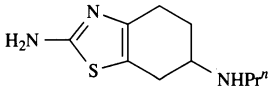
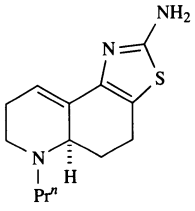


72

related 2-amino-L-(+)-tartrate]. Thus an absolute stereochemical correlation with 5-hydroxy-2-aminotetralins is seen, rather than with 7-hydroxy derivatives (p. 175). The DA agonist **72**a also includes a 2-aminothiazole unit. It proved selective for D-2 presynaptic sites as judged by its ability to reverse γ-butyrolactone induced accumulation of rat striatal DOPA and other tests.[153] The *R*-(+)-antipode was the eutomer in these tests, as it was in binding assays for D-2 sites (IC_{50} nM vs. [^{3}H]spiperidone: dextro 860, levo 2243, apomorphine 24). Models relating (+)-**72**a to *R*-(−)-apomorphine were proposed. Racemic material was resolved via the



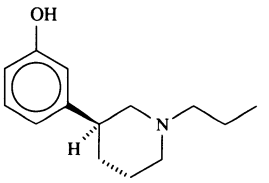
72a *R*-(+) shown

isobutyramide and optical purity (>98 %ee) established by HPLC of corresponding *R*- or *S*-ditoluoyltartrates (absolute configuration by X-ray analysis of the N-methyl derivative).

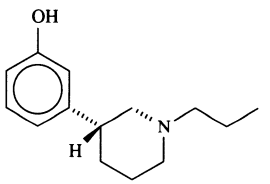
6.11. Models[14]

Most attempts to correlate the absolute stereochemistries of various dopamine ligands with receptor interactions have followed the conceptual model of Freeman and McDermed (p. 177). Kaiser, in his extensive account of dopamine ligands,[4] reviewed the use of this model in explaining the affinity differences between a variety of homochiral dopamine agonists and their distomers; most of these examples have been discussed earlier in this chapter.

Neumeyer's group has focused on correlations among antipodal pairs which differ not only in affinity but also in intrinsic activity. Thus the agonist set of eutomers shown in Fig. 6.5 may be superimposed in regard to juxtaposition of: (1) the hydroxyl equivalent to *meta*-hydroxyl of the phenethylamine pharmacophore, (2) the N-alkyl group, and (3) the ammonium hydrogen ($^+$NH) or nitrogen lone pair—all features considered key structural components for interaction at DA receptors.[138,139] All structures employed were close to those preferred conformationally. Corresponding antipodes of each of these compounds, which behave as DA antagonsits rather than agonists (see pages 172, 179, and 184) may be superimposed in a similar manner. To fit antipodes of 3-PPP to this scheme (3*R*-agonist, 3*S*-blocks postsynaptic DA receptors, potency levels low, page 184), the molecules must adopt a "planar" conformation (**73** for *S*; **74** for *R*) which is unfavorable by



(*S*, antagonist)
73



(*R*, antagonist)
74

3 kcal/mol over lowest-energy conformers. The authors propose that payment of this energy penalty may account for the relative weakness of 3-PPP antipodes in DA-ligand assays. Neumeyer is also a coauthor of a paper reporting a wide range of dissociation constants for DA ligands measured for high- and low-affinity sites of porcine pituitary tissue.[140] [^{3}H]-Spiperone was used to label D-2 sites and K_D (high) values were obtained by omitting NaCl from the incubation medium. 100 nM saline is known to assist in converting high-affinity D-2 receptors to the low variety kind which have a reduced affinity for DA. A tetrahedral model was proposed to account for affinity differences—this had two hydrogen bonding sites separated by about 8 Å and directed between 15° and 30° off orthogonal to the surface of the receptor. Several steric obstacles were required to account for the inactivity of certain of the test compounds.

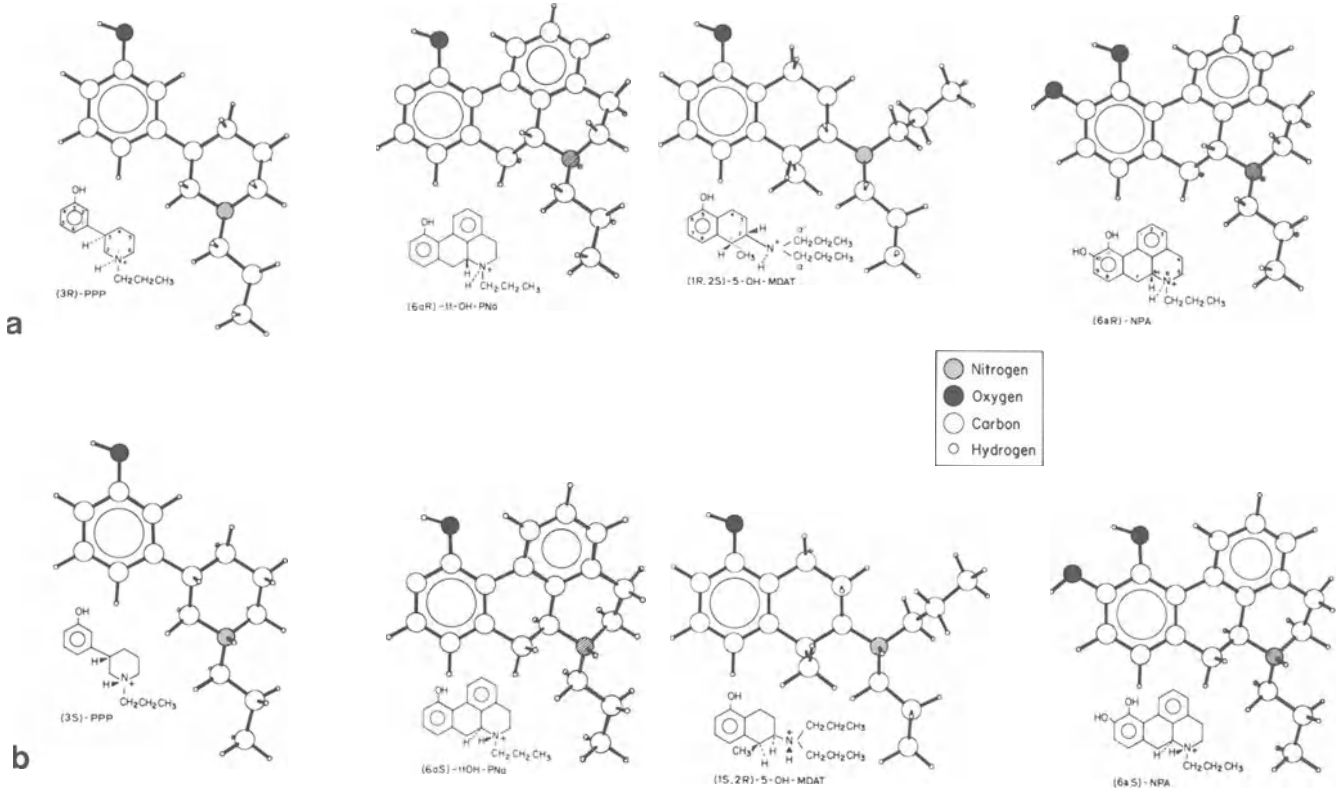
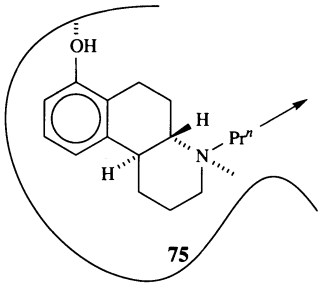


FIGURE 6.5. (a) Dopamine agonists. The conformation for (3*R*)-PPP [τ(C2′ – C1′ – C3 – C2) = 150°] is that which best superimposes onto other DA agonists. Energy-minimized structure for (6a*R*)-NPA. Preferred conformation for (1*R*,2*S*)-5-OH-MDAT with τ(C3 – C2 – N1 – C_α) = 180° and an arbitrary conformation of the *N*-propyl groups. (b) Enantiomers reported to be postsynaptic DA antagonists. The conformation for (3*S*)-PPP [τ(C2′ – C1′ – C3 – C2) = 210°] is that which best superimposes onto the other DA antagonists. Energy-minimized structure for (6a*S*)-NPA. Preferred conformation for (1*S*,2*R*)-5-OH-MDAT with τ(C3 – C2 – N1 – C_α) = 180° and an arbitrary conformation of the *N*-propyl groups (after Neumeyer *et al.*).[139]

Wikström and his colleagues have also sought correlations amongst DA agonists by molecular superimpositions and activity implications in regard to McDermed's model.[119,125] When the more potent antipodes of agonists were similarly positioned in regard to the aromatic nucleus with its *m*-hydroxy (or equivalent structure—see later) and basic nitrogen atom of the phenethylamine pharmacophore, two main positions were found for the N-substituent—these were *upward* or *downward* in terms of Fig. 6.6. The upward position was considered to be less restricted than the downward mode since it could accommodate groups larger than *n*-propyl.

The *upward* group comprises the 4a*S*,10b*S*-7-hydroxybenzo[*f*]quinoline (**58**/**75**) and the 3-*S*-antipode of 3-PPP (**42**/**76**). These compounds also correlate with the 2-*S*-5-hydroxyaminotetralin (**77**) if it is assumed that one *n*-propyl sub-



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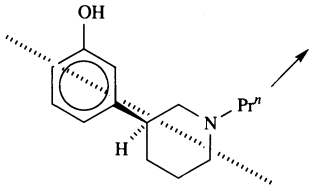

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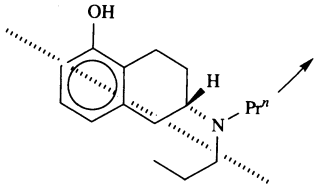

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stituent of the amino function is equivalent to the piperidine ring of the other two compounds. Dopaminergic activity is retained in **75** and **77** when *n*-propyl is

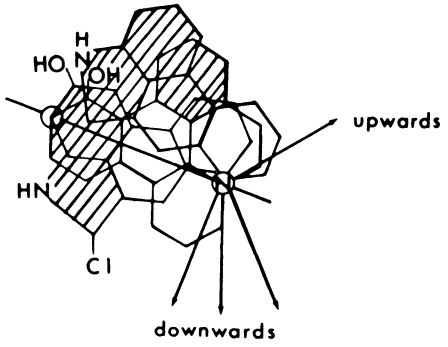
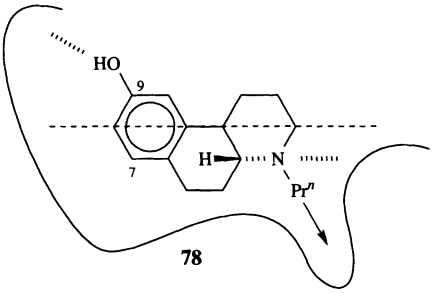


FIGURE 6.6. Dopaminergic structures superimposed (resolved monophenolic 2-aminotetralins).[119]

replaced by *n*-butyl[123,92], indeed the racemic *n*-butyl derivative exceeds the potency of the *n*-propyl derivative **75**.

The *downward* group has more members. It includes the 4a*R*,10b*R*-9-hydroxybenzo[*f*]quinoline **78**, the 3-*R*-antipode of 3-PPP **79**, and the 2*R*-7-hydroxy-2-aminotetralin **80** (cf. McDermed's manipulations of the 2-*S*-(5-OH)- and



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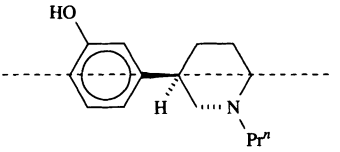

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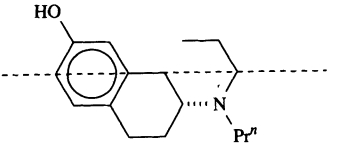
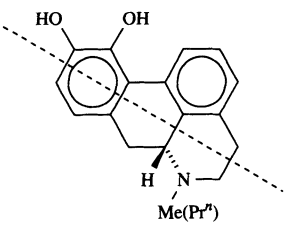


80

2-*R*-(7-OH)-aminotetralins, page 176). The *N*-*n*-butyl analogue of the benzo-[*f*]quinoline **78** lacks dopaminergic activity (racemate)[119] while, in reserpinized rats, compounds with *N*-alkyls larger than Pr*n* were more potent in their *S*- than in their *R*-forms.[92] 6a*R*-(−)-Apomorphine **81** and various 5-*R*-ergolines **82** also conform to this arrangement (with the assumption that the pyrrolylethylamine moiety of the latter molecule represents the DA pharmacophore).[134] Other members of this group are the 6a*R*-oxaergolines **48** and the pyrazole **46** (see pages 186 and 187).

N-*n*-Butyl analogues of (−)-apomorphine, the ergolines, and oxaergolines are feeble DA agonists or inactive.[114,142,143]



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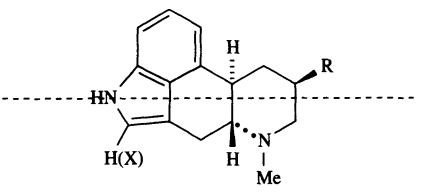
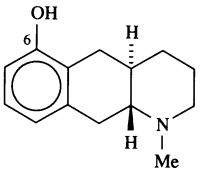


82

Subsequent to Wikström's paper, Seiler[112] reported the 6-hydroxyoctahydrobenzo[*g*]quinoline **83**. This compound is clearly an analogue of apomorphine and the inactivity of the corresponding N-butyl derivative places this too within the N-R "downward" group. Superimposition of this set of agonists produces a three-dimensional total volume of active structures which may be regarded as the complement of the DA-receptor structure volume (Fig. 6.6). Well-defined portions of

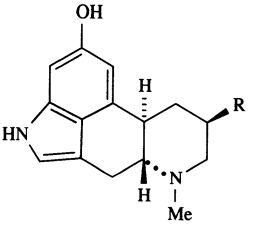
this structure are as follows: (1) an aromatic nucleus with a *m*-OH phenolic function, a pyrrole or pyrazole ring in lieu; (2) a proper distance (extended



83

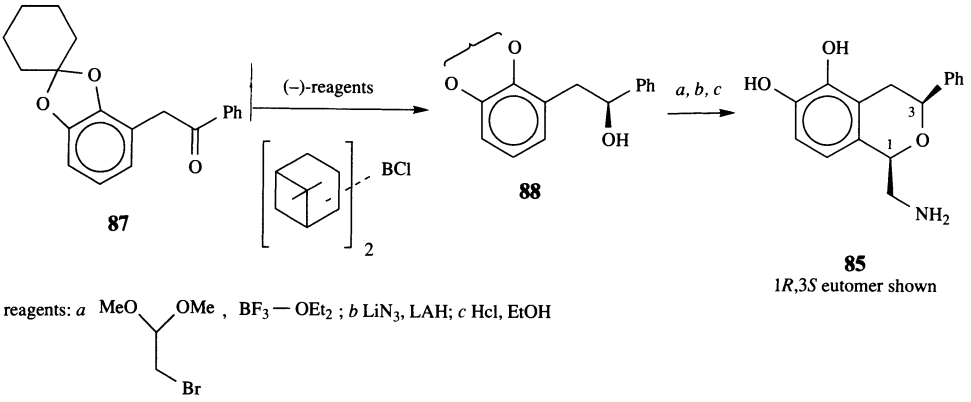
phenethylamine of α-conformational type, page 171) between the aromatic nucleus and the basic nitrogen; (3) two main positions for the N-substituent(s) (upward or downward in the sense of Fig. 6.6), one of which (downward) is sterically restricted to accommodate maximally an *n*-propyl group or a piperidine ring, but not *n*-butyl—the upward direction has less restricted demands and can tolerate *n*-butyl; (4) the stereochemistry of the chiral carbon next to basic nitrogen is critically important—in all active structures of Fig. 6.6 the direction of the C-N bond is *beneath* the plane of the paper.

Seiler's group[112,144,145] have made similar proposals to those of the Swedish workers. In the case of the ergolines they suggest that the increase in activity seen after metabolic hydroxylation at C-13[146] is due to a change from the "quasi-α-rotameric" to the more efficient β-rotameric orientation acquired by this change (see **84**).

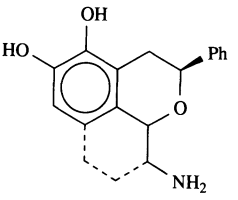


84

A D-1 agonist, too recently described to have been included in the described models, is the benzopyran (**85**, A68930) developed by Abbott Laboratories.[147] The 1*R*,3*S*-antipode (K_i 1.6 nM) was twice as effective as its racemic mixture in displacing [^{125}I]SCH23982 (D-1 selective ligand, page 223) and 40 times more so than the D-1 agonist SKF38393 (page 93), while the K_i of 1*S*,3*R*-**85** was very high (7200 nM). The same potency relationships obtained in a test for stimulation of adenylate cyclase. The 1*R*,3*S*-eutomer was virtually inactive in corresponding D-2 asays. In a behavioral test (rat rotation model), 1*R*,3*S*-**85** was potent in the whole animal at sc dose levels of 0.2 to 2.03 μmol/kg, while its antipode failed to elicit contralateral rotations at a 20 μmol/kg dose level. Activity fell drastically when the 3-phenyl substituents was removed. In this respect some analogy with DA agonists based on benzazepine may be indicated—perhaps analogues with one of the phenolic hydroxyls replaced by a halogen may prove to be a D-1 antagonist (see



page 223). On the other hand, the benzopyran derivative may relate to the 2-aminotetralin class of DA agonist (see **86**) (NPr^n_2 analogues of **85** have yet to be reported).

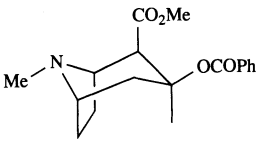


86

Stereoselective procedures were used to obtain 1*R*,3*S*-**85** and its antipode. Reduction of the ketone **87** with the (+)- or (−)chlorodiisopinocampheylborane reagent developed by Brown[148] gave the *R*- and *S*-alcohols **88**, respectively, in 75% yield and 98 %ee. Reaction of **88** with bromoacetaldehyde dimethylacetal under BF_3–OEt_2 catalysis gave the 1,3-*cis*-isochroman product exclusively, convertible to **85** of known geometry.

6.12. Cocaine and Dopamine

Natural levo cocaine (**89**), well-known for its power to potentiate adrenaline by preventing access of the neurotransmitter to storage sites in the adrenergic neuron, has also been found to inhibit the uptake of dopamine in striatal tissue of rodents and humans. Action in this last respect has been related to the affinities of cocaine and several cocaine-like compounds for binding sites in striatal tissue as well as their potencies as psychomotor stimulants.[149] Radiolabeled [^{123}I] analogues of **90** have been described and a 3α/3β binding comparison of 3-(4-iodophenyl)



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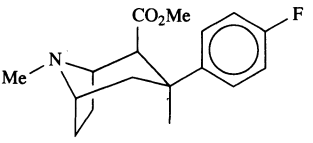
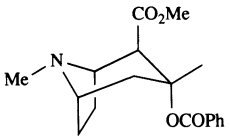


90

derivatives presented.[156] Because natural cocaine is one of eight possible stereoisomers, Carroll *et al.*[150] evaluated the power of all forms to inhibit the binding of tritiated WIN 35428 (**90**), an analogue of cocaine which binds more effectively than the parent alkaloid to striatal sites.[151] Natural *R*-cocaine (IC_{50} 0.102 μm) proved 155 times more potent than its antipode. The second most active isomer was the allo form **91** (IC_{50} 6.16 μm), followed by the antipode of this com-



91

pound (IC_{50} 9.82 μm). There was evidence from Hill coefficients (>1) that the allo isomers acted in a noncompetitive manner. The paper described methods for the synthesis of all isomers; relative configurations were based on ^{1}H-NMR data.[152]

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7

Dopamine Antagonists

7.1. Introduction

It was recognized in the 1960s that chlorpromazine and other antipsychotic agents act by the blockade of dopaminergic receptors (Snyder[1] and references cited therein). Hence this section, in effect, presents an account of stereochemical influences upon the activity of drugs used to treat psychotic disorders (*Martindale* **29**, 709).

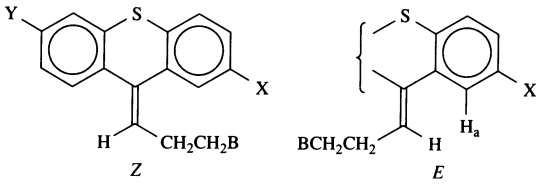
A variety of antipsychotic agents with ability to block DA-receptors have been discovered and many of these are dissymmetric in both a geometric and chiral sense. Work on DA antagonists has been especially fruitful in regard to the identification of receptor subtypes, since it has led to ligands specific for D-1 and D-2 receptors. Apart from the phenylbenzazepine series, there are few direct structural analogies between DA agonists and antagonists.

7.2. Thioxanthenes

These derivatives, designed as analogues of phenothiazines such as chlorpromazine, provide a series of *E*/*Z* geometrical isomers (**1**) (Table 7.1). Products in clinical use are generally the *Z*-forms although mixtures of variable isomeric composition have been reported in the past, e.g., flupenthixol 50% Z,[2] thiothixene 37% *Z*, 100% in capsule formulation.[3] The USP 1985 specifies a *Z*-configuration for chlorprothixene and thiothixene and includes limit tests for the *E*-isomers. Wan Po and Irwin[4] have reviewed analytical methods and developed an efficient HPLC procedure for the quantification of *E*/*Z*-mixtures of this kind using promazine as the internal standard. They used this procedure to demonstrate light-induced $Z \rightarrow E$ isomerization.[5]

Synthetic routes lead to mixtures of *E*/*Z*-isomers which may be separated by physical techniques. Configurations of the more potent *Z*-isomer have been established by X-ray crystallography (Table 7.1). An NMR method using the shift reagent tris(dipivalomethanato)europium has also been described[13]; the H_a proton (see *Z*-**1**) of a *Z*-isomer is close to the amino function and its resonance signal

TABLE 7.1.
List of Thioxanthenes in Clinical Use



1

	X	Y	B	References to X-ray study
(a) Chlorprothixene	Cl	H	NMe_2	6,7
(b) Fluprothixene	CF_3	H	NMe_2	6,7
(c) Thiothixene	SO_2NMe_2	H	$N(CH_2)_4NMe$[a]	8
(d) Flupenthixol	CF_3	H	$N(CH_2)_4N(CH_2)OH$[a]	9,10
(e) Clopenthixol	Cl	H	$N(CH_2)_4N(CH_2)OH$[a]	11
(f) Pifluthixol	CF_3	F	$N(CH_2)_4N(CH_2)OH$[a]	12

[a] Piperazine derivatives.

moves downfield when the base complexes with shift reagent, e.g., by 0.57 ppm at 60 MHz for *Z*-chlorprothixene—the signal remains unresolved within the aromatic multiplet in the spectrum of the *E*-isomer. A method based on NOED spectra has also been reported; irradiation of the methylene protons adjacent to the alkenic carbon of **1** leads to enhancement of the H_a resonance.[14]

Hyttel *et al.* of Lundbeck (Denmark) have presented an extensive review of the biochemical and pharmacological profile of *E*/*Z*-thioxanthenes which include company results.[15] A selection of these data are given here. In binding experiments *Z*-flupenthixol (**1d**) exceeded the affinity of its *E*-isomer for both agonist and antagonist sites of a variety of brain tissues. Radioligands included tritiated haloperidol, spiroperidone, *Z*-flupenthixol (**1d**) and pifluthixol (**1f**), sulpiride, dopamine, and ADTN. Variations in *Z*/*E*-affinity ratios were wide (Table 7.2).

TABLE 7.2.
Affinity Ratios of *Z*- and *E*-Flupenthixol (1d) in Dopamine Receptor Binding

Tissue	Ligand[a]	*Z*/*E*-Affinity ratio	Ref.
Rat striatum	[^{3}H]haloperidol[b]	23	16
Calf striatum	[^{3}H]haloperidol[b]	150	17
Bovine retina (D-1 prep.)	[^{3}H]spiroperidol[b]	118 (low overall affinity)	18
Rat striatum	[^{3}H]sulpiride[b] (*rac* mixture)	78	19
Calf striatum	[^{3}H]dopamine	44	20
Rat striatum	[^{3}H]ADTN[c]	67	21
Rat striatum	[^{3}H]-*Z*-flupenthixol	273	16
Rat striatum	[^{3}H]-*Z*-pifluthixol	1310	22

[a] The *Z*-isomer has the highest affinity in all cases.
[b] D-2 selective.
[c] 2-Aminotetralin derivative (Chapter 6, page 174).

TABLE 7.3.
Binding Constants of Two Pairs of *E*/*Z*-Thioxanthenes[25]

	K_i (nM) vs. [^{3}H] SCH 23390 (D-1)	vs. [^{3}H]spiperidone (D-2)
Z-Flupenthixol (**1d**)	4.3	0.8
E-Flupenthixol	907	94
Z-Pifluthixol (**1f**)	2.9	1.0
E-Pifluthixol	95	73

Affinity was highest when an antagonist ligand was used; *Z*-flupenthixol displaces D-2 selective and nonselective (thioxanthenes) ligands with similar ease and is judged equally active at D-1 and D-2 receptors (chlorpromazine has a tenfold preference for D-2 sites).[23,24] Binding K_i values of a pair of *E*/*Z*-thioxanthenes versus [^{3}H]-SCH 23390 (D-1 specific, see page 224) and [^{3}H]spiperidone confirm these observations (Table 7.3).

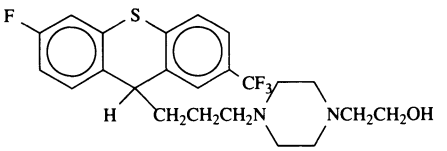
The D-1 receptor activity of flupenthixol is affirmed by its inhibition of DA-sensitive adenylate cyclase. In most experiments the *E*-isomer was without inhibitory action, e.g., rat corpus striatum K_i values (nM); *Z*-**1d** 1.0, *E*-**1d** > 5000.[26] Flupenthixol also blocks α_1-adrenoceptors, 5-HT$_1$, 5-HT$_2$ and histamine H-1 receptors, but higher concentrations than those effective against DA receptors are required and degrees of stereoselectivity are low.[15] A dosage of 0.1 mg/kg ip of *Z*-**1d** elevated rat plasma prolactin (a D-2 effect) while the *E*-isomer was 245 times less active.[27] Likewise *Z*-**1d** exceeded the potency of its *E*-isomer (at least 100-fold in general) in a variety of behavioral and pharmacological tests (Table 7.4).

Similar data and findings of stereoselectivity are available for clopenthixol (**1e**), chlorprothixene (**1a**), fluprothixene (**1b**), thiothixene (**1c**), and piflutixol (**1f**).[15] *Z*-Pifluthixol has the highest affinity for DA receptors among the thioxanthene group and typically lacks receptor selectivity. By including 30-nM spiroperidone in the assay, [^{3}H]-*Z*-pifluthixol exclusively labels D-1 receptors in rat corpus striatum.[16,22,31] In DA-receptor binding tests *Z*/*E*-affinity ratios were high, e.g., calf striatum vs. [^{3}H]haloperidol 750,[31] rat striatum vs. [^{3}H]-*Z*-flupenthixol 197.[16] The *Z*/*E*-ratio was lower (22) in a test for inhibition of DA-stimulated adrenylate cyclase.[16]

TABLE 7.4.
Flupenthixol Geometrical Isomers (1d): Behavioral and Pharmacological Tests

Inhibition of:	ED_{50} (mg/kg) *Z*-(**1d**)	*E*-(**1d**)	Ref.
Amphetamine stereotypy rats ip	0.07	> 100	28,29
Apomorphine stereotypy rats ip	0.3	> 80	28,29
Conditioned avoidance response rats sc	0.028	> 5	30

Reduction of pifluthixol gives the chiral derivative tefluthixol (**2**) which is less active than its precursor in binding and adenylate cyclase tests.[16] Data on its antipodes have not been reported.



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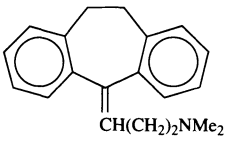
7.3. Butaclamol and Related Benzocycloheptapyridoisoquinolines

Because of its relative rigidity the pentacyclic derivative butaclamol **9** has proved of special value to studies of dopaminergic receptors. An interesting account of the development of butaclamol as an antipsychotic agent has been given by Humber in *Chronicles of Drug Discovery*.[32]

Chemistry. In work based on variants of the antidepressant imipramine (**3**), the



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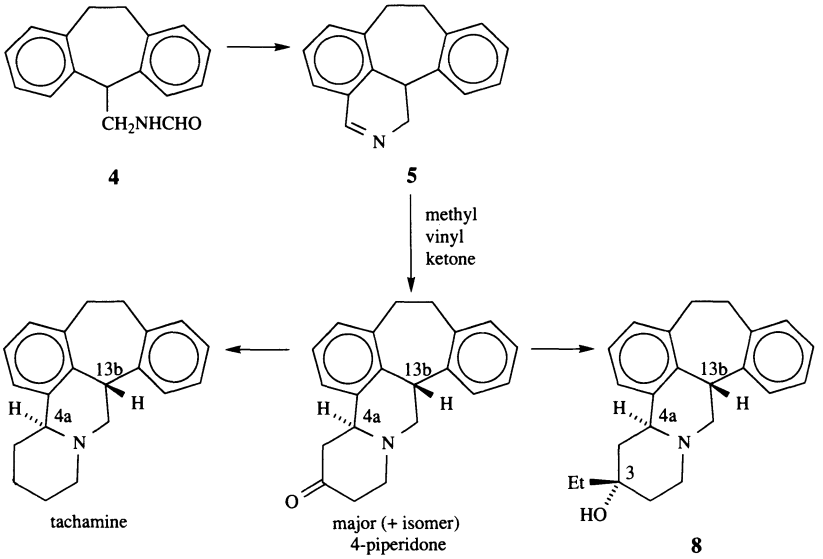
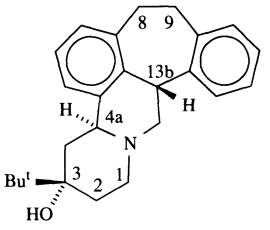
formamide **4** led to the Schiff's base **5** and 4-piperidone **6**. The products **7** (tachamine) and the 3-ethyl-3-hydroxy derivative **8** proved active in behavioral tests—the *t*-alcohol **8** was as active as chlorpromazine in antagonizing stereotyped



4 **5** tachamine **7** major (+ isomer) 4-piperidone **6** **8**

behavior in rats given amphetamine. The 3-*t*-butyl (butaclamol) and 3-isopropyl analogues of **8** proved more effective in the behavioral test and were subsequently developed as antipsychotic agents. Minor products of the reactions were isomeric forms of tachamine and butaclamol. The stereochemistry of the ring junction of major products was first established from ^{1}H-NMR data (coupling magnitudes of the benzylic proton at C-13b with the C-14 protons) and C-3 by synthesis of the axial (α)-Et analogue of **8** (3-Et) by a stereoselective route, confirmed later by X-ray crystallography.[33] When racemic butaclamol was resolved, all of the *in vivo* and *in vitro* activity of the mixture was found to be due to the dextro isomer, of absolute configuration 3*S*,4a*S*,13b*S*,[33] as depicted in **9**. In fact the stereoselectivity



9 3*S*, 4a*S*, 13b*S*-(+)-butaclamol

of butaclamol isomers at DA receptors is the highest seen for isomeric DA antagonist pairs. A large collection of relevant data is presented in the Hyttel *et al* review.[15] Some binding parameters for (+)- and (−)-butaclamol are shown in Table 7.5. Butaclamol displays a greater affinity for D-2 than D-1 antagonist sites and antipodal ratios are correspondingly higher. As usual, agonists (DA and others) are less well displaced than antagonists.

Dextro butaclamol, but not its antipode, inhibits DA-sensitive adenylate cyclase[16] and reverses the presynaptic decrease in DOPA accumulation caused by apomorphine.[38] The same isomer (0.1 mg/kg) markedly increased prolactin levels in rats while its levo antipode had no effect at 20 mg/kg.[27] α-Adrenoceptors discriminate between antipodal butaclamols but bind to (+)-**9** less well than do DA receptors.[39] In a variety of behavioral tests diagnostic of DA-antagonists, (+)-**9** proved a potent agent while its antipode was feeble or inactive (references cited by Hyttel *et al.*[15]).

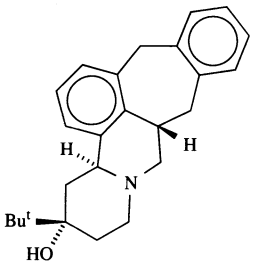
Isomers of the 3-isopropyl analogue of **9** likewise differed in potency in DA-antagonism tests. The more potent dextro antipode (dexclamol, same configuration as dextro butaclamol) inhibited [^{3}H]-spiroperidol binding in rat striatal tissue 150-fold more effectively than its levo isomer, i.e., it showed a stereoselectivity

TABLE 7.5.
Binding Studies with Antipodal Butaclamols

Tissue	Radioligand	(+):(−) Affinity ratio	Ref.
Rat striatum	[^{3}H]haloperidol	7,333	16
Calf striatum	[^{3}H]spiroperidone	14,285	34
Bovine retina (D-1)	[^{3}H]spiroperidone	176	35
Rabbit renal artery (D-1)	[^{3}H]spiroperidone	92	36
Rat striatum	[^{3}H]-*Z*-flupenthixol	8,970	16
Calf striatum	[^{3}H]dopamine	160	37

of a lower order than found for butaclamol.[40] The 3-cyclohexyl and 3-phenyl analogues of butaclamol also display stereoselectivity for [^{3}H]-spiroperidol binding sites (900- and 1400-fold, respectively, with dextro the more effective).[40]

Remarkably isobutaclamol (**10**), a benzo-[5,6]-cycloheptane analogue of butaclamol, is also a DA antagonist of similar potency as the original molecule. Its resolution, absolute configuration, and crystal structure have been reported.[41-44]



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The more potent dextro isomer has the same configuration as (+)-butaclamol. Some antipodal comparison data are as follows: inhibition of amphetamine stereotypy in rats (ED_{50} mg/kg) (+)-**10** 0.3, (−)-**10** > 25[42]; displacement of [^{3}H]-spiroperidone from calf striatum (K_i) (+)-**10** 2−4, (−)-**10** 350.[40]

The ring-opened analogue **11** of butaclamol was far less effective than the parent in binding and *in vivo* tests.[45]

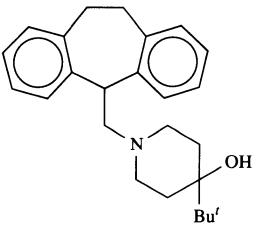

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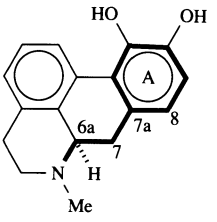
TABLE 7.6.
Parameters Associated with the Conformations of the Phenethylamine Moieties in (+)-Butaclamol and (−)-Apomorphine[32]

Parameter[c]	(+)-Butaclamol		(−)-Apomorphine[a]
	Conformer A[a]	Conformer B[b]	
$\tau(C_{13a}-C_{13b}-C_{14}-N)$	−169°	−155°	—
$\tau(C_{13}-C_{13a}-C_{13b}-C_{14})$	17°	5°	—
$\tau(C_{7a}-C_7-C_{6a}-N)$	—	—	178,178°
$\tau(C_8-C_{7a}-C_7-C_{6a})$	—	—	−146,−135°
Distance of N from ring A plane	0.19 Å	−0.9 Å	−0.9,−1.23 Å
Distance of N from center of ring A	5.1 Å	5.1 Å	5.12,5.09 Å

[a] Derived from crystallographic data.
[b] Derived from measurements on Dreiding models.
[c] See formulas **12** and **13** for ring numbering of dihedral angles.

Two stable conformers of (+)-butaclamol are possible, interconvertible by rotation about the C-8/C-9 bond of the bimethylene bridge that links the two aromatic rings (A and B of Fig. 7.1). In conformer A one of the 9-H protons is close to the 13b-proton, while in conformer B one of the 8-H protons and 13b-H are proximate. The solid state conformation of *rac*-butaclamol HCl is that of the A-form[33]; some authors favor this as the biologically active conformation.[46,47] However, in a search for correspondence between (−)-apomorphine and (+)-butaclamol, close agreement between structural parameters of the two molecules was found in regard to their phenethylamine moieties provided butaclamol was in its B-conformation (Table 7.6).

In terms of the molecules orientated as in **12** and **13**, the nitrogen atom lies behind the plane of ring A and, in similar degree, in the two structures (in con-



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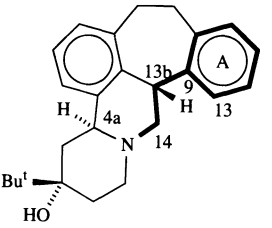

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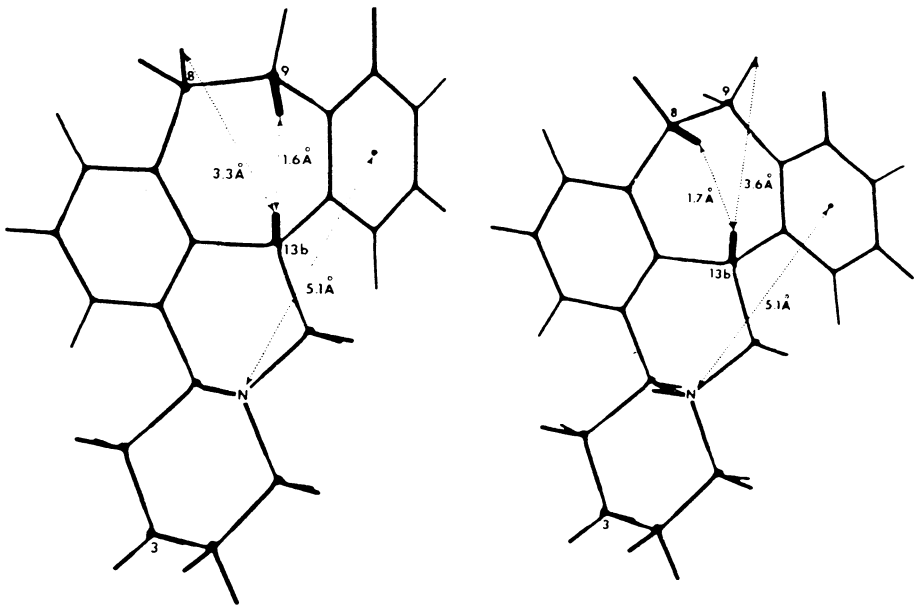
former A of Fig. 7.1, nitrogen is placed just above this plane). It was shown[48] that the phenethylamine groupings of (−)-apomorphine and conformer B of



Butaclamol conformer A

Butaclamol conformer B

FIGURE 7.1. Shadowgraphs of Dreiding models of the nuclei of (+)-butaclamol conformers; the distances shown are from measurements on Dreiding models (after Humber).[32]

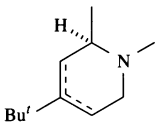
(+)-butaclamol can be aligned with aromatic rings A coplanar and concentric and with the nitrogen atoms coincident. The authors defined a neuroleptic pharmacophore from the data of Table 7.6 and showed it to be present (potentially or actually) in a wide range of dopaminergic ligands.[49]

Two low-energy conformers are also possible for isobutaclamol—the analogue of butaclamol in which ring A is displaced to straddle the central cycloheptane ring. In conformer B, with one of the 8-H protons in close proximity to 13a-H, the out-of-plane displacement of nitrogen with respect to ring A (0.9 Å) is the same as in (+)-butaclamol. Although the distances between nitrogen and the center of ring A differ markedly (5.1 Å for butaclamol, 6.4 Å for the isoanalogue, both B conformers), the two molecules may be superimposed with the exception of three carbon segments of ring A which lie in the same plane and are immediately adjacent to each other. It was concluded that the dopamine receptor contains a planar aromatic ring binding site that has the dimensions of at least two adjacent benzene rings.

Very recent X-ray,[101] NMR, and theoretical studies[102] have provided further details of the conformation and internal flexibility of butaclamol hydrochloride, and have shown that the salt exists as solute in DMSO as an equilibrium mixture of two conformations which differ in their stereochemistry about the ring junction that contains the nitrogen atom (4a-H/N: or N^+–H *t* 80:*c* 20).[103]

Since the DA-blocking activities of butaclamol and its analogues are dependent in large degree on the presence of a *t*-butyl or similar bulky hydrocarbon group attached equatorially to position 3 of the skeleton, an accessory lipophilic binding site has been included in the model of the DA receptor devised by these authors (site H).

Anhydro (**14**) and desoxy (**15**) analogues of *rac*-butaclamol all had similar potencies in the prevention of amphetamine-induced stereotypy[49] and hence must all have suitable placed *t*-butyl groups for interaction at site H. Such cannot be the case for the inactive analogues **16** (*t*-butyl assigned a pseudoequatorial orientation in a ring E boat) and **17** (eq-*t*-butyl displaced to the 2-position of ring E).



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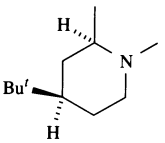

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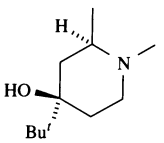

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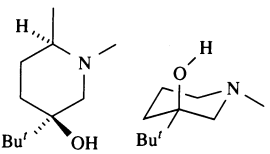


17

Tollenaere and colleagues[50] carried out a series of superimpositions between dexclamol and several neuroleptic agents of diverse structure in a search for spatial correlations among the group. Direct comparison of geometric characteristics derived from X-ray crystallographic data was not very revealing in terms of identification of a common pharmacophore. However, satisfactory fits between dexclamol and several different types of neuroleptic could be achieved utilizing conformations that mostly approached those of minimum energy. The examples of benperidol (a butyrophenone) and pifluthixol (a thioxanthene) are shown in Fig. 7.2. A barrier of only 2 kcal/mol was computed for the rotation around the bond linking the C=O and phenyl ring of benperidol, necessary to bring it in line with ring A of dexclamol.

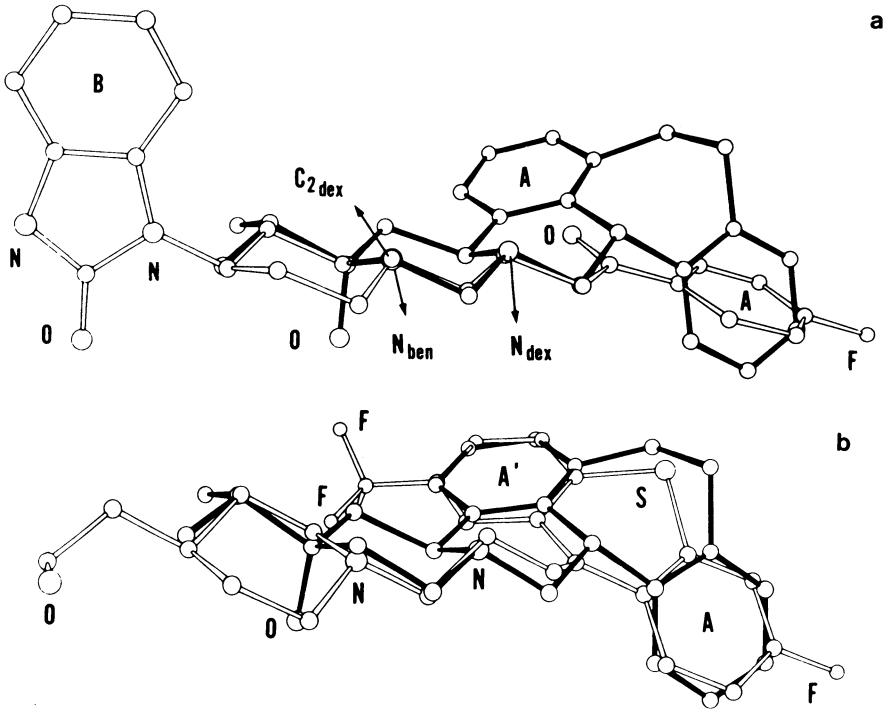


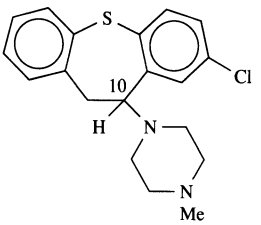
FIGURE 7.2. Fit between dexclamol and benperidol (a), and dexclamol and pifluthixol (b). Solid lines denote dexclamol (after Tollenaere *et al.*).[50]

It is noteworthy that dexclamol is in the conformation form A in these operations, i.e., as observed in the solid state.[51] Thus the model so derived differs from that of Humber's group, who employed a type-B conformer. Tollenaere's model rests on the assumption that C-2 of dexclamol occupies a configurational region in which the tertiary nitrogen atom of potent neuroleptics is normally found. It would be of interest to examine the fit of novel dopamine antagonists discovered since 1980 to the two models.

The stereoselectivity of σ-receptor sites toward antipodal forms of butaclamol is opposite to that of DA receptors, as judged by GPI binding data vs. [^{3}H](+)-SKF 10047 (see page 461): K_i (nM) values were 38 for the levo and 1300 for the dextro isomer.[52]

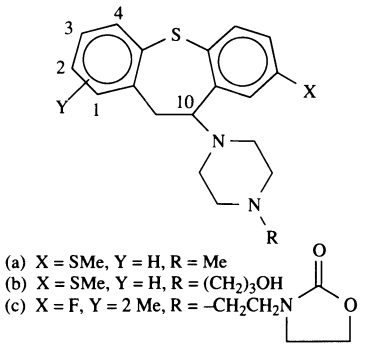
7.4. Dibenzo[*b*,*f*]thiepins and Related Compounds

This group of antagonists, of which octoclothepin (**18**) is the best known example, has structural similarities to phenothiazine and reduced thioxanthene (**2**)



18

antipsychotic agents. Octoclothepin **18** and its three analogues methiothepin **19**a, oxyprothepin **19**b, and **19**c have been resolved and the configurations of dextro isomers established as 10*S* by X-ray diffraction.[40,53-56] For configurational comparisons with other neuroleptics see Refs. 53, 57, and 58.



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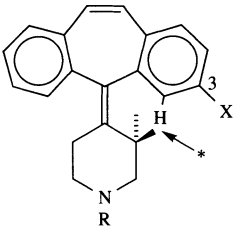
The usual biochemical and behavioral tests show that octoclothepin **18** is a potent DA receptor antagonist. Although (+)-octoclothiepin has only an 11-fold higher affinity than its antipode for [^{3}H]-spiroperidone binding sites in calf striatal membranes,[40] high degrees of stereoselectivity were found for stereotypy blockade and catelepsy.[59,60] In the most recent study of enantiomers of octoclothepin, of assured optical purity by use of the chiral shift reagent *R*-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol, antipodal ratios were reported in a series of *in vivo* animal models.[99] The *S*-(−)-isomer was the eutomer in most tests (37 × dextro form in the rat catalepsy test) but its antipode proved the more potent *vs* quipazine (× 1.7) and in a flexor reflex test (× 2.7) in rats. Receptor binding of *rac*-**18** followed the order α_1-adrenoceptor > 5-HT_2 > D-2 > D-1 with the *S*-isomer the more tightly bound except for 5-HT_2 sites, which showed no stereoselectivity. Ratios of IC_{50} values were modest and greatest (× 3.1) for α_1-sites. Data on antipodal forms of **19**a–**19**c are lacking, apart from a report that those of methiothepin (**19**a) have equal high affinities for 5-HT_2 sites.[100]

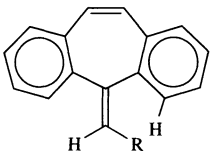
In addition to its neuroleptic activity, the racemate of octoclothepin is also a potent inhibitor of NA uptake.[61] Speculations on the antipodel potency ratio in this respect have been made[47] which were later revised when it was established that *S*-**18** was over 300 times more effective than the *R*-antipode.[99]

Two further groups of dopaminergic antagonists share the tricyclic feature of octoclothepin of a pair of aromatic rings linked by a 7-membered ring (see also butaclamol). The 5H-dibenzo[*a*,*d*]cycloheptenes **20** are analogues of cyproheptadine (Chapter 11).

Substitution in the 3-position of the system results in atropisomerism,[62] i.e., a state of chirality which arises as a result of barriers to conformational interconvertions. In a dibenzo[*a*,*d*]cycloheptene system, interconversion of conformers is opposed by nonbonded interactions between R and H as shown in **20**c. Hence when the two aromatic rings are nonidentical (as in **20**) stable antipodes may be isolated. The absolute configuration of one of the levo atropisomers (**20**a) was

determined by X-ray diffraction and shown to be pR_a, pS_b; other isomers were related to (−)-**20**a from their CD spectral features.[63] The ability of *rac*-**20**b to block the conditioned avoidance response in squirrel monkeys and apomorphine-induced stereotyped behavior in rats resided in the levo antipode: ED_{50} (mg/kg) for test in rats *rac*-**20**b 9.8, (−) 3.8, (+) > 60, haloperidol 0.32.[64] The racemate **20**b





20c

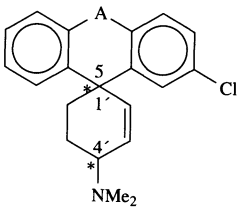
(a) X = Br, R = Me; (b) X = SCF_3, R = $CH_2cC_3H_5$

20

*Barrier to conformational interconversions

had moderate affinity for DA receptors (vs. [^{3}H]spiroperidone), with the (−)-antipode binding about 100-fold more effectively than (+)-**20**b.[40,63] A similar antipodal ratio was found in tests for inhibition of DA-stimulated adenylate cyclase in carp retina,[65] hence (−)-**20**b has affinity for both D-1 and D-2 receptors. Levo **20**b was also the more potent antipode vs. the adrenoceptor antagonist [^{3}H]-WB 41401 (page 130) but reverse stereoselectivity was found in regard to affinity for muscarinic receptors vs. [^{3}H]quinuclidine benzilate.[63]

Spiranes **21** related to the cycloheptadines **20** have also been investigated.[57] All four isomers of **21**a and **21**b have been isolated[66] and configurations of the



(a) A = CH_2CH_2; (b) A = CH = CH

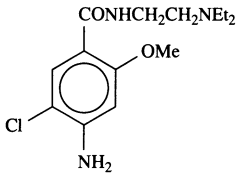
21

more active forms established by X-ray crystallography. In tests for inhibition of apomorphine-induced behavior in rats, 5,1′-*S*, 4′-*R* levo isomers had far greater potencies than other isomers. In the solid state conformation the NMe_2 group is directed toward the chlorine atom in an arrangement analogous to that of the *Z*-thioxanthene molecule (page 210).

7.5. Benzamides

The starting point for this group is the antiemetic agent metoclopramide (**22**). Although this compound behaves as a DA antagonist (notably at the chemorecep-

tor trigger zone and in the gut; it also reverses apomorphine- and amphetamine-induced stereotyped behaviors in animal models), it only weakly displaces [^{3}H]-haloperidol from its binding sites and fails to inhibit DA-sensitive adenylate cyclase.[67] The related compound sulpiride **23** (Dolmatil) has been developed as a DA antagonist of D-2 specifity. It is a particularly potent inhibitor of the dopaminergic control of prolactin release (a D-2 feature).



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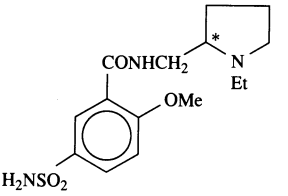
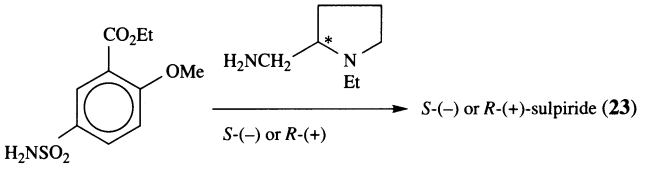


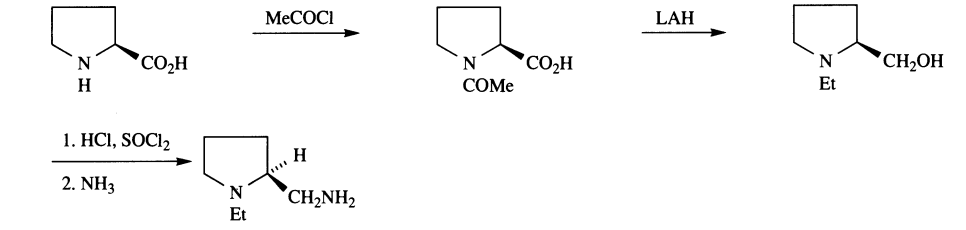
23 Sulpiride

Antipodal forms of sulpiride are accessible by stereospecific synthesis[68] by a process in which the ethyl benzoate **24** is treated with *S*-(−)- or *R*-(+)-*N*-ethyl-2-aminomethylpyrrolidine. The absolute configuration of the *S*-(−)-antipode of the pyrrolidine reagent was established by its chemical derivation from *S*-(−)-proline (Scheme 7.1).



24

The eutomer is the levo isomer of *S*-configuration. In binding experiments (−)-sulpiride had relativley low affinities for DA-receptor sites compared with other groups of antagonists, but with selectivity for D-2 sites. Its dextro antipode failed to bind to DA sites. Thus in competition with D-2 ligands ([^{3}H]-haloperidol, spiroperidone, and domperidone) it had IC_{50} values in the range 100–360 nm,[69-71] and 25,000 and above vs. thioxanthene ligands which bind to D-1 and D-2 sites (D-2 blocked?) (IC_{50} = 3600 nM vs. apomorphine).[72] The presence of sodium was essential to binding—thus its interaction with D-2 receptors differs from that of other neuroleptics which bind in the absence of sodium, although less effectively.[72,73] The results of Billard *et al.*[25] confirm the greater affinity of *S*-sulpiride

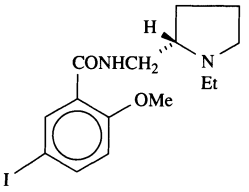


Scheme 7.1.

for D-2 sites, but the *R*-antipode is preferred at D-1 sites although both isomers interact only feebly (Table 7.7).

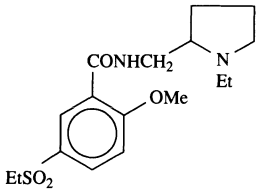
The absence of D-1 receptor stimulation is seen in the failure of sulpiride to inhibit DA-sensitive adenylate cyclase.[15] Both antipodes caused an increase in prolactin levels in man.[74] The evaluation of antipodes of sulpiride in behavioral tests is hindered by the limited passage of these components across the blood brain barrier (octanol/water partition coefficient 0.07).[67] When a central route of administration was employed (injection into nucleus accumbens), (−)-sulpiride was 260-fold more potent than the (+)-isomer as an inhibitor of ADTN-induced hyperactivity.[75] One puzzling result is the fact that *R*-(+)-sulpiride is a potent and selective antagonist of renal vasodilatory DA_1 receptors.[76] Bass and Robie[77] showed this action to be stereoselective only in regard to presynaptic receptors (*S* 130 × *R*)—antipodal forms were equipotent at postsynaptic receptors (effects measured after pretreatment with phenoxybenzamine).

The [^{125}I] analogue of sulpiride in its *S*-antipodal form **24a**, is recommended as a selective radioligand for dopamine D-2 receptors.[78] Binding IC_{50} values (nM) of *R*- and *S*-**24a** versus [^{125}I] *S*-**24a** in striatal homogenates of rat brain were 113 and 1.5, respectively.

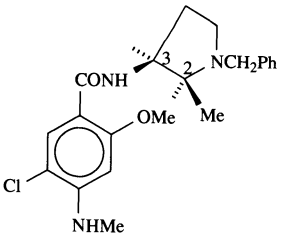


24a

The pharmacological profile of sulfopride (**25**), the ethyl sulphonyl analogue of sulpiride, mirrors the original compound. Affinity for DA receptors (D-2) is restricted to levo **25**.[15,79] Data on the DA-receptor selectivity of the 3-pyrrolidino benzamide **26** (YM-09151-2) are conflicting—early reports claimed preferential



25 Sulfopride



26 YM-09151-2

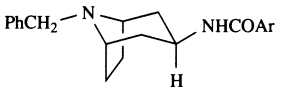
TABLE 7.7.
Binding of Sulpiride Antipodes to Rat Striatum[25]

	K_i (nM)	
	vs. [^{3}H]SCH-23390 (D-1)[a]	vs. [^{3}H]spiperidone (D-2)
R-Sulpiride	30,000	1102
S-Sulpiride	100,000	10

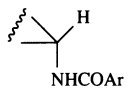
[a] 5-phenylbenzazepine derivative.

action at D-1 sites[80] but later studies pointed to antagonism of D-2 receptors.[15] Reports on stereoisomers are scarce—*cis*-**26** was about 8 times more effective than the *trans*-isomer in preventing apomorphine stereotypies in rats.[81]

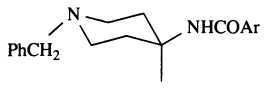
Hadley[67] reported some tropane analogues of benzamide antagonists of DA. The equatorial NHCOAr derivative **27a** was far more potent (ED_{50} 0.005 mg/kg) as an antiemetic in the dog than its axial analogue **27b** (ED_{50} 0.025 mg/kg) and superior to the related piperidine **28** (Clebopride, ED_{50} 0.01 mg/kg).



27a



27b



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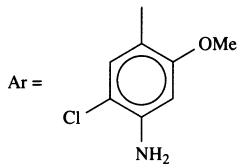
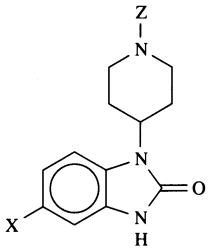




28′

29

(a) X=H, Z=$(CH_2)_3COC_6H_4p$-F
(b) X=Cl, Z=$(CH_2)_3COC_6H_4p$-F
(c) X=H, Z=$(CH_2)_3CH(C_6H_4p\text{-}F)_2$

A model of the central dopamine receptor based on the probable conformation of the potent tropane **27a** (which exceeds butaclamol in activity) has been advanced.[67] In its H-bonded form the NHCOAr unit of **27a** (see Fig. 7.3)

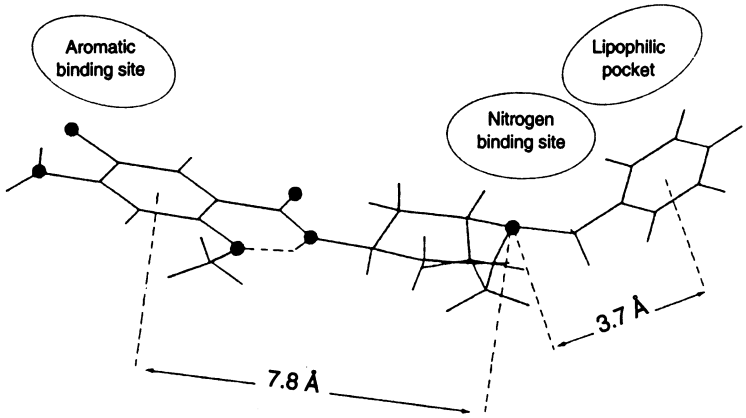


FIGURE 7.3. Model of the central dopamine receptor based on the tropane antagonist **27a** according to Hadley.[67]

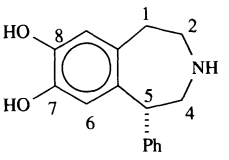
resembles the 4-piperidyl-benzimidazolinone features of the neuroleptic agents benperidol (**28**'a), pimozide (**28**'c), and halopemide (**28**'b).

In related indolizines **29**, the equatorial 2-phenyl derivative was a potent antagonist of apomorphine-induced climbing in mice (ED_{50} 0.024 mg/kg) while its axial phenyl analogue was ineffective in this respect.[82]

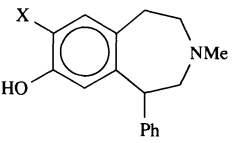
7.6. Halogenated Benzazepines

The 5-phenylbenzazepine **30** has already been described as a potent D-1 selective agonist (Chapter 6, page 193).

Remarkably, in a SA relationship which relates to that between isoprenaline and DCI, replacement of 8-hydroxy of the DA pharmacophore by halogen (Cl, Br, I) produces potent antagonists of DA receptors with retention of specificity for D-1 sites.[83,84] Binding K_i values (nM) for the antipodal 8-chloroderivates **31**a in rat striatum were 0.3 for *R*-(+) (SCH 23390) and 192 for *S*-(−) (SCH 23388) versus [^{3}H] SCH 23390. Neither antipode was effective in displacing the D-2 ligand [^{3}H]spiperidone (K_i values were 760 for the *R*- and 988 for the *S*-isomer).[25] Although Kebabian *et al.*[85] state that D-1 receptors are selective for the *R*-antipode of the 8-bromo derivative **31**b (SKF 83566), the reference they quote[86] refers to the ability of *R*- and *S*-antipodes to antagonize serotonin-induced constriction of rabbit thoracic aorta (pA_2 values were 7.95 for the *R*- and 5.80 for the *S*-isomer).



30 (+)-eutomer shown



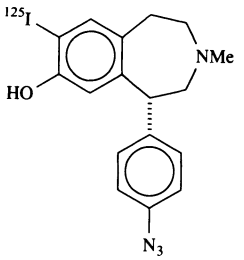
(a) X = Cl; (b) X = Br; (c) X = I

31

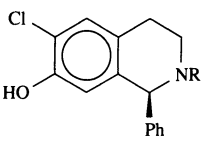
Tritiated forms of **31**a and **31**b (*R*-isomers) are valuable as D-1 receptor probes, while *R*-**31**c iodinated with [^{125}I] is particularly sensitive in this regard because of its higher specific activity.[85] In binding experiments[87] employing this [^{125}I] radioligand, relative affinities of *R*- and *S*-di-OH/NH **30** (agonist) were 706: 1, and of R- and *S*-**31**b (bromo analogue) 320: 1.

The corresponding photoaffinity probe *R*(+)-I-MAB **32** has now been developed.[88] Its affinity for [^{3}H] SCH 23390 binding sites of canine striatal membranes was 100-fold greater than that of the *S*-(−)-antipode. When striatal membranes were incubated with *R*-(+)-**32** and photolyzed, electrophoresis (sodium dodecyl sulfate-polyacrylamide gel) and autoradiography revealed three distinct bands of apparant M_r 74,000, 62,000, and 51,000, previously reported after the use of *rac*-**32**.[89,90]

While eutomers of antipodal phenylbenzazepines have a benzylic chiral center of *R*-configuration whether they be agonists (Chapter 6, page 193) or antagonists, those of tetrahydroisoquinolines (**33**) related to the antagonists **31** display reverse *S*-geometry at the same center.[91] Ring contraction led to a fall in binding affinity



32 R-(+)-I-MAB

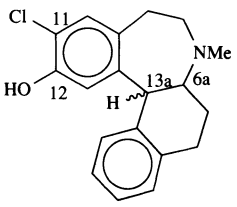


(a) R = Me; (b) R = H
33
S-antipode shown
(X-ray evidence of configuration)

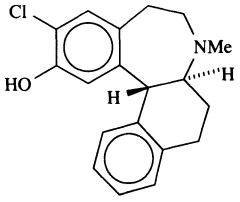
but selectivity for D-1 sites was maintained; binding K_1 (nM) values vs.[^{3}H] SCH 23390 for **33**b were: *S*, 6.6; *R*, 442; SCH 23390, 0.43, and inhibitory K_i (nM) values for conversion of [^{32}P]-ATP to [^{32}P]-cAMP were: *S*, 6.58; R, 568; SCH 23390, 0.47. Least-squares-fitted SYBYL comparisons [MM2(85) program] revealed that *S*-(+)-**33** antipodes possessed a much better fit to *R*-**31** isomers than did the *R*-(−)-enantiomer.[92] The same authors have presented an extensive conformational analysis and molecular modeling investigation of the tetrahydroquinolines **33** and related compounds.

Berger *et al.*[93] obtained some interesting results when they cyclized **31** to the tetracyclic molecule **34**. The chemistry involved a ring-closure reaction which produced a separable mixture of 11,12-dimethoxy analogues of **34**. One product was shown by X-ray crystallography to be the *cis*-isomer and hence its partner must have *trans*-geometry. Racemic *trans*-**34** (O-methylated) was resolved by use of (+)- and (−)-ditoluoyltartaric acids, and the two antipodes converted to corresponding *cis*-diastereoisomers by base-catalyzed epimerization at C-13b.

Enantiomeric purity was established by HPLC using β-cyclodextrin as a component of the mobile phase, and absolute stereochemistries determined by X-ray analysis of the levo antipodes of the *cis*- and *trans*-diastereoisomers. Binding affinities of the diastereoisomers **34** are listed in Table 7.8. The (−)-*trans*-isomer **35**



34



35
(–)-6a*S*, 13b*R*

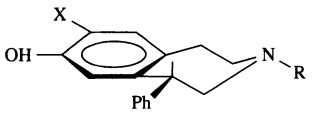
had significantly greater D-1 affinity and selectivity, and a configuration at C-13b which corresponded with the C-5 configuration of its noncyclized parent **31**. The inactivity of the *cis*-derivatives may be accounted for by the fact that one of the substituents on the azepine ring must assume an axial orientation in either of the readily convertible near-chair conformers, in contrast to the *trans*-series where both substituents are rigidly orientated equatorially.

In an extensive conformational analysis exercise using MM2 calculations, a

TABLE 7.8.
D-1 and D-2 Receptor Affinities of Some Benzo[*d*]naphth[2,1-*b*]azepines (34)[93]

Isomeric form of **34**	K_i (nM)	
	vs. [^{3}H] SCH 23390 (D-1)	vs. [^{3}H]spiperidone (D-2)
rac-cis	473	9,073
(+)-*cis*	898	16,317
(−)-*cis*	513	3,476
rac-trans	3.3	4,115
(−)-6a*S*,13b*R* (**35**)	1.9	514
(+)-6a*R*,13b*S*	531	3,046
SCH-23390	0.4	648
R-(+)-**31**a		

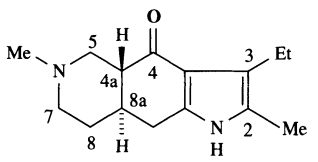
Danish group[94] concluded that the most probable receptor-bound conformation of both the antagonist **31**a and the DA-agonist SKF38393 (**30**) is a chair with an equatorially oriented phenyl substituent as in **35a**—in this arrangement the two aromatic rings are approximately orthogonal. The constrained analogue **35** cannot adopt alternative chair of twist conformations and an arrangement of type **35a** proved to be its lowest-energy form.



35a

7.7. Miscellaneous Antagonists

The pyrrolo[2,3-*g*]isoquinoline **36** was designed on the basis of a hypothetical mode of the interaction of antipsychotic agents with the dopamine receptor and knowledge of the structure of the clinical agent molindone (**37**).[95] Doses producing 50% block of avoidance response in rats were (mg/kg): (−)-**36** 0.49, (+)-**36** > 6.0; molindone 3.6; haloperidol 0.35. The more potent antipode of **36** had a 4a*R*,8a*R*



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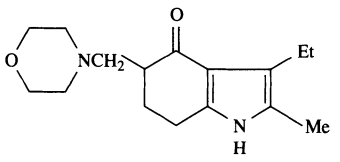


37

configuration (by X-ray analysis) as predicted (see below). Four binding regions were proposed for Olson's model involving:

1. interactions with protonated nitrogen, e.g., an ionized carboxylate function ($^+$NH....$\bar{O}_2$C−);

2. π–π stacking interaction between an aromatic amino acid unit and an aromatic feature of the ligand, e.g., ring A of dexclamol;
3. similar interaction with a second aromatic feature of the ligand (e.g., ring B of dexclamol and other tricyclic derivatives) or a carbonyl group as present in butyrophenone neuroleptics and atypical agents such as molindone and the benzamides;
4. interactions at an auxiliary lipophilic site as proposed by Humber (p. 216) which binds bulky alkyl and aryl groups and a variety of spiropiperidine and benzimidazolone groups seen in butyrophenones.

Figure 7.4 illustrates these sites—much of these arguments correlate with the superimposition results of Tollenaere (page 217).

The 4a,8a-*trans* ring fusion of **36** assures that the aromatic ring, nitrogen lone pair (or $^{+}$NH), and carbonyl groups are fixed in an orientation that optimizes receptor interactions which bind sites 1–3. From these modeling operations a 4a*R*,8a*R*-configuration was predicted for the eutomer of **36**, as proved to be the case.

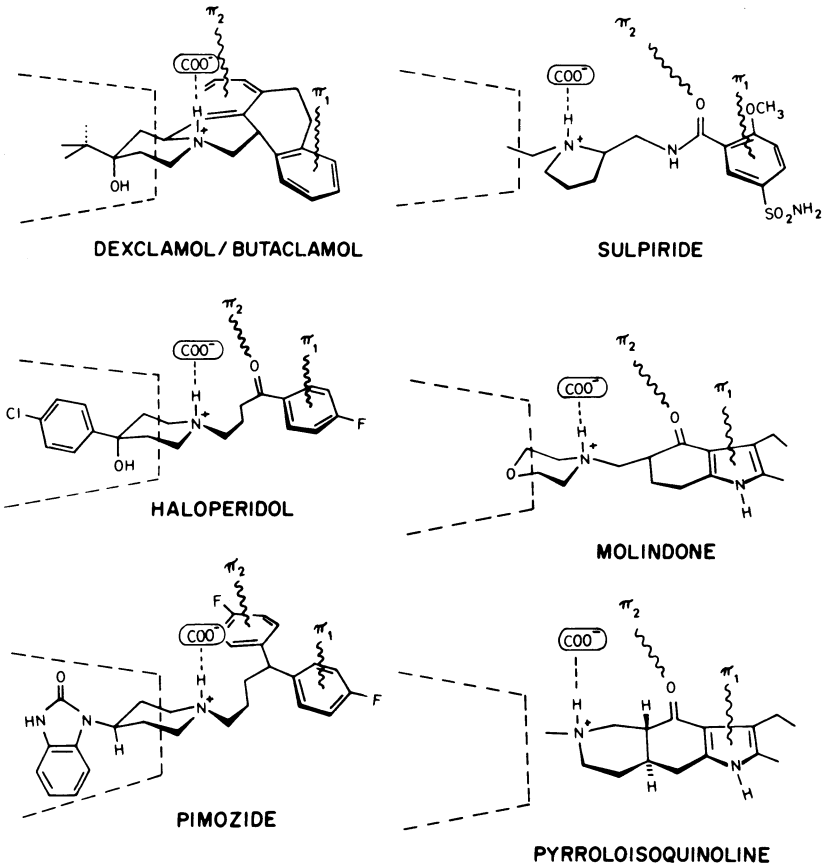
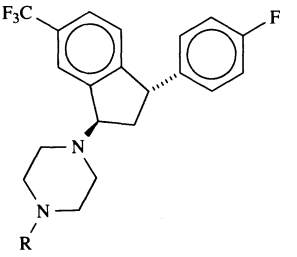


FIGURE 7.4. Receptor interactions of diverse antipsychotic drugs: aromatic groups (π_1, π_2), carboxylate group (COO^-), and auxiliary lipophilic binding site (dashed lines) (after Olson *et al.*).[95]

Another class of neuroleptic agent is based on 1-piperazino-3-phenylindans, and represented by the compound tefludazine **38**a which has a *trans*-arrangement of 1,3-substituents.[96] The *cis*-racemate lacked activity while that of the *trans* was confined to the 1*R*,3*S*-enantiomer (configuration by X-ray crystallography).[97] Some data are shown in Table 7.9. Unpublished results from the Lundbeck company are included in the review of Hyttel *et al.*[15]



(a) R = $(CH_2)_2OH$ (tefludazine); (b) R = Me

38

The fact that the ED_{50} for DA antagonism of the racemate **38**b (CF_3 replaced by F) was greater than twice that of the (+)-eutomer attained significance when it was discovered that the neuroleptic distomer (levo 1*S*,3*R*) was a potent inhibitor of the uptake of DA and other neurotransmitter amines into synaptosomes (Table 7.10).

When structural comparisons were made between antipodal forms of tefludazine and octoclothepin **18**, the X-ray structure of the potent neuroleptic *S*-(+)-octoclothepin corresponded to the 1*S*,3*R*-enantiomer of the indan derivative (inhibitor of amine uptake) rather than the 1*R*,3*S*-neuroleptic antipode. This unexpected result was rationalized by means of a conformational analysis of the two compound, details of which are complex and not easily summarized,[47] (see Ref. 99 for the most recent discussion of this point).

TABLE 7.9.
Activity Data for Tefludazine and its Isomers[96, 99]

	Antagonism of methylphenidate-induced stereotypies in mice ED_{50} (mol/kg po)	Displacement of tritiated	
		Haloperidol IC_{50} (nM)	Spiperidone
t-rac (**38**a)	0.07	8.8	19
t-(+)[a]	0.15	not tested	14
t-(−)	8.6	not tested	590
c-rac (**38**a)	> 80	not tested	not tested
t-rac (NMe)[b]	11 (*cis* > 95)	57 (*cis* 2500)	95
t-(+)	2.5	22	33
t-(−)	42	780	420
Flupenthixol	0.14	3	—
Chlorpromazine	16	12	—
rac-Octoclothepin	6.7	3	—

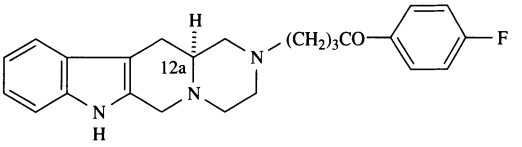
[a] Configuration 1*R*,3*S*.
[b] Structure (**38**b, R = Me) with CF_3 replaced by F.

TABLE 7.10.
Inhibition of Neuroamine Uptake by Tefludazine and its N-Methyl Analogue[61, 96]

	Synaptosomal uptake inhibition IC_{50} (nM)		
	DA	NA	5-HT
rac-**38**a (tefludazine)	520	660	7000
1*R*,3*S*-(+)[a]	2200	15,000	not tested
1*S*,3*R*-(−)[b]	130	730	not tested
rac-NMe[c]	180	45	2000
1*R*,3*S*-(+)	1800	13,000	4000
1*S*,3*R*-(−)[b]	88	32	630

[a] Ref. 99.
[b] Neuroleptic distomer.
[c] Structure (**38**b, R = Me) with CF_3 replaced by F. Data on *rac-cis* analogue: 110 (DA), 97 (NA), 6100 (5-HT).

The pyrazinopyridoindole (**39** centbutindole), which incorporates a butyrophenone side chain, has properties characteristic of a DA antagonist (e.g., prevention of amphetamine-induced stereotyped behavior in guinea pigs, enhanced DA turnover in corpus striatum).[98] It displaces [^{3}H]spiperidone binding in calf striatal membranes with the levo isomer twenty times more effective than the dextro



39
(−)-12a*S*-eutomer shown

form.[40] In tests on antipodal pairs (derived synthetically from *S*- and *R*-tryptophan) the (−)-12a*S*-isomer proved to be the eutomer, and this isomer could be superimposed satisfactorily on (−)-6a-*R*-apomorphine as a result of the correspondence of 12a and 6a geometry, respectively.

The review of Hyttel *et al.*[15] on chiral neuroleptics includes a few further examples not discussed here.

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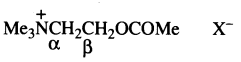
8

Cholinergic Agonists

8.1. Introduction

Much of this chapter and the following one on cholinergic antagonists revolves around acetylcholine (**1** ACh) and its analogues. Acetylcholine was the first neurotransmitter to be reported and its discovery dates back to Loewi's classic work on paired frog hearts carried out in the early 1920s. The dual aspects of the



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pharmacological effects of ACh, namely, those mimicked by muscarine and those by nicotine, were quickly recognized and implied the existence of at least two types of cholinergic receptor (muscarinic mAChR and nicotinic nAChR). The development of specific ligands and pharmacological models has led to the definition of four subtypes of mAChR (M_1, M_2, M_3, and M_4)[1] and much progress has been made in isolating and characterizing these receptors together with nAChR by application of recombinant DNA techniques[2,3] (see Ref. 4 for a general account of the cholinergic field).

It is now the best part of a century since Cushny[5] reported a potency difference between the muscarinic antagonists hyoscyamine and the racemic mixture atropine, but interest in the stereochemical aspects of ACh and its congeners did not really blossom until the 1960s. This interest included the evaluation of chiral substrates for acetylcholinesterase (AChE), the enzyme responsible for terminating the action of ACh.

The topic of cholinergic agents has been extensively reviewed since the late 1950s, and several of these works deal specially with stereochemical influences.[6-12]

8.2. Methylated Analogues of Acetylcholine

Chiral analogues of ACh produced by α- or β-methylation of its bimethylene chain in respect to nitrogen provided some of the earliest evidence of steric influ-

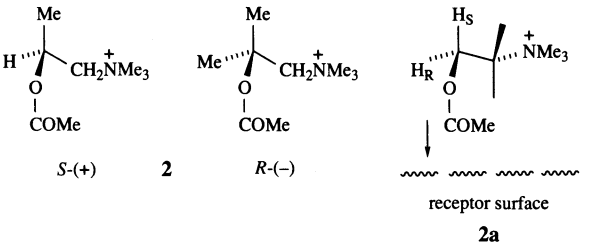
TABLE 8.1.
Muscarinic Activities of Methylated Acetylcholines[17]

Substituent	Form	No. of moles ≡ 1 mole of ACh as agonist in the GPI	(+)/(−) Activity ratio
α-Me	*RS*	49	
	S-(−)	232	8 (11)[c]
	R-(+) (eutomer)	28	
α-Me (N^+Me_2Et)	*RS*	264	
	S-(−)	1980	11 (22)
	R-(+) (eutomer)	170	
β-Me[d]	*RS*[a]	1.58	
	S-(+) (eutomer)[b]	1.01	240 (270)
	R-(−)	240	

[a] Methacholine.
[b] *S*-(+)-hydrolized by AChE at about half the rate of ACh; *R*-(−) is a weak inhibitor of the enzyme[14]; Belleau and Lavoie[15] found the absorption of *R*-(−) to the enzyme to be exothermic ($\Delta H°$ −4.6 kcal/mol), while that of the eutomer was endothermic ($\Delta H°$ +2.2 kcal/mol).
[c] Ratios in parentheses from cat blood pressure assay.
[d] pD_2 values reported by Chang and Triggle[18] were: *S* 7.24 and *R* 4.22 (*S*/*R* ratio 1000).

ences on cholinergic activity. β-Methylation produces an agent selective for muscarinic sites and of similar potency to the parent ACh, while α-methylation leads to a ligand selective for nicotinic sites.[13] The high potency of β-methyl ACh may in part be attributed to its resistance to attack by acetylcholinesterase (AChE)[14] (footnote *b* to Table 8.1.)—ACh and the α-methyl homologue are hydrolyzed at similar rates. The 1935 report of Major and Bonnett[16] of (+)-β-methyl ACh having about 100 times the muscarinic activity of its antipode in a fall of blood pressure test was reinvestigated during the 1960s by groups led by Beckett and Ariëns, respectively. Some data of Beckett *et al*[17] are shown in Table 8.1.; these reveal the feeble muscarinic properties of the α-methyl derivatives, the potent activity of *S*-(+)-β-methyl ACh (≡ACh), and the high antipodal activity ratio of the β-methyl isomers. Ellenbroek *et al.*[19] reported a similarly high *S*/*R* affinity ratio (320) for antipodal β-methylacetylcholines (pD_2: *S* 6.9, *R* 4.4, ACh 7.0) tested on rat jejunum. Since both antipodes behaved as full agonists (i.a. = 1) the authors considered that differences in potency must be related only to differences in affinity, i.e., while methyl in the *S*-(+)-antipode **2** has little influence on affinity, it is an interfering factor in receptor occupation by the *R*-(−)-isomer.

These results suggest that the ACh molecule approaches its receptor in such a manner that the *S*-prochiral β-proton is remote from the receptor surface. This approach mode is depicted in **2a**, shown with the O/N functions in an antiplanar–anticlinal disposition (see later).

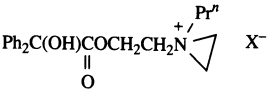


2a

When H_S is replaced by methyl [as in *S*(+)-**2**] ligand–receptor binding is not obstructed; the same replacement of H_R [giving *R*-(−)-**2**] positions methyl close to the surface, where it impedes binding.

In later work, however,[20] a discrepancy was found between the *S*/*R* affinity ratio (180) of antipodes for muscarinic sites of rat heart, and the ratio of their abilities to inhibit release of [^{3}H]-noradrenaline (650) from sympathetic nerves of rat heart electrically stimulated. This result pointed to the *S*-isomer having a greater intrinsic activity than the *R*-form in its action at adrenergic sites, in addition to its greater affinity.

Ringdahl[21] subsequently determined the relative efficacies of the antipodal β-methacholines by application of a method developed by Furchgott and Bursztyn[22] which involves measurement of agonist responses before and after inactivation of a fraction of the receptors with an irreversible antagonist. Ringdahl used propylbenzilycholine mustard **3** to inactivate the receptors rather than



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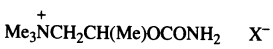
dibenamine, originally employed. Data for ACh and antipodal methacholines at GPI sites, with AChE inhibited by diisopropylfluorophosphate, were:

	pD_2	pK_D	e_r[a]
ACh	7.44	5.77	1.0
S-(+)-Methacholine	7.37	5.70	0.60
R-(−)-Methacholine	4.50	3.21	0.28

[a] e_r denotes the relative efficacy.

Thus the markedly lower activity of *R*-methacholine is a consequence of both its lower affinity ($1/617 \times S$) and lower intrinsic activity ($0.4 - 0.5 \times S$) as compared with its antipode. Birdsall *et al.*[23] identified two binding sites in rat cerebral cortex occupied by [^{3}H]PrBCh (*N*,*N*-dimethyl-*N*-propyl-2-aminoethylbenzilate) and measured the respective low (K_L) and high (K_H) affinity of a number of agonists from their ability to displace this ligand. The authors suggest that the K_L/K_H ratio reflects agonist efficacy and reported values of 100 for ACh, 80 for *S*-methacholine, and 11 for *R*-methacholine. Rat-forebrain binding parameters for methacholines vs. the tritiated dioxolane (**20**, page 240) have also been reported[24]: pK_i 6.96 (*S*-methacholine), 4.24 (*R*-methacoline), 8.06 (ACh) (AChE inhibited).

Antipodes of bethanechol (**4**, β-methylcarbamoylcholine) have also been examined.



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The *S*-(+)- and *R*-(−)-isomers prepared by De Micheli *et al.*[25] displayed a eudismic ratio of 740 at rat jejunum sites with the *S*-antipode the eutomer. Schwörer *et al*[26] reported a ratio of 915 using guinea-pig intestinal muscle. The

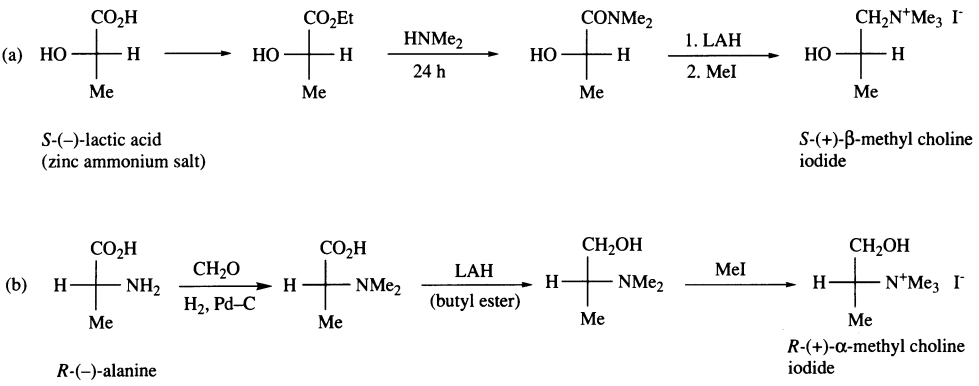
claim that cardiac tissue was relatively insensitive to bethanechol compared to *S*-methacholine was confirmed by Buhl *et al.*[27] and attributed to the absence of high-affinity binding sites in this tissue. Rat jejunum contains both high- and low-affinity sites (high 13.6%; low 86.4%).

In all tissues the affinities of *S*-(+)-bethanechol exceeded those of the *R*-(−)-isomer (vs. [^{3}H]QNB).

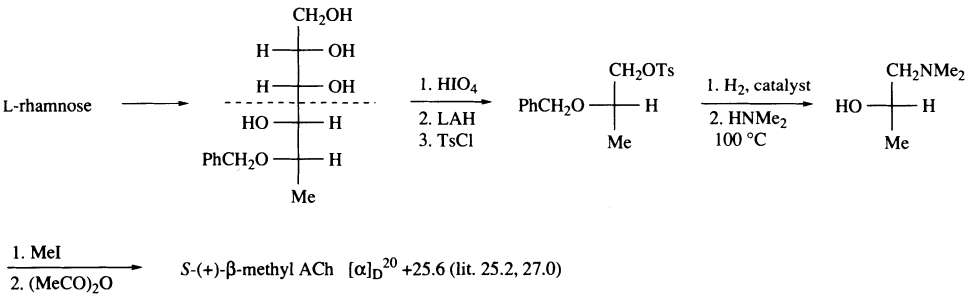
The stimulation of presynaptic receptors by certain muscarinic agonists inhibits the stimulation—evoked release of ACh from peripheral and central nerves.[28] Only the *S*-antipode of bethanechol was effective in reducing the outflow of [^{3}H]ACh.[28] The unexpected greater inhibitory action of ***RS*-4** was attributed to the weak inhibition of AChE by the *R*-isomer (***S*-4** is substrate for the enzyme).

The question of indirect action (release of ACh) may need to be considered in work on bethanechol, since its parent carbachol has been shown to act, at least in part, in this manner (see Chap. 10, p. 327). Racemic α-methyl ACh, although a feeble agonist at GPI sites (49 × less potent than ACh), retains half the activity of ACh at frog rectus nicotinic sites (*rac*-β-MeACh is 180 times *less* potent than ACh in this test).[29] Lesser[30] compared the potencies of *R*-(+)- and *S*-(−)-α-methyl ACh antipodes in a variety of nicotinic preparations. He found relatively small differences in potency of the two isomers and no consistent degree of stereospecificity, which ranged from 0.4–2.12. Thus the *R*-antipode was the more potent at the frog rectus abdominis and chick biventer muscles, and the *S*-isomer at cat superior cervical ganglia and in the atropinized cat blood pressure preparation. Similar results were obtained when chick biventer muscle was employed in the presence of the acetylcholinesterase inhibitor physostigimine—evidence of the noninvolvement of AChE.

Chemistry. Antipodes of β-methyl ACh were originally obtained by a process involving resolution of 1-dimethylaminopropan-2-ol.[16] Isomers used by Beckett's group were prepared from *R*- and *S*-lactic acid by a process which established absolute stereochemistry (Scheme 8.1a). A related stereospecific sequence starting from *R*- and *S*-alanine led to antipodes of α-methylacetylcholine (Scheme 8.1b). Ellenbroek[32] (quoted in Ref. 19) used similar synthetic methods to obtain these materials. More recently, syntheses based on sugars have been reported—the conversion of L-rhamnose into *S*-(+)-β-methyl ACh is outlined in Scheme 8.2.[33]



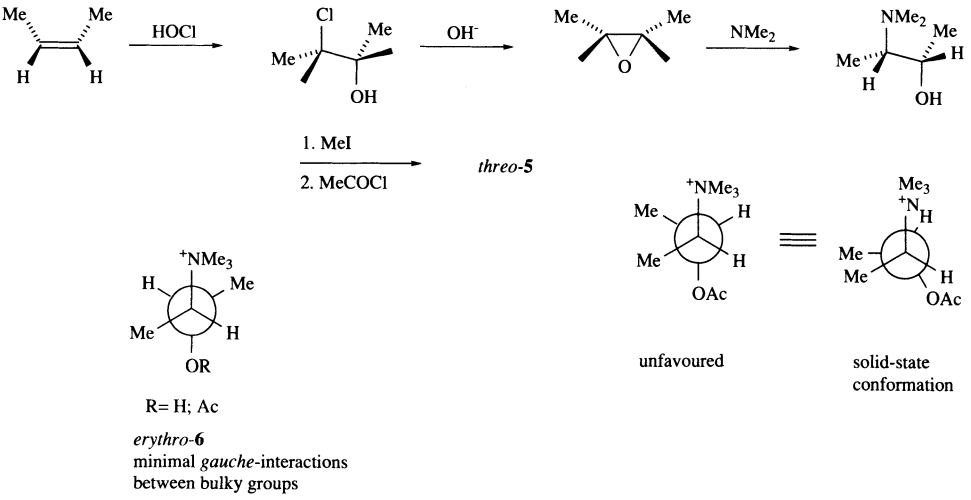
Scheme 8.1. Stereospecific syntheses of *S*(+)-β-methyl- and *R*(+)-α-methyl-choline.[31]



Scheme 8.2. Synthesis of *S*-(+)-β–methyl ACh from L-rhamnose .[31]

The two *rac*-diastereoisomers of α,β-dimethyl Ach have been examined.[34] The more active *erythro*-isomer had 14% of the muscarinic potency of ACh and was almost completely resistant to AChE; the *threo*-isomer was inert as a cholinergic agent and a poor substrate for AChE (see also discussion of ACh analogues based on decalin, page 262).

Chemistry. Erythro-**6** and *threo*-**5** were obtained from *trans*- and *cis*-butene, respectively (Scheme 8.3). Configurations anticipated on mechanistic grounds were supported by IR evidence for the precursor aminoalcohols (dilute solutions were used to eliminate intermolecular effects): *cis*-derived—intramolecularly bonded OH band only (a free OH band requires the unfavored conformer **5**); *trans*-derived—free and bonded OH bands seen (conformer **6** favored). Activity differences between the diastereoisomers were interpreted in terms of the high energy of the antiplanar N/O *threo*-conformers. However, in solid state the *erythro*-isomer adopts a synclinal N/O conformation ($^{+}N\text{-}C_3\text{-}C_2\text{-}O_1$ dihedral angle 76°) while the *threo*-isomer had a configuration similar to the antiplanar form (Scheme 8.3) with a dihedral angle of 143°.[35]



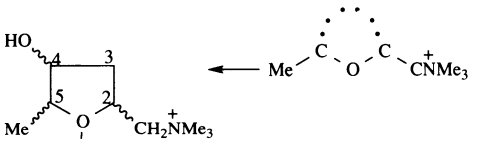
Scheme 8.3. Conversion of *cis*-butene to *threo*-α,β-dimethyl ACh (*trans*-butene gives the *erythro*-isomer).

Dimethyl substitution of ACh either in the α,α or β,β positions markedly reduces, but does not abolish, the muscarinic action of the parent (see **7**).[36]

		Potency (GPI, ACh = 1)		
$+ \alpha \beta$				
$Me_3NCH_2CH_2OCOMe$	α-Me (*RS*)	0.0339	β-Me (*RS*)	0.622
7	α,α-di-Me	0.0025	β,β-di-Me	0.001

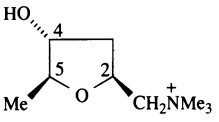
8.3. Muscarine and Its Analogues[37]

Interest in the stereochemistry of muscarine, a natural product found in the fungus *Amanita muscaria* (fly agaric), preceded and overlapped work on methylated acetylcholines. The similarities between the actions of ACh and muscarine on smooth muscles and glands was recognized early in the century[38] and gave rise to the definition of "muscarinic" actions of ACh to distinguish them from effects on ganglia and voluntary muscles mimicked by nicotine. Investigations of muscarine proved difficult due in part to its low natural occurrence, e.g., 0.0002% in *A. muscaria*, and its structure and absolute configuration were not establish until the late 1950s, and confirmed by stereospecific synthesis.[39] The natural (+)-isomer forms one of eight stereoisomers of 2-trimethylammoniummethyl-4-hydroxy-5-methyltetrahydrofuran **8**. It may be regarded as a cyclic analogue of ACh in



8

which the β- and carbonyl carbons are linked by a bimethylene bridge. Steric requirements for activity among these isomers are stringent, demanding that the 5-methyl and 2-$CH_2\overset{+}{N}Me_3$ substituents be *cis* to each other and *trans* to the 4-OH group. Of the enantiomorphic pair having this geometry, only the 5*S*,4*R*,2*S*-isomer[(+)-muscarine **9**] is highly potent, the (−)-form being several hundred times



9 2*S*,4*R*,5*S*-(+)-muscarine

less active (Table 8.2).* All other isomers are only feebly active, the next most potent of those tested being *rac*-epiallomuscarine which share the *trans*-$OH/CH_2\overset{+}{N}Me_3$ geometry of muscarine itself. A point of immediate interest is the

* An amusing account of how the distinction between natural and synthetic muscarine by polarimetry forms a key element of the plot of Dorothy L. Sayer's novel *The Documents in the Case* has been given by Edwin Crundwell.[41]

TABLE 8.2.
Activities of Muscarine and Its Isomers[40]

Compound	Substituent geometry		Form	No. of moles ≡ 1 mole ACh (cat blood pressure)
	$CH_2{}^+NMe_3$/Me	$CH_2{}^+NMe_3$/OH		
Muscarine	*cis*	*trans*	*RS*	1.0
			2*S*, 4*R*, 5*S*	0.5
			2*R*, 4*S*, 5*R*	350
Epimuscarine	*cis*	*cis*	*RS*	350
Allomuscarine	*trans*	*cis*	*RS*	200
Epiallomuscarine	*trans*	*trans*	*RS*	120

identity of the C-2 configuration of muscarine and the *S*-(+)-eutomer of β-methyl acetylcholine.

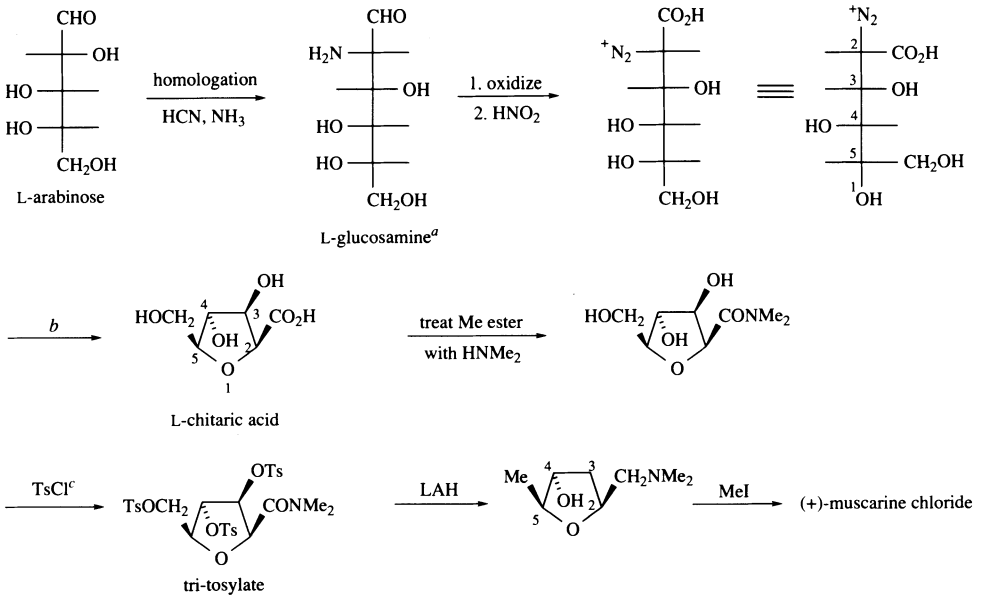
Test on frog heart and rectus abdominis muscle quoted by Wilkinson[39] shown in Table 8.3 illustrate the failure of natural and racemic muscarine to act at voluntary muscle sites, the approximately double potency of natural (+) compared to synthetic (*rac*) material, and the drastic fall in potency that follows removal of one of the N-methyl groups of muscarine. The results also demonstrate the greater sensitivity of muscarinic compared to nicotinic sites toward ACh. (See Ref. 204 for 1992 reports on stereoisomeric muscarines)

Chemistry. While the relative stereochemistry of natural muscarine has been confirmed by X-ray crystallography,[42] its absolute geometry rests on its synthesis from various sugars of known configuration as reviewed by Morrison and Scott.[43] The original synthesis of Hardegger and Lohse[44] is the best known (Scheme 8.4). A key step involved the conversion of L-glucosamine (derived from natural L-arabinose) to L-chitaric acid. Reduction of the derived tri-tosylate was remarkable in bringing about amide to amine conversion at C-2, reductive removal of two of the tosyl groups (at C-3 and C-5, $CH_2OTs \rightarrow Me$), and cleavage of the third (at C-4). The absolute configurations 2-*S*, 4-*R*, 5-*S* for (+)-muscarine follow from this series of interconversions.

TABLE 8.3.
Effects of Muscarine and Related Compounds on Frog Heart and Rectus Abdominis Muscle[39]

	Concentration of sample frog heart[a] (muscarinic)	(μg/ml bath-volume) frog rectus submaximal concentration[b]
rac-muscarine iodide	0.032	> 500
(+)-muscarine iodide	0.018	> 500
(+)-normuscarine HCl	5.0	—
Acetylcholine chloride	0.002	5

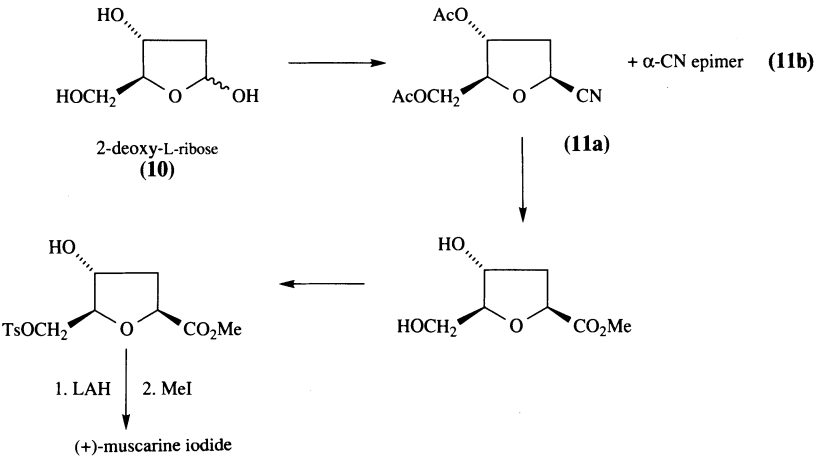
[a] Concentration which produces decreases in amplitude and rate of beat of both auricle and ventricle equivalent to those given with 0.002 μg/ml ACh.
[b] Concentration giving one-quarter of the maximum concentration of the isolated frog's rectus abdominis muscle.



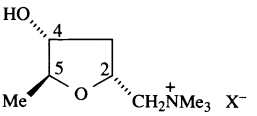
[a] Separated from concurrently produced diastereomer.
[b] Cyclizes with retention of configuration.
[c] Ts = p-$C_6H_4Me{\cdot}SO_2$.

Scheme 8.4. Hardegger and Lohse's synthesis of (+)-muscarine.[44]

Pochet and Huynt-Dinh[45] also devised a synthesis of (+)-muscarine from L-arabinose (Scheme 8.5). 2-Deoxy-L-ribose **10**, obtained in five steps from L-arabinose, gave the diastereoisomeric nitriles **11a** and **11b**, one of which (**11a**) led to (+)-muscarine by the route shown. The nitrile **11b** was converted in the same manner to (−)-allomuscarine **12** which, along with (−)-epimuscarine (2*S*, 4*S*, 5*S*), has also been isolated from natural sources. Use of 2-deoxy-D-ribose provided a route to (−)-muscarine.



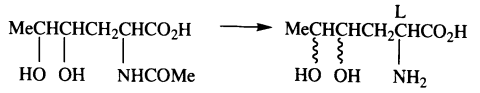
Scheme 8.5. Synthesis of (+)-muscarine from 2 deoxy-L-ribose.[45]



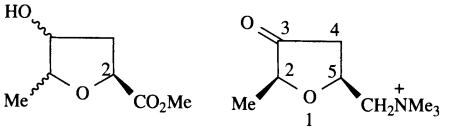
12 (–)-allomuscarine (2*R*,4*R*,5*S*)

Whiting, Au-Young and Belleau[46] described a nonsugar-based synthesis which exploited the selectivity of hog kidney acylase for L-amino acids. Treatment of *trans-N*-acetyl-*rac*-crotylglycine with performic acid gave the mixture **13**, which yielded 25% of the L-α-amino acid **14** after incubation with the acylase-l-enzyme. Cyclization of the acid (HNO_2, MeOH) gave the tetrahydrofuran **15** of known configuration at C-2 which yielded two products on further elaboration, one of which was (+)-muscarine (2 × ACh in GPI assay). The other product was assumed to be an *allo*-muscarine, which had very weak activity in the GPI test.

The muscarinic activity of *rac*-muscarine is retained at high levels (elevated in some tests) when oxidized at C-3 to muscarone (**16**, shows 2*S*,5*S*-isomer) (Table 8.4), suggestive of an improved analogy with ACh.[39,47]



13 **14**



15 **16** (+)-muscarone

(note ring number change)

TABLE 8.4.
Cholinergic Activities of Muscarone and Related Compounds[39, 47]

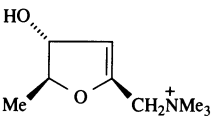
Compound	Equipotent molar ratio (ACh = 1)				
	Cat blood pressure (M)[a]	Rabbit ileum (M)	Frog heart (M)	Blockade of cat superior cervical ganglion (N)	Frog rectus (N)
rac-Muscarone	0.12	0.13	4	0.1	0.5
2*S*,5*S*-Muscarone (+)-**16**	0.25	0.15	—	—	2.0
2*R*,5*R*-Muscarone (−)-**16**	0.10	0.06	2.5	0.05	0.5
rac-allo-Muscarone (*trans*-2-Me/5-$CH_2N^+Me_3$)	0.25	0.28	6.1	0.075	0.2
rac-4,5-Dehydromuscarine (**17**)	0.76	—	61	—	—
rac-4,5-Dehydro-epimuscarine (**18**)	1.5	—	120	—	—

[a] Denotes muscarinic test; N denotes nicotinic test.

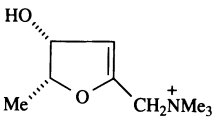
8.4. Muscarone Analogues and Cyclopentane Derivatives

Remarkably, muscarone behaves as a potent nicotine agonist, superior in fact to ACh. In addition, both muscarinic and nicotinic receptors display little selectivity for antipodes of muscarone, in sharp contrast to the large potency difference between (+)- and (−)-muscarine at muscarine sites (cf. similar observations made for α-methyl ACh at nicotinic sites, page 234. The data of Table 8.4 show a fewfold preference of nicotinic sites for the 2*R*,5*R*-antipode of muscarone, i.e., that related to the *distomer* of muscarine. Belleau and Puranen[48] have commented on this fact in terms of the effect of replacing the hydrogen-bonding donor function (CHOH) of muscarine for the acceptor function (C=O) of muscarine (see also Ref. 49).

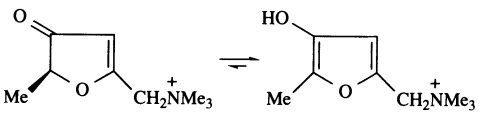
In muscarones, *cis*-2-Me/5-CH_2NMe_3 geometry is no longer a prerequistite for high potency since *rac-allo*-muscarone shows high nicotinic activities in all the tests of Table 8.4 (see Ref. 204 for antipodal data). Racemic 4,5-dehydromuscarine **17** and 4,5-dehydro-*epi*-muscarine **18** provide another pair with potencies (muscarinic) little influenced by configuration. Racemic 4,5-dehydromuscarone **19** is also a potent cholinergic agonist (equipotent with *rac*-muscarone in muscarinic tests, more effective in the frog rectus test).[39] Beckett *et al.*,[50] have shown by ^{1}H-NMR that **19** exists in equilibrium with the corresponding enol in D_2O at pD 5.



trans-**17**



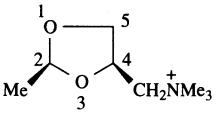
cis-**18** (one antipode shown)



keto **19** enol

8.4.1. Muscarine Analogues

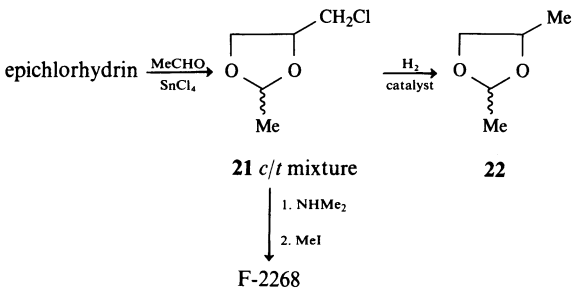
The 1,3-dioxolane **20** represents an analogue of muscarine in which the CHOH fragment of the natural product has been replaced by oxygen. Its cholinergic properties were discovered during the 1930s (Fourneau's compound F2268). In 1962 Triggle and Belleau[51] showed that the product of the literature procedure was a mixture of geometrical isomers (co-crystalline) and that the *cis*-member was



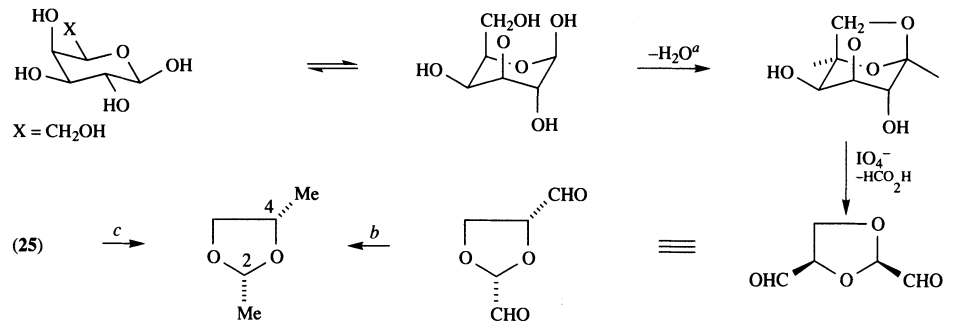
20 *cis*-4*R*-isomer shown

five times as active as the *trans*-isomer when both were tested in the racemic form and equal in activity to ACh (GPI assay). Belleau and Puranen[48] later resolved the *cis*-isomer and demonstrated a potency difference of 100 between the antipodes. The eutomer was the 4*R*-isomer **20** related in configuration to the C-2 center of (+)-muscarine (note that the sequence rule alters the steric designation at C-4); its potency was remarkable—six times ACh in the GPI. In a subsequent report on all four diastereoisomers, observed potencies relative to ACh (100) were: *cis*-2*S*,4*R* 500; *trans*-2*R*,4*R* 50; *trans*-2*S*,4*S* 25; *cis*-2*R*,4*S* 8.5.[51]

Chemistry. The precursor of F-2268 is the 4-chloromethyldioxolane **21**—Triggle and Belleau[51] converted this to the 2,4-dimethyl derivative **22** which proved to be an isomeric mixture separable by GC into two components (61:39 ratio). The major product corresponded physically (IR, NMR) with the *cis*-derivative **23** prepared stereospecifically from D-galactose (Scheme 8.6). The periodate cleavage step in the sequence has been described by Hann and Hudson.[52]



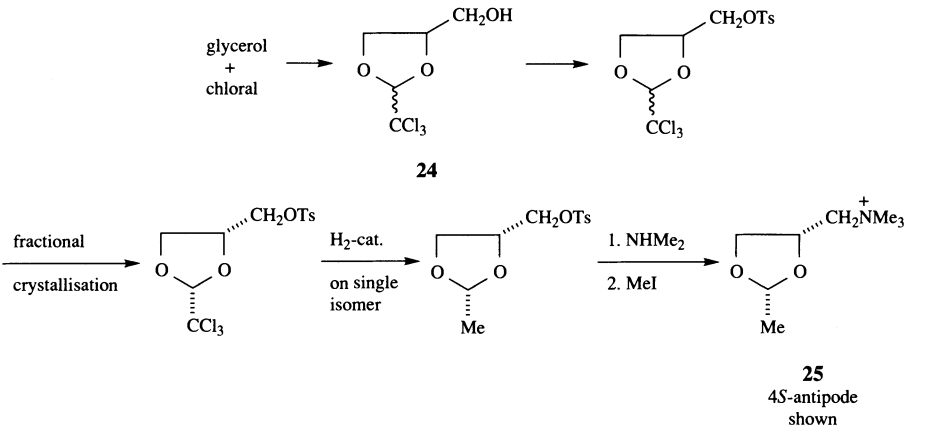
The free base derived from F-2268 by N-demethylation (using LAH) was likewise proven by GC to be a 61:39 isomeric mixture. The quaternary product (F-2268) was thus an isomeric mixture but inseparable, because of molecular com-



[a]Low-pressure pyrolysis.
[b]Reagents: 1. $NaBH_4$; 2. TsCl; 3. LAH (Ref.51).
[c]Via lower mp tosylate of **24**.

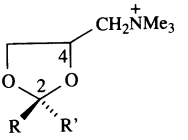
Scheme 8.6. Conversion of β-D-galactose to the *cis*-2*R*,4*S*-dimethyldioxolane (**23**).[52]

plexation. Use of chloral instead of acetaldehyde in the cyclization reaction gave 4-hydroxymethyl isomers **24**, which formed tosylates that could be separated by fractional crystallization. The lower-melting tosylate was correlated chemically with the reference *cis*-2,4-dimethyl derivative **23** (Scheme 8.6), and to the quarternary salt **25** which must also be of *cis*-configuration. The *trans*-analogue of **25** was obtained from the *t*-tosylate. Both antipodal forms of *cis*-**20** were obtained from D-isopropylidene glycerol[53] by elegant functional group manipulations (Scheme 8.7).

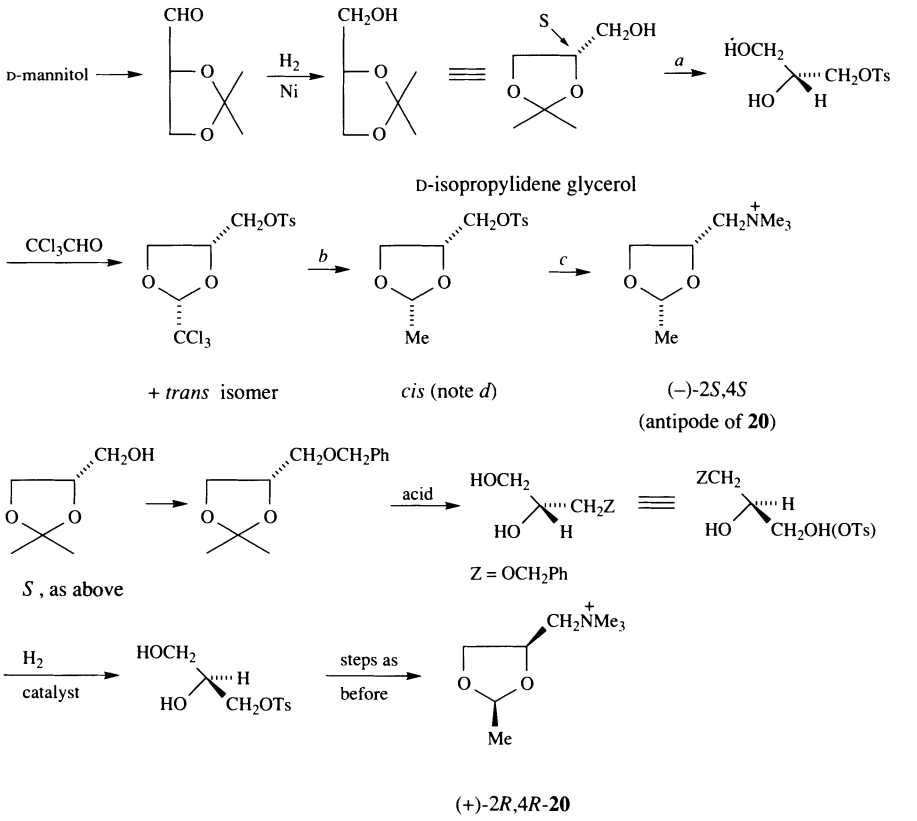


Chang and Triggle[54] confirmed the 100-fold potency difference between 4*R*-**20** and its 4*S*-antipode and found that 2,2-dialkyl analogues of the dioxolane **20** were much weaker agonists that the parent and that stereospecificity was much reduced (Table 8.5). In the 4-*R* series, progressive alkyl substitution caused a decrease in pD_2 values but did not affect the intrinisic activity. In the 4-*S* series, little change in the pD_2 value occurred after the initial major fall but a progressive

TABLE 8.5.
Muscarinic Activities of Some 1,3-Dioxolanes[54]



R	R′	Antipode (C-4 config.)	pD_2 (GPI)	Intrinsic activity	*R*/*S* ratio
Me	H	*R*	8.64	1	104
Me	H	*S*	6.02	1	
Me	Me	*R*	5.37	1	8.4
Me	Me	*S*	4.44	1	
Et	Et	*R*	4.92	1	3.2
Et	Et	*S*	4.42	0.73	
Pr^i	Pr^i	*R*	4.03	1	0.4
Pr^i	Pr^i	*S*	4.42	0.53	

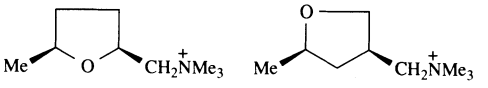


[a]1. TsCl, 2. mild acid.
[b]Separate *cis*-isomer, H_2-catalyst.
[c]1. NMe_2H, 2. MeI.
[d]Configuration confirmed by IR comparisons with authentic *rac*-material.

Scheme 8.7. Stereospecific synthesis of antipodes of the dioxolane **20** from D-mannitol.[48]

decrease in i.a. was found. These results have been discussed in terms of the polar and nonpolar requirements of the receptor.[8c]

The two desethers of the *cis*-dioxolane **26** were less active at GPI sites than the parent, but with little difference between the 1- and 3-regioisomers; simple removal of 2-Me abolished activity (EPMR 966, ACh = 1)[51,55].



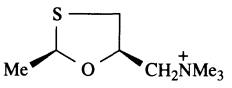
1-desether
EPMR (ACh) 17.5
GPI

26

3-desether
EPMR 22.8 (0,7 for **20**)
all *rac*-materials

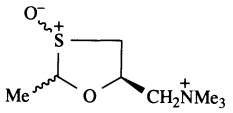
An Italian group have extended the work on F-2268 to corresponding 1,3-oxathiolanes.[56] The *cis*-derivative **27** proved potent at both muscarinic

and nicotinic sites[57] and several times more effective than its *trans*-analogue (Table 8.6). All four *rac*-diastereoisomers of the sulfoxide **28** were less active than the sulfide **27**, the potency fall being least in the case of the 2*c*, 3*t*, 5*r* (reference)



27

(one anitipode shown)



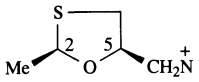
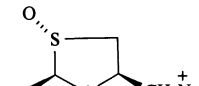
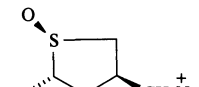
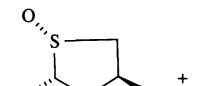
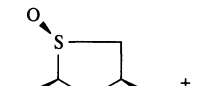
28

(sulfoxides)

27a is α-sulfoxide; see Table 8.7

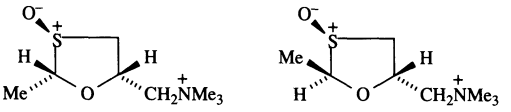
isomer. The *epiallo*-analogue was the more potent of the remaining three (cf. epiallomuscarine, Table 8.2)—evidence of the prime importance of a *trans*-configuration at chiral centers 3 and 5. The sulfone (SO_2) analogue of **27** and its *trans*-partner were generally of low potency (the *cis*-derivative was one-quarter as active as ACh at frog rectus sites).

TABLE 8.6.
Cholinergic Activities of Some 1,3-Oxathiolanes[56]

Compound (all *rac*)	Relative geometry	EPMR[a] GPI	Frog rectus
	*cis*[c] *trans*	0.43[b] 6.0	1.25[b] 27.5
	2*c*,3*t*,5ref	1.05	13.6
	2*t*,3*c*,5ref (allo)	370	330
	2*t*,3*t*,5ref (epiallo)	27	330
	2*c*,3*c*,5ref (epi)	90	9.7
	*cis*-sulfone *trans*-sulfone	165 400	4 114

[a] Equipotent molar ratio (≡ 50% level of effect of ACh).
[b] Revised values quoted by Gulatieri *et al.*[65]
[c] EPMR 0.3 for *rac*-dioxolane **20** relative to *rac*-muscarine.[57]

Chemistry. Treatment of methyl 2-hydroxy-3-mercaptopropionate **29** with acetaldehyde gave a separable mixture of 2-methyl-5-carbomethoxy-1,3-oxathiolanes **30** of configurations based on NMR evidence (deshielding influence of 5-CO_2Me on 2-Me and 2-H) (Scheme 8.8). Each isomer **30** was converted to the corresponding quaternary salt **27** by standard methods. The configuration of the more potent *cis*-derivative was later confirmed by X-ray crystallography.[58] Sulfoxides were obtained by treating bases corresponding to **27** (quats) with H_2O_2–acetic and the two diastereoisomers separated by chromatography.[59] Prolonged oxidation gave the 3,3-dioxides (sulfones). Assignment of sulfoxide geometry relative to that of C-2 and C-5 was based on chemical shifts induced by various reagents. Thus, since benzene is known to associate with the positive end of a solute dipole, its shielding effects should be greater on proton groups *trans* to oxygen than *cis*, because oxygen forms the negative end of the S-O dipole. The method worked best for 2-Me and 2-H protons. Data for the sulfoxide pair (**31**) derived from the *cis*-oxathiolane are shown.



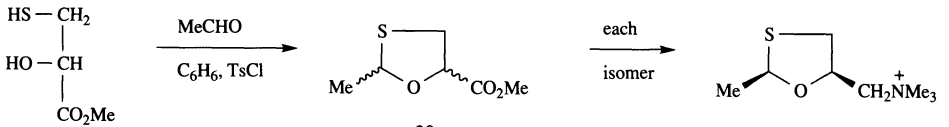
31

Δ-values[a]	
2-Me 0.37	2-Me 0.07
2-H 0.06	2-H 0.62

[a] $\Delta = \delta_{CDCl_3} - \delta_{C_6H_6}$ (EM 390 Varian Spectrometer).

Resolution of *rac*-**27** (*cis*, as base) was carried out by use of D-(+)- and L-(−)-di-toluoyltartaric acid (optical purity established by use of a chiral shift reagent, $Eu(tfc)_3$; 2-Me resolved in antipodal spectra).[59] Use of *S*-(+)- and *R*-(−)-2-hydroxy-3-mercaptopropionic acids in the sequence of Scheme 8.8 provided materials of known absolute configuration. These acids have been related to *S*- and *R*-lactic acids by desulfurization.[60]

Pharmacological data on resolved materials are shown in Table 8.7 (carbachol reference). The most potent muscarinic agent was (+)-**27** (2*R*,5*R*) which has the same absolute geometry as (+)-muscarine and the (+)-*cis*-dioxolane **20** (both



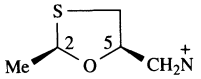
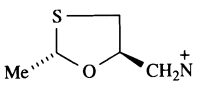
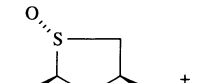
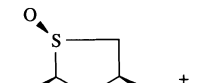
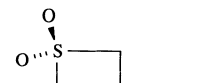
29

30
c/t mixture, separate
cis 2-Me 1.64 ppm 2-H 5.28 ppm
trans 1.57 ppm 5.48 ppm

27
from *cis*-**30**

Scheme 8.8. Synthesis of *cis*- and *trans*-1,3-oxathiolanes.[56]

TABLE 8.7.
Cholinergic Activities of Some Resolved 1,3-Oxathiolanes[49, 59]

Compound	Stereochemistry	EPMR (relative to carbachol) GPI	Frog rectus	Eudismic ratio
 *cis*-**27**	*rac*	0.08	2.8	170 (M)
	(−)-2*S*,5*S*	7.1	24	20 (N)
	(+)-2*R*,5*R*	0.04	1.2	
 *trans*	*rac*	1.3	4.2	1.5 (M)
	(−)-2*S*,5*R*	2.0	2.8	1.6 (N)
	(+)-2*R*,5*S*	3.0	4.5	
 **27a**	*rac*	1.3	45	133 (M)
	(−)-2*R*,3*R*,5*R*	2.0	33	3.5 (N)
	(+)-2*S*,3*S*,5*S*	159	120	
	*rac*	209	41	3.4 (M)
	(−)-2*S*, 3*R*, 5*S*	213	510	24 (N)
	(+)-2*R*,3*S*,5*R*	660	21	
	*rac*	218	15	1 (M)
	(−)-2*R*,5*R*	170	6.5	12 (N)
	(+)-2*S*,5*S*	168	76	
(+)-muscarine		0.4	> 124	

eutomers). It was remarkably potent (10 × muscarine) with a high eudismic ratio (170). However, its antipode (−)-2*S*,5*S*-**27** still acted as a full agonist and was only 7 times less potent than carbachol. The corresponding *trans*-antipodes were nearly as potent as the reference compound and displayed little enantioselectivity, cf. *trans*-antipodes of the related dioxolane (page 241). Thus although the affinities of the (−)-*cis*- and (+)/(−)-*trans*-1,3-oxathiolanes are lower than that of the most potent isomer, their efficacies must be significantly high. All isomers except the (−)-*cis*-derivative approached the potency of carbachol as nicotinic agonists and exhibited relatively low eudismic ratios—results reminiscent of the behavior of muscarone (such data on related dioxolanes have not been traced).

Sulfoxidation lowers both the muscarinic and nicotinic potencies of the oxathiolanes. It is significant, however, that the most potent antipode of the *cis*-series (Table 8.7) is related in configuration to (+)-muscarine, i.e., S-O of the sulfoxide appears to mimic the role of 3-OH of muscarine at the receptor. In the *cis*-3-SO/5-CH_2NMe_3 isomers potency was low (as in *epi*muscarine), the

TABLE 8.8.
Muscarinic Potency and Affinity of 1,3-Oxathiolane Eutomers in Various Tissues[61]

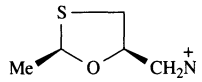
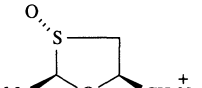
Tissue[b]	(+)-**27** (sulfide)[a]		(−)-**27a** (sulfoxide)[a]	
	$-\log ED_{50}$	$-\log K_D$	$-\log ED_{50}$	$-\log K_D$
GP ileum	8.06	6.87	6.95	5.38
GP atrial force	7.54	5.59	6.66	5.22
GP atrial rate	7.12	5.79	6.36	5.29
Rat urinary bladder	6.59	5.73	5.81	4.88

[a] See Table 8.7 for structures.
[b] All classified as M_2 receptors.

most active being the (+)-isomer at nicotine sites (1/21 × carbachol). The adverse influence of the presence of an S-O function *cis* to 5-CH_2NMe_3 was also apparent from the feeble action of corresponding sulfones (SO_2) at GPI sites—nicotinic sites were less sensitive as judged by the reasonable potency of the (−)-2*R*,5*R*-isomer (6.5 times less active than carbachol). Note also that the nicotinic potencies of the sulfoxide pair (-)-2*R*,3*R*,5*R* and (+)-2*R*,3*S*,5*R*, which differ only in S-O configuration, are close. Muscarinic enantioselectivity was low in the 1,4-*cis*-sulfoxide and absent in the sulfone, while modest values were recorded for nicotinic sites.

The potencies and affinities (negative logarithm of dissociation constant) of a series of antipodal pairs of oxathiolane derivatives have been compared in a series of tissues[61] all considered to be of the M_2 subtype. The eutomers (+)-**27** (sulfide) and (−)-**27a** (sulfoxide; see Table 8.7) showed the following order of potency: GP ileum > GP atria-force > GP atria-rate > rat urinary bladder, as did *rac*-muscarine. Numerical data are shown in Table 8.8. Affinity values ($-\log K_D$) are considered of greater significance in providing evidence of receptor heterogeneity, but only if differences of at least 0.5 are observed. On these grounds the M_2 receptors of GP ileum differ from those in GP atria and rat bladder. Enantioselectivities provided further evidence of such differentiation. When potencies are considered, the enan-

TABLE 8.9.
Enantioselectivity of Oxathiolanes in Various Tissues[61]

		Guinea-pig ileum	*Atria* force	Rate	Rat bladder
 (+)-eutomer	$\frac{ED_{50}(-)}{ED_{50}(+)}$	170	147	195	167
	$\frac{K_D(-)}{K_D(+)}$	179	25	16	144
 (−)-eutomer	$\frac{ED_{50}(+)}{ED_{50}(-)}$	132	19.9	14.4	25.2
	$\frac{K_D(+)}{K_D(-)}$	53	8.2	13.5	15.1

tioselectivities of the *cis*-sulfide **27** do not differ significantly from the affinity ratio (Table 8.9). However, when affinities are compared the values of GP ileum and rat bladder are markedly greater than those for GP heart (rate and force). In the case of the *cis*-sulfoxide **27a** both ratios of affinities and potencies point to a differentiation of GP ileum from the other tissues studied, i.e., provide evidence for subtypes of M_2 receptors.

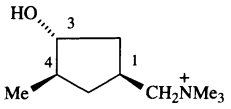
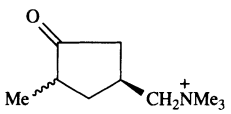
Antagonists in the 1,3-dioxolane and oxathiolane series are described in Chapter 9, page 297).

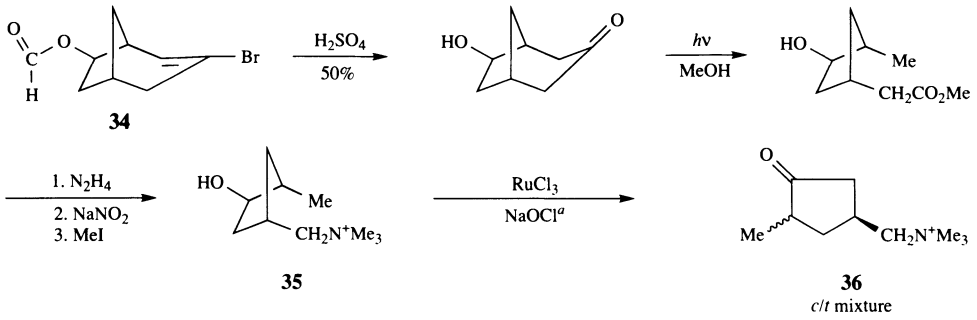
8.4.2. Cyclopentane Derivatives

Surprisingly, the carbocyclic analogue **32** (see Table 8.10) of muscarine that retains the relative configuration of the natural product is reasonably active at GPI sites and, although of low potency (1/75 × ACh), more active at nicotinic sites than the parent (Table 8.10). Potency levels were much lower in epi, allo, and epiallo congeners. The muscarone analogue **33** (see Table 8.10), available as a non-separable mixture of *c*/*t*-isomers, proved to be a potent muscarinic and nicotinic agonist (≡ACh).

Racemic deoxymuscarine **35** was obtained from the bromoformate **34** (Scheme 8.9) and oxidized to the deoxy muscarone/allo muscarone mixture **36** with

TABLE 8.10.
Cholinergic Activities of Some Cyclopentane Analogues of Muscarine and Muscarone

Compound (all *rac*)	EPMR (ACh = 1) GPI	EPMR (ACh = 1) Chick biventer or frog rectus	Reference
 **32**	5–10	75	63, 64
epi 3*c*,4*c*,1ref	500	395	65
allo 3*c*,4*t*,1ref	100	99	
epiallo 3*t*,4*t*,1ref	335	2040	
 *c*/*t* mixture **33**	1.0 0.4	0.8	63, 66
Muscarine	1.0	50	63
Muscarone	0.13	0.5	
allo-Muscarone	0.29	0.2	

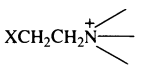


Scheme 8.9. Synthesis of carbocyclic analogues of muscarine and muscarone.[62,63]

RuO_4. Cyclohexanes related to some of the more potent cyclopentane derivatives had low orders of muscarinic potency.[67]

8.5. Conformational Studies

The neurotransmitter amines acetylcholine, dopamine, histamine, and serotonin are all achiral molecules of type **37** in which a charged amine function is



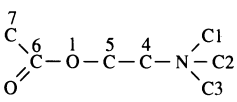
37

separated from a molecular feature X by a bimethylene chain. As a result of free rotation about the central C-C bond all of these neurotransmitters may associate with their receptors in a variety of conformations ranging from synplanar to antiplanar arrangements (see Chapter 2, page 18). The question of receptor conformation attracted attention at an early stage of the post-World War II investigations of cholinergic receptors stimulated by the need to account for the duality of action of ACh, and is even more relevant today with increasing evidence of the existence of subspecies of muscarinic and nicotinic sites.

As outlined and defined in Chapter 2 (page 18), the torsion or dihedral angle parameter (τ) most conveniently describes the three-dimensional shape of a molecule.

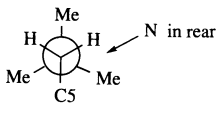
All cholinergic molecules studies contain the unit **38** (or a close variant) and the four values required to define its stereochemistry are given. Of these, τ_1 and τ_4

r_1 C5–C4–N–C3
r_2 O1–C5–C4–N
r_3 C6–O1–C5–C4
r_4 C7–C6–O1–C5

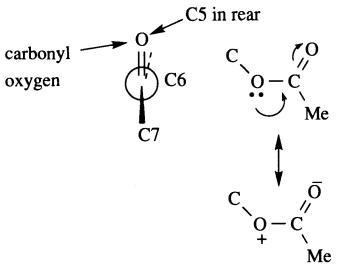


38

usually fall close to 180° on account of a preference for the fully staggered conformation (**39**) and for a near-planar ester unit (**40**).[68] Values of τ_2 and τ_3 are there-

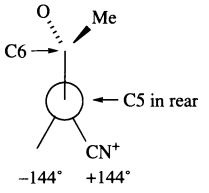


39

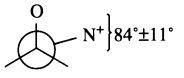


40 (after Casy)[11]

fore of most interest, particularly τ_2 because this defines the relative dispositions of the acyl group and quaternary head, functions both vital to pharmacological properties in ACh and its congeners.[69] Many torsion angle values have been recorded from X-ray analyses. Magnitudes of τ_2 and τ_3 observed in crystals of a variety of muscarinic agents are shown in Table 8.11. With the exception of ACh bromide and the 1,3-dioxolane, τ_3 values fall in the range $180° \pm 36°$; this means that in the majority of cases the acetyl (Me-C=O) portion of the molecule is set well away from the quaternary head (**41**) [+N–C6 distance in pm (1 Å = 100 pm): ACh Cl^- 440 ($\tau_3 - 167°$); ACh Br^- 412 ($\tau_3 + 79°$)].[70]



41 (after Casy)[11]



42

The torsion angle relating $^+NMe_3$ to OCOMe (τ_2) commonly has a value in the range 73 – 94° so that the N and O functions are more or less synclinal (**42**).

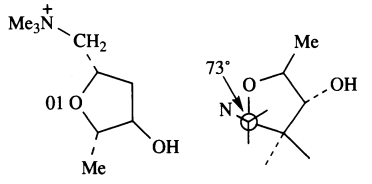
TABLE 8.11.
Certain Torsion Angles Observed in Crystals of Muscarinic Agents[70]

Compound	τ_2	τ_3
ACh bromide	+77	+79
ACh chloride	+85	−167
(+)-Muscarine iodide	+73	+144
(+)-*cis*-2-Methyl-4-trimethylammonium-methyl-1,3-dioxolane iodide	+94	+103
5-Methylfurmethide iodide	+83	+174
	+81	+176
(+)-β-Methyl ACh iodide	+87	−143
(+)-α-Methyl ACh iodide	+89	+167
	−150	−179
erythro-α,β-Dimethyl ACh iodide	+76	−155
Carbachol	+178	−174
(+)-*trans* 2-Acetoxycyclopropyl-1-trimethylammonium iodide	+137	+147

It turns out, in fact, that most compounds comprising the molecular feature O-C-C-N^+, where the charged group is quaternary nitrogen or a protonated base and the oxygen function is hydroxy or acyloxy, prefer the synclinal N/O arrangement in the solid state, e.g., L-α-glycerophosphorylcholine $CdCl_2.3H_2O$, choline chloride,[71] and lactoylcholine iodide.[81] An electrostatic interaction between the charged nitrogen group and the ether oxygen of the acyloxy function is probably an important stabilizing factor which leads to a preference for the synclinal rather than the antiplanar (sterically favored) conformation (see later).

Some molecules of this type do, however, display a preferred antiplanar–anticlinal conformation. These include the potent agonists carbamyl chloride[72] ($\tau_2+178°$, stabilized by several hydrogen bonds) and (+)–*trans*-2*S*-acetoxycyclopropyl-1*S*-trimethylammonium iodide[73] ($\tau_2+137°$, fixed by the rigidity of the three-membered ring), and the weakly active thio (τ_2 171° for bromide) and seleno (τ_2 175° for iodide) analogues of ACh in which ether oxygen is replaced by the bulkier and less electronegative sulfur or selenium atom.[74]

In five-membered cyclic analogues of ACh, synclinal N/O conformations are found for L-(+)-muscarine iodide (**43**) ($\tau_2+73°$),[42] (+)-*cis*-2*S*-methyl-4*R*-trimethylammoniummethyl-1,3-dioxolane iodide (**44**) ($\tau_2+94°$),[75] and 5-methylfurmethide (**45**) (τ_2+81 or $+83°$).[76] In the furan derivative an antiplanar N/O conformation is seriously destabilized by interactions between $^+NMe_3$ and 3-H of the heterocyclic ring. Surprisingly, τ_2 in crystals of L-(+)-muscarone iodide (**46**) is $+162°$[77]; the shapes of the tetrahydrofuran rings of muscarone and muscarine also differ and this may lead to a reduction in steric interactions between $^+NMe_3$ and the 3-methylene group in the antiplanar N/O arrangement.



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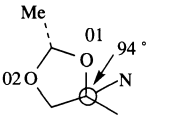

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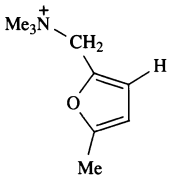

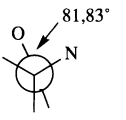

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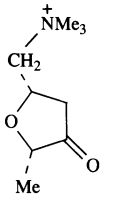
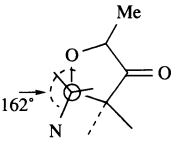




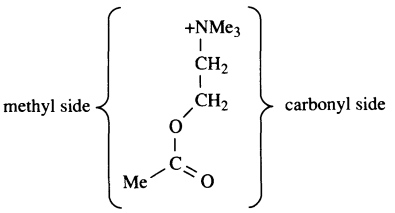
46

After Casy.[10]

In addition to the muscarinic agents already described, the nicotinic agonists 1,1-dimethyl-4-phenylpiperazine (DMPP),[78] nicotine,[79] α-methyl ACh,[80] and lactoyl choline[81] have also been examined by X-ray diffraction. Chotia and Pauling have compared the crystal structures of these molecules and noted several

common features.[78] From these they propose that the conformation of ACh relevant to the nicotinic receptor is one with τ_2 approximately 75° and τ_3 near 180°. The NCCN and $NCC_{Ar}C_{Ar}$ torsion angles of DMPP and nicotine respectively are taken to be the equivalent of O1-C5-C4-N (τ_2) of the choline molecules. Arguments of this kind are open to challenge, however, since there is good evidence that many nicotinic agents including nicotine itself and DMPP act indirectly through release of endogenous ACh (most muscarinic agents are believed to act directly at the receptor).

From the evidence of solid state conformation, Chotia[82] considers that the conformations of ACh at muscarinic and nicotinic sites are similar and explains the differing actions of the neurotransmitter in terms of receptor interaction with either the methyl side (muscarinic effects) or carbonyl side (nicotinic effects) of the molecule (**47**). He argues that the carbonyl group is either blocked or absent in potent muscarinic agents such as ACTM, β-methylACh, the dioxolane (**44**), and muscarine while the reverse obtains in nicotinic agents. Similar arguments have been advanced by Pullman.[107] The crystal structure of oxotremorine has also been reported.[83]



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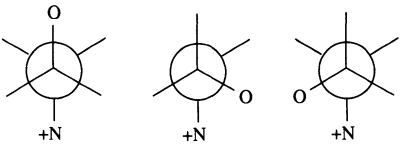
8.6. NMR (Chiefly ^1H) Studies of Conformation

In the cholinergic field, most of the NMR evidence complements the results of the X-ray diffraction studies. This agreement may be a result of the molecules being stabilized by strong intramolecular interactions that are not seriously disturbed by solvents. Analysis of spectra run in the presence of the receptor from *Torpedo californica*, however, supports a preferred anticlinal–antiperiplanar conformation for the ligand when bound to nAChR, as described in detail in Chapter 10.[84]

NMR evidence of the conformation of compounds containing the unit **37** (see page 249) is restricted to the torsion angle O1-C5-C4-N (τ_2). In molecules like ACh the three possible staggered conformations relevant to τ_2 are **48–50**). The results of NMR analyses establish the relative populations of the three forms and identify favored conformations if any exist. All rotamers are freely interconverting because

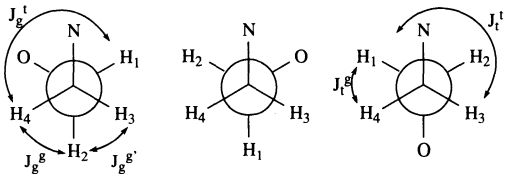


48 **49** **50**

of the low bariers to rotation between the members and NMR provides a picture of the time-averaged conformation at room temperature (or at higher or lower temperatures, if desired). Conclusions reached are less precise than those of X-ray crystallography. Thus preferred conformations may usually be defined no closer than synclinal or antiplanar, but on the other hand the NMR spectroscopist must take into account a mobile system rather than a fixed one as presented by a crystal lattice.

Some detail of specific cases will now be given. All analyses are based on the fact that the extent of spin–spin coupling between two protons vicinally disposed as in H-C-C-H (^3J) depends on their stereochemical orientation as defined by the appropriate torsion angle, i.e., the $^3J/\cos^2\phi$ relationship of Karplus (Chapter 2, page 23). Estimates of ^3J values associated with torsion angles of 60° and 180° may be made by the aid of data on model compounds of fixed geometry and by taking into account the effects of the electronegativity of substituents attached to the H-C-C-H fragment.[85] Comparison of experimental and predicted coupling constant values then leads to the conformational conclusion.

The 4-proton bimethylene system of ACh and its analogues is described as AA′XX′ or AA′BB′ depending on whether the chemical shift difference between the methylene pairs is large or small.[86,87] Rapid interconversion of conformers by rotation about the C-C bond renders each geminal pair of protons chemically equivalent but does not lead to a single averaged vicinal coupling constant except when the conformer populations are equal. Hence two distinct ^3J values arise because the extents of coupling between A and X (or B) and A′ and X (or B) differ, and the values obtained are the population weighted average of contributions from the three staggered conformations **51–53** (the only ones likely to be significant).



(a) (a) (b)

51 **52** **53**

Fractional populations: $2a + b = 1$

$J = J_{1,4} = aJ_g^t + aJ_g^{g'} + bJ_t^g$

$J' = J_{1,3} = 2aJ_g^g + bJ_t^t$

J_g^t denotes a *trans* coupling in an N/O synclinal conformation while J_t^g denotes a gauche coupling in an N/O antiplanar conformation etc. (after Casy)[11]

Since the system is not first order, $J_{1,3}$ and $J_{1,4}$ may not be derived directly from the spectrum (Fig. 8.1). The line pattern does, however, provide estimates of the sum (N) and difference (L) of the two ^3J values and these may be refined by means of an iterative calculation and computer program[88] giving: N 9.49 Hz and L 4.43 Hz.[89] Evidence about the sign of L (unknown from the spectral analysis) is derived from Abraham and Pachlers' relationship where $\sum E$ is the sum of the electronegativities of the substituents attached to the C-C fragment concerned.[90] Substitution of positive and negative values of L into this equation gives $\sum E$ equal to 15.5 and 17.5, respectively. The value calculated using Huggins electronegativity

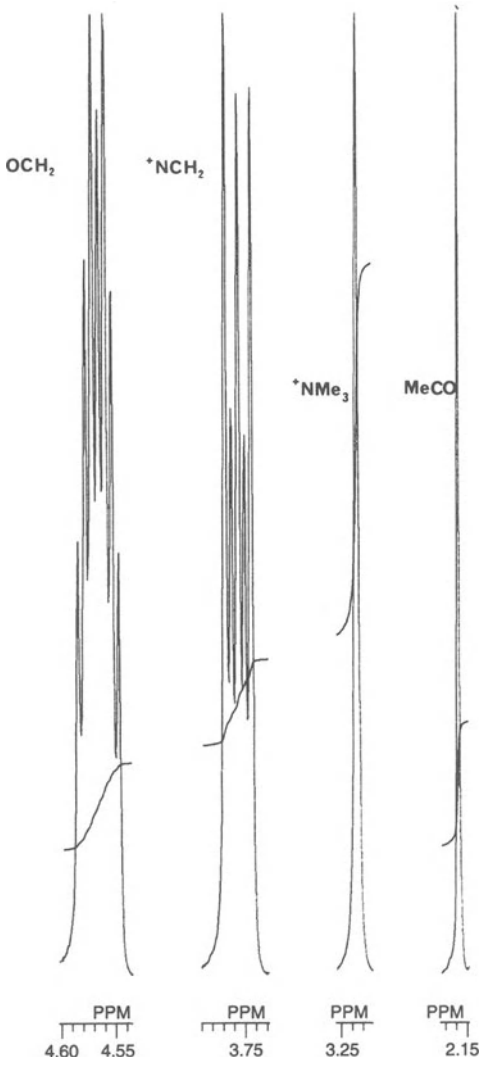


FIGURE 8.1. Part of the 400-MHz ^{1}H-NMR spectrum of acetylcholine chloride in D_2O at 20° C. The OCH_2 methylene multiplet (lower field near 4.57 ppm) is more complex than that of the $^+NCH_2$ signal (near 3.76 ppm) as a result of coupling between ^{14}N and the β-protons. The spectrum remains second order in spite of being run at 400 MHz; the original analysis was based on 60-MHz data.[89]

values[91] is 15.3, hence L is probably positive. Since $N+L=2J$ it follows that $J=7.0$ and $J'=2.5$ Hz. These results are now compared with values anticipated if (i) antiplanar and (ii) synclinal conformers be favored (and assuming $J_g{}^t=J_t{}^t$ and $J_g{}^g \cong J_g{}^{g'} \cong J_t{}^g$).

$$\tfrac{1}{2}N+\tfrac{1}{6}L=17.97-0.80\sum E$$

(i) Antiplanar conformation favored (see **53**)

$$J_{1,4}=J^g \qquad \text{and} \qquad J_{1,3}=J^t$$

In this case the smaller coupling (2.5 Hz) could correspond to J^g, but the larger (7.0 Hz) is abnormally low for J^t even allowing for substituent effects.

(ii) Synclinal conformations favored (see **51** and **52**)

$$J_{1,3}=\tfrac{1}{2}(J^g+J^g)=J^g \qquad \text{and} \qquad J_{1,4}=\tfrac{1}{2}(J^t+J^g)$$

Here the 2.5 Hz coupling is consistent with the J^g value while $J=7.0$ Hz approaches the expected value of $\frac{1}{2}(J^t+J^g)$, e.g., about $(10+3)/2=6.5$ Hz. On this basis J^t has a value (11.5 Hz) within the normal range.[89]

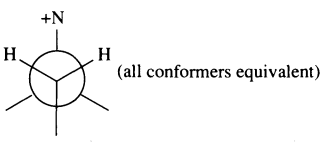
Partington *et al.*[92] used a similar approach to that of Culvenor and Ham—a table of ^3J values was compiled by combining electronegativity relationships with Spragg's analysis of the low-temperature spectrum of morpholine.[93]

Cushley and Mautner[94] carried out similar analysis of the spectra of acetylthiocholine and acetylselenocholine in D_2O. The ^3J values obtained (11.59 and 5.00 Hz for the thio and 12.62 and 4.83 Hz for the seleno analogue) were of magnitudes typical of J^t and J^g coupling, respectively, hence these analogues must exist almost exclusively in the antiplanar conformation in solution. The spectrum of acetylthiocholine has also been analyzed by other groups.[95,92]

Rotamer populations of ACh and its relatives may also be calculated by use of the magnitude of coupling between ^{14}N and a β-proton (N-C-C-H).[92,96] An advantage of the method is that these couplings may be measured directly from the spectrum, e.g., from the OCH_2 multiplet of choline bromide[92]; the higher-field NCH_2 band is less complex because N-C-H coupling is near zero. If only two constants J^g_{NH} and J^t_{NH} are assumed, J_{NH} for the ethyltrimethylammonium ion **54** (2.1 Hz) is made up as follows:

$$J_{NH}=\tfrac{1}{3}(J^t+2J^g)=2.1 \text{ Hz}$$

J_{NH} is close to 0.7 Hz, because values in this range are seen in spectra of choline derivatives shown to have strongly preferred N/X rotamers (e.g., thioacetylcholine **55**), whence $J^t_{NH}=4.9$ Hz (if the NH coupling constants have the same sign). If synclinal conformations are favored for ACh, J_{NH} will be made up of the average of J^g_{NH} AND J^t_{NH}; and the observed value of 2.5 Hz is close to that anticipated: $(0.7+4.9)/2=2.7$ Hz.



(all conformers equivalent)

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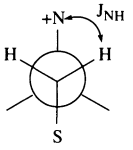

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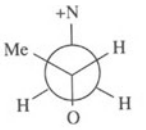
8.6.1. Methylacetylcholines

Analysis of the 8-line multiplet due to the CH_2 protons of β-methyl ACh (**56**) by Bible's method[97] gave values of 9.7 and 1.5 Hz for the two ^3J couplings that operate in the CH_2CH fragment of the molecule.[98] The orders of magnitude of

$$Me_3\overset{+\alpha}{N}CH_2\overset{\beta}{C}HMeOCOMe$$

56

these values are typical of *trans* and *gauche* vicinal coupling, respectively, hence either **57** or **58** is the preferred conformation. Comparison of the methylene ^3J values and the β-methyl chemical shift with those of rigid models[98] and use of



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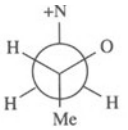

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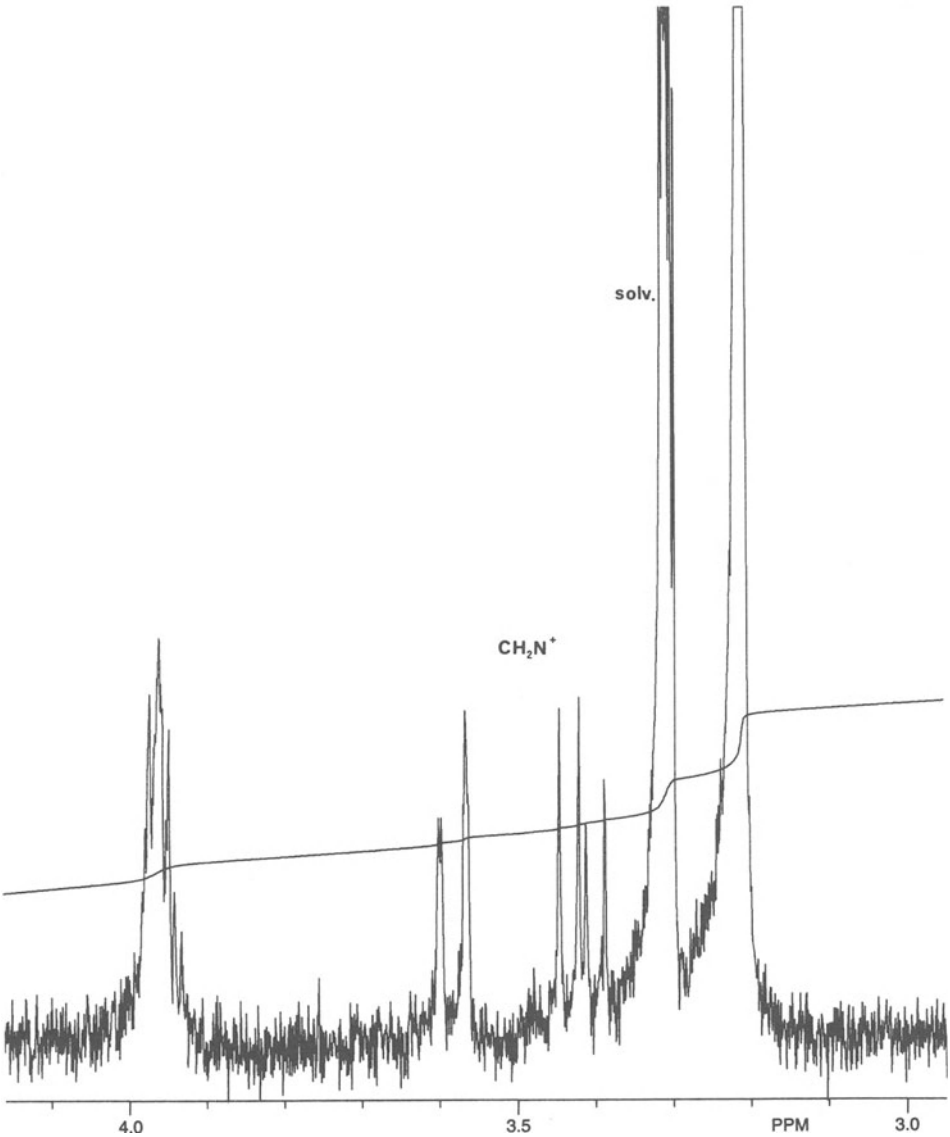


FIGURE 8.2. Part of the 400-MHz ^{1}H-NMR spectrum of *rac*-muscarine chloride (1 mg in CD_3OD, 130 scans). The methylene proton signals of the $CH_2{}^+NMe_3$ side chain appear near 3.58 and 3.42 ppm, respectively.

other arguments[92,96] leads to the selection of **58**. First-order analysis of the methine resonance of α-methyl ACh (CH_2CH) yields ^3J values of similar magnitude (4.6 and 4.2 Hz); this fact shows that conformational preferences among α-methyl ACh conformers are low.[98] The 2-Hz coupling between the methylene protons and ^{14}N supports this conclusion.[92,96]

As must already be clear from the X-ray diffraction and NMR studies outlined so far, the solid state conformation of a cholinergic agonist is very similar to its preferred conformation as a solute in D_2O. This holds true for β-methylACh ($\tau_2 + 85°$, β-Me and ^+N antiplanar, τ-152°)[80] while the crystalline α-methyl isomer exists in two forms, a synclinal and a near-antiplanar N/O conformer (τ_2 +90° and −148°).[99] Carbachol ($Me_3N^+.CH_2.CH_2.O.CO.NH_2$, synclinal as solute in D_2O)[92,100] is exceptional in being antiplanar in the solid state[72] but its unusual crystal form is considered to be stabilized by intermolecular hydrogen bonds that are unique to the carbamate.

The prevalence of synclinal N/O conformers among ACh and its congeners is unexpected on steric grounds, but may be attributed to an electrostatic interaction between the positive charge of the onium group and the partial negative charge on the ether oxygen—an intramolecular interaction which operates most effectively when the two functions concerned are synclinal. IR evidence for such an interaction is provided by the fact that the carbonyl stretching mode of ACh is at a higher wave number than that of the corresponding tertiary amine and of ethyl acetate.[98,101,102] In thiocholines no significant electrostatic interaction can develop, since sulfur is of low electronegativity, and the preferred conformation (antiplanar) is governed by van der Waals repulsions.

Studies of conformation and electron distribution in nicotine and ACh by ^{13}C NMR have also been reported.[103]

The failure to observe NOE enhancement of the 2-Me signal of muscarine in D_2O on irradiation of $^+NMe_3$ has been advanced as evidence that solid and solute conformations of muscarine are similar, i.e., with the quaternary head projecting away from the ring (see X-ray structure).[104] ^1H-NMR spectral data confirm this view (Fig. 8.2). The methylene protons of the $CH_2{}^+NMe_3$ side chain of muscarine gave rise to an 8-line resonance with vicinal (^3J) couplings of about 10 Hz and <2.5 Hz, respectively, i.e., magnitudes consistent with conformation **43** for which ^3J values typical of vicinal proton pairs of dihedral angles (1) 180° and (2) near 60° are to be anticipated.

8.7. Molecular Orbital (MO) Calculations of Conformation

Use of quantum chemical methods in the cholinergic fields has recently been reviewed.[105] Kier has been particularly active in applying MO calculations to pharmacologically active molecules[106] including cholinergics, and he uses the Huckel molecular orbital (HMO) method. His calculations for ACh lead to energy minima for the torsion angles τ_2 (80°), τ_3 (180°), and τ_4 (plateau between 120 and 240°), hence his model for the preferred conformation corresponds closely to the solid state molecule. Other groups, using different methods of calculation, arrived at a similar conclusion.[107,108] The energy barrier to rotation over the τ_2 range 80° (synclinal O/N) to 180° (antiplanar) is low; the antiplanar conformer is

approximately 3 kcal less stable than the synclinal arrangement[107] so conformer interconversions will be rapid down to about 50 K.[109] The NCCO torsion angles for muscarine and muscarone were 60° and 120° respectively in lowest-energy conformations,[110] values which correlate reasonably well with X-ray diffraction data. Calculations have also been made on β-methyl ACh,[111] oxotremorine,[112] and nicotine[107,113] although it is recognized that neither of the last two agents is likely to have direct cholinergic actions (see pages 314 and 327, and Ref. 114). Pullman *et al.*[107] calculated the net total ($\sigma+\pi$) electronic charges in ACh and muscarine and found that (i) the $^{+}$N atom of both compounds is in fact nearly neutral (0.06 eu), cf. data on $^{+}NH_3$ of protonated histamine,[115] (ii) most ($\approx 70\%$) of formal positive charge is distributed among the three N-methyl groups—more to be hydrogen than the carbon atoms, and (iii) the carbonyl and ester oxygens of ACh bear similar net total charges while the intermediate carbon carries an appreciable excess of positive charge, viz:

$$\underset{\text{O}}{-0.25} \quad = \quad \underset{\text{C}}{+0.32} \quad - \quad \underset{\text{O}}{-0.24} \text{ eu}$$

These studies have been extended to other ACh derivatives.[116,117]

8.8. Conformationally Restrained Analogues of Muscarinic Agonists

Although X-ray diffraction, NMR, and molecular orbital studies concur in identifying the preferred conformations of ACh and many of its active analogues as those with synclinal nitrogen and oxygen functions, there is no guarantee that such forms represent the conformation adopted by the agonist at the cholinergic receptor, i.e., the "active" conformation. Barriers to rotation in molecules such as ACh are low and easily overcome by energy derived from the thermal motion of the molecules or (in cases where barriers are higher) perhaps by that released on formation of the agonist–receptor complex itself.[118] Data on conformationally restrained analogues of ACh, in which the dispositions of onium and acetate functions are more or less "frozen" in relation to those in the flexible parent molecule, therefore provide clearer information about the active conformation. In most molecules of this class barriers to conformational change are much higher than those of the acyclic forms, hence the geometry of the molecule established in vitro is unlikely to be altered significantly on drug–receptor interaction. The drawback to this approach is that the analogue must inevitably be larger than ACh itself and the skeleton used to restrict movement of the N and O functions will almost certainly impede binding of the pharmacodynamic groups to the receptor surface. Even a single methyl group may profoundly affect the activity of ACh, e.g., α-*S*, α-*R*, and β-*R*-methyls cause 232-, 28-, and 240-fold drops in the muscarinic potency of ACh, respectively, although methyl is not detrimental in this sense when present in the β-*S*-orientation[17] (see page 232). Except for analogues based on cyclopropane, all rigid or semirigid derivatives of this type examined so far contain a far greater array of atoms than one additional carbon and potencies observed are generally of a much lower order than that of ACh. (See also a critique of the use of restrained analogues by Mutschler and Lambrecht.)[119]

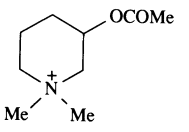
If the derivatives yield dose response curves parallel to those of ACh and are able to reproduce the maximal response to this agonist when applied in high enough dose levels, the additional molecular features may be assumed to influence the affinity of the molecule for the receptor rather than its intrinsic activity. A modification of the intrinsic activity is also revealed if the derivative is shown to have partial agonist or antagonist properties, as is well illustrated by the results of altering the acyl feature of ACh.[120]

Within a series of analogues of similar molecular dimensions and skeleton, however—and especially if a diastereoisomeric group is under study—activity variations may reasonably be assumed to have conformational significance even if the potencies recorded are of a low order. It must be emphasized that any conclusions drawn from the results about the active conformation of ACh rest on the assumption that the analogues in question act directly at the muscarinic site. It is unfortunate that comprehensive evidence on this point is rarely presented.

The review of conformationally restrained analogues which follows is subdivided into derivatives of ACh and muscarine. In each division isomer type and the relevant pharmacology are discussed, including a brief summary of some of the synthetic and stereochemical methodology.

8.8.1. Analogues of ACh

Schueler[121] first drew attention to the possibility of the muscarinic and nicotinic effects of ACh being mediated by different conformational isomers of the flexible molecule. He examined the 3-piperidyl acetate **59** and the morpholine derivative **60** as models of antiplanar and synclinal N/O conformations of ACh,



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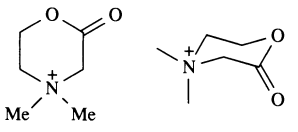


60

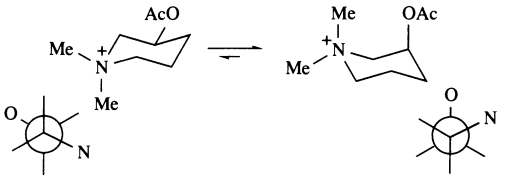
respectively. Both were feebly active in muscarinic and nicotine assays, with **59** the more potent. The validity of this comparison is doubtful, however, because of the disparity of skeletal arrangements of atoms in the two molecules. Later **59** was found to have 1/660 and its 4-piperidyl analogue 1/300 the muscarinic potency of ACh (effects on rat blood pressure, blocked by hyoscine HBr),[122] while ileum experiments also confirm the muscarinic properties of **59**.[123,124] Antipodal forms of **59** and the corresponding *t*-amine hydrochloride have been examined by Lambrecht (Table 8.12).[125] All forms were far less potent than ACh at muscarinic sites but had intrinsic activities close to one. *S*-Antipodes were a fewfold more effective than *R*-isomers. Absolute stereochemistries were based on the configuration of *R*-(+)-3-hydroxypiperidine, obtained by stereospecific synthesis from mannitol.[126] ^{1}H-NMR evidence as outlined below (dimensions of 3-H resonance, 19 Hz in D_2O) showed the axial 3-OCOMe chair **62** to be favored over the equatorial chair **61**—the ratio 67 (axial):33 (equatorial) was calculated for the methiodide. In the spectrum of the hydrochloride salt, signals due to each protonated epimer are resolved.[127]

TABLE 8.12.
Muscarinic Activities of Racemic and Antipodal Forms of 3-Acetoxy-1-methylpiperidine HCl and Methiodide[125]

Compound	pD_2(GPI)[a]	i.a.	*R*/*S* ratio
rac-HCl	4.32	1.06	12.5 (5)[b]
R	3.58 (2.99)[b]	0.84	
S	4.67 (3.68)	1.07	
rac-MeI	5.00	1.02	13.4 (3.3)[b]
R	4.19 (3.40)	0.96	
S	5.32 (3.92)	1.02	
ACh	8.00	1.00	

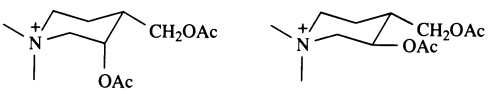
[a] Effects blocked by atropine sulfate (1×10^{-7} M) but not by hexamethonium (3×10^{-4} M).
[b] GP atrium.[119]

The results thus favor either antiplanar (**61**) or synclinal (**62**) active conformations with possible preference for the latter on population grounds (cf. results for corresponding thia analogues discussed later).



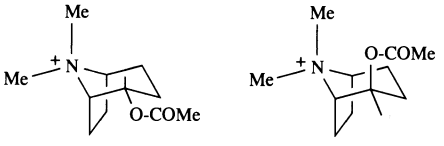
cis-**61** *trans*-**62**

The activity of the *cis*-diacetate (**63**) (1/100 ACh) and inactivity of the *trans*-isomer (**64**) in muscarinic tests has been cited as evidence that synclinal N/O conformers are the receptor-bound species of ACh agonists.[124] The fact that the isomeric diacetates are far more likely to differ in their 4- rather than 3-substituents orientations invalidates this argument.[122]



cis-**63** *trans*-**64**

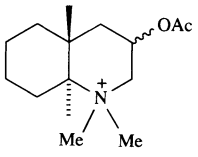
The comparative pharmacology of **65** and **66** may reasonably be studied because these tropyl acetates differ only in their N/O geometry. It was reported[128]



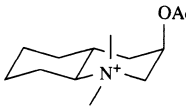
65 **66**

that the antiplanar form **65** (DL- or L-isomer) is 50–100 times less active than ACh while L-**66** (synclinal) has no spasmogenic properties when tested at the same concentration levels (rat sigmoid colon preparation). The synclinal analogue **66** was, however, more potent in nicotinic tests performed on the atropinized cat (rise in blood pressure: 0.5–1.0 mg/kg **66** ≡ 1.0–2.0 mg/kg **65**; at same dose levels **66** caused contraction of the nictitating membrane while **65** was ineffective; 0.25 mg/kg ACh produced positive responses in both tests).

The decahydroquinolines **67** reported by Smissman and Chappell[129] are ACh analogues similar in type to the tropanes **65** and **66**, all being related to 3-piperidinol with the onium nitrogen forming part of the ring system. Only the synclinal form **68** had agonist properties (guinea pig ileum, molar potency = 0.02 with ACh = 1) but its affinity for the receptor appeared to be less than that of the antiplanar isomer **69** on the basis of study of the blockade of ACh-induced contractions (**69** caused 60% inhibition at 50 μg/ml while at least 100 μg/ml were required



67



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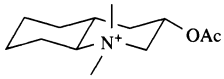
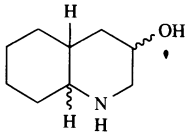


69

for blockade by **68**). Neither isomer was active in tests for nicotinic activity. True AChE (from eel) hydrolyzed the equatorial isomer about one-fifth as fast as it did ACh, while the axial derivative inhibited the enzyme.[129,130]

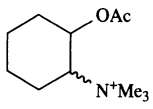
The decahydroquinolines **67** were derived from reduction products of 3-hydroxyquinoline.[129] Of the three isomers **70** isolated, one was assigned a *cis*-ring



70

juncture since its ^{1}H-NMR spectrum displayed a one-proton signal at δ 2.82 (m, $W_{1/2}$ 9 Hz) typical of the C-10 methine resonance of *cis*-decahydroquinoline. The 10-H signal was obscured in the spectrum of *trans*-decahydroquinoline and in those of the remaining isomers **67** which must therefore be *trans*-derivatives. The stereochemistry at C-3 followed from the dimensions of the C-3 proton signals (δ 3.70 $W_{1/2}$ 21 Hz for equatorial OH and δ 3.72 $W_{1/2}$ 7 Hz for axial OH isomer); see below.

Cyclic analogues of ACh containing the full trimethylammonium cationic head were first described in studies of *cis*- and *trans*- 2-trimethylammoniumcyclohexyl acetate (**71**) and the corresponding cyclopentyl derivatives (**72**) as substrates for



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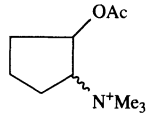
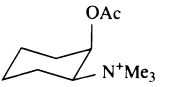


72

AChE.[131] The muscarinic properties of the cyclohexyl pair have been reported, however[132]; *R,S-trans*-**71** was about 400–1000 times less potent than ACh while the *cis*-racemic mixture was inactive at all concentrations used (Table 8.13). The 1*R*,2*R*(−)-*trans*-enantiomer was 4 times as active as the racemic mixture while the (+)-*trans*-isomer was much weaker (8×10^{-4} mol/l produced a contraction which was less than 50% of the ACh maximum). The superiority of trans-**71** to the *cis*-racemic mixture has been confirmed.[123,133]

A potency difference between *cis*- and *trans*-**71** is unexpected in terms of relative dispositions of nitrogen and oxygen functions in the isomeric pair. Their preferred conformations are **73** *(cis) and* **74**a *(trans)*[134,137] and O-C-C-N torsion



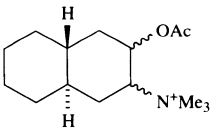
73

(a) R = H; (b) R = *t*-Bu

74

angles must be close to 60°, i.e., the N and O substituents are synclinal in both isomers. However, inversion of the *trans*- but not the *cis*-derivative produces a conformation which is antiplanar with respect to $^{+}NMe_3$ and OCOMe; hence, the active form of *trans*-**71** may be the unfavored N^aO^a conformer (see later). The *cis*-form was inactive as a substrate for bovine AChE while the *trans*-isomer was hydrolyzed at a very slow rate.[132]

Fusion of 2-trimethylammoniumcyclohexyl acetate to a second cyclohexyl ring prevents ring inversion provided the ring junction is *trans*. The decalins **75** are ACh



75

TABLE 8.13.
Muscarinic Potency of Racemic and 1*R*,2*R*-*trans*-2-Trimethylammoniumcyclohexyl Acetate[132]

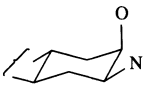
Compound	Effective concentration (mol/l) inducing concentration of GPI[a]
ACh	1.3×10^{-8} to 1.6×10^{-6}
rac-trans-**71**	6.1×10^{-6} to 1.6×10^{-3}
1*R*,2*R*-*trans*-**71**[b]	1.5×10^{-6} to 4.0×10^{-4}

[a] Maximum concentration produced by ACh at 5.1×10^{-5} mol/l. Atropine (1×10^{-9} mol/l) inhibited all active compounds to a similar degree. Hexamethonium (9.9×10^{-5} mol/l) had no effect on the contraction produced by *rac-trans*-**71**.

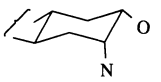
[b] Configuration by synthesis from 1*S*,2*R*-2-hydroxycyclohexane carboxylic acid.

analogues of this type and the four *R,S*-isomers provide three synclinal (**76–78**) and one antiplanar N/O (**79**) disposition. Low orders of muscarinic activity were shown by the N^aO^a and N^aO^e isomers, the former being distinctly the more potent (Table 8.14).[135]

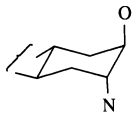
76 N^eO^e



77 N^eO^a

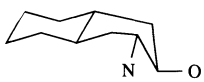


78 N^aO^e

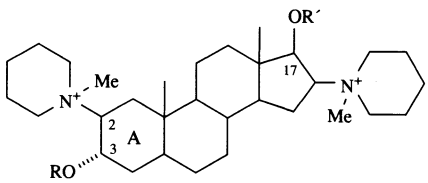


79 N^aO^a

It may be argued that skew-boat conformations such as **80** are likely to be favored over the chair **79** in order that nonbonded interactions of the OCOMe and $^+NMe_3$ groups be reduced (this is the case, in fact, for ring A of the steroid **81** in both the solid[136] and solute [137] condition), but NMR and X-ray diffraction evidence show that the antiplanar conformation **79** is maintained (see below).



80



R = COMe, R′= H or COMe
2- and 3-substituents axial in chair conformation
81

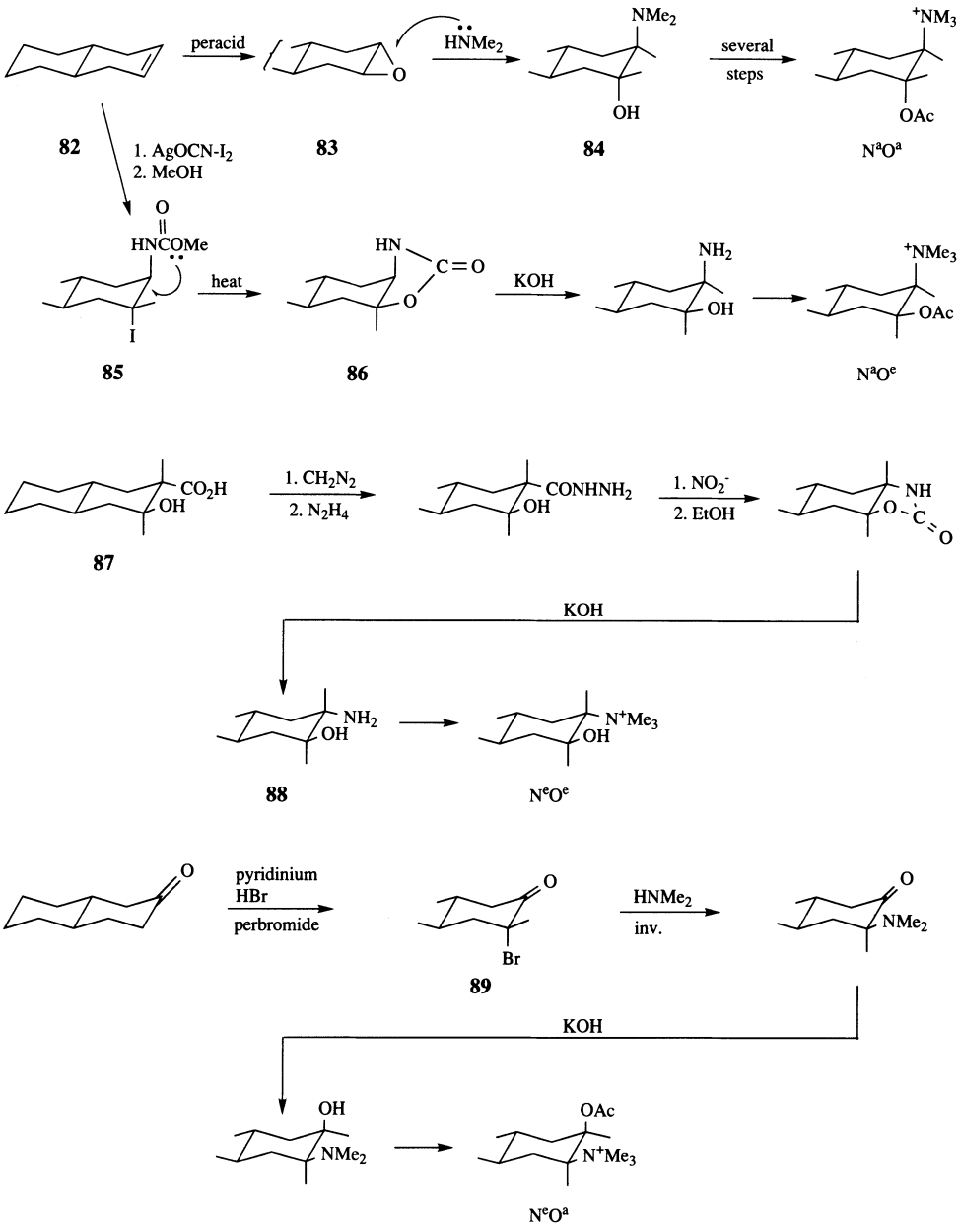
A variety of methods, utilizing reactions of established stereochemistry, were used to prepare the four decalin analogues **76–79**.[135] Treatment of *trans*-Δ^2-octalin **82** with a peracid gave the 2,3-oxide **83** which was opened in a *trans*-manner to form 3-(axial)-dimethylamino-2-(axial)-*trans*-decalol (**84**), the precursor of the N^aO^a ACh analogue (Scheme 8.10). *Trans*-addition of silver cyanate-iodine to the

TABLE 8.14.
Pharmacological Data on Some Decalin Analogues of ACh[a] (135)

Compound	Equipotent conc. (μg/ml)[b]	Relative molar potency (ACh = 100)[c]
N^aO^a (**79**)	50	0.052
N^aO^e (**78**)	500–1000	0.0026–0.0052
N^eO^e (**76**)	inactive at 1500	—
N^eO^a (**77**)	inactive at 1800	—
erythro-**6**	1.7	1.2
threo-**5**	67	0.029
ACh Cl^-	0.0125	100

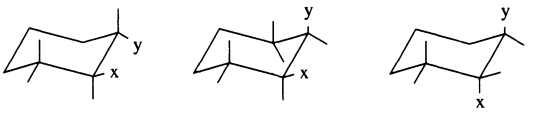
[a] Relative rates of hydrolysis by true AChE (ACh = 100):N^aO^a (**79**) 14.6; all other isomers negligible with the N^eO^a form (**77**) an inhibitor; *erythro*-**6** negligible, *threo*-**5** 9.[135]
[b] GPI test, all iodides except ACh.
[c] Corrected values (Smissman, private communication).

alkene **82** gave the urethane **85**, which on heating formed the cyclic analogue **86** with inversion of the C-2 center. Subsequent reaction of **86** led to the N^aO^e isomer (Scheme 8.10). A Curtius rearrangement of the hydrazide derived from 3-(equational)-carboxy-*trans*-2-(equatorial)-decalol (**87**) led to the aminodecalol precursor **88** of the N^eO^e isomer. Finally, the N^eO^a isomer was obtained by a route involving the bromination of *trans*-2-decalone which gave the 3(axial)-bromo derivative **89**



Scheme 8.10. Synthesis of four isomeric decalin analogues of ACh. [135]

exclusively and the displacement of bromine with inversion by dimethylamine (Scheme 8.10). Information about the conformation of decalyl and cyclohexyl ACh analogues was obtained from the dimensions of the N-C-H and O-C-H methine ^{1}H-NMR signals.[135,137] The four combinations of vicinal couplings possible for the methine protons of 1,2-disubstituted cyclohexanols are shown below together with their ranking in terms of outer line separations; this order is based on typical ^3J values for six-membered alicycles.[138] Model values were obtained from the spectra of six-membered derivatives which had clearly defined preferred conformations, such as **89a** and **89b** (in the first case the N-substituent is markedly larger



2aa + 1ae > 1aa + 2ae (X) > 2ea + 1ee (Y) ≡ 2ee + 1ea
(a = axial, e = equatorial)

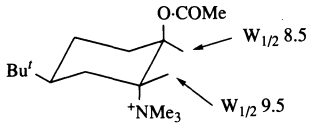
1–H $W_{1/2}$ 7 Hz
2–H $W_{1/2}$ 18 Hz

89a

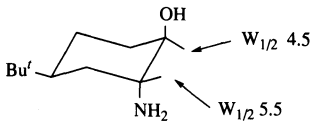
1–H bw* 24 Hz (in DMSO-d_6)
2–H bw* 24,5 Hz

89b (after Casy)[11]

than OH and so takes the equatorial orientation). Dependent on the particular combination of coupling involved, either the terminal line separation (bw*) or the width at half the maximum height ($W_{1/2}$) of the signal provided the best guide to the conformation.[137] Combinations containing an aa component lead to the larger methine signal dimensions. In the *t*-butylcyclohexyl derivatives (**90**), etc., the ^{1}H-NMR evidence showed that deviations from preferred chair conformations only arose in quaternary salts which required an axial trimethylammonium group. In the N^aO^a analogue (**90**) of special interest, the 1- and 2-methine signal dimensions, although clearly of equatorial rather than axial type, were larger than those seen in the spectra of analogues with smaller axial substituents, such as **91**, and this



90



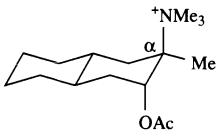
91 (after Casy)[11]

evidence together with chemical shift data was taken to indicate that a flattened ring was preferred with the onium group bent away from the alicyclic ring. The most active decalin methiodide (**79**) with similar methine resonance dimensions as **90** does in fact have a solid state conformation in which the $^+NMe_3$ group is bent away from the ring to give a N-C-C-O torsion angle of 148°.[139]

Pharmacological data on diastereoisomers of α,β-dimethyl ACh

(Me_3N^+.CHMe.CHMe.O.COMe I^-) (page 235) were originally considered to correlate with those for the decalin isomers (Table 8.14) in that the more active *erythro*-isomer was expected to have a preferred N/O antiplanar conformation on steric grounds while the corresponding *threo*-conformer would be unfavored.[135] Subsequent X-ray diffraction analysis showed, however, that the *erythro*-isomer adopted a synclinal N/O conformation in the solid state while that of the *threo*-isomer was similar to the antiplanar form.[139] No solute conformation studies have been reported. If antiplanar N/O conformers are in fact, the active species, differences in the muscarinic potencies of these diastereoisomers are more probably determined, not by the population variations of the *erythro*- and *threo*-rotamers but by differences in the orientations of the two methyl substituents in the antiplanar forms.

Subsequent results concerning α-methyl- (**92**) and β-methyl- (**93**) (proposed models for *erythro*-species) and α, β-dimethyl- (**94**) (model for *threo*-species) derivatives failed to clarify this issue because the *threo*-analogue proved the most active muscarinic agent and was not a substrate for AChE, as were **92**, **93**, and *threo*-α,β-dimethyl ACh (all slowly hydrolyzed).[140] A further puzzle is the fact that methyl substitution raises the potency of the methyl-free derivative **79** (a direct comparison was not made), but it is significant that all the active decalins have antiplanar–anticlinal N/O conformations.



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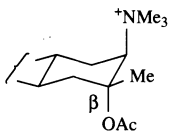

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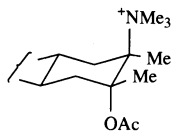
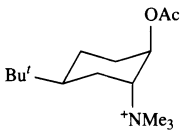


94

A tertiary butyl substituent provides a conformational restraint in 2-trimethylammoniumcyclohexyl acetates that is an alternative to the use of the *trans*-decalin skeleton. This approach was adopted in the hope of obtaining data complementary to that of Smissman and, perhaps, achieving higher orders of potency.[137] Although the last aim was not realized, the N^aO^a isomer **95** was found

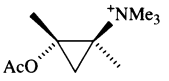


95

to possess significant, albeit weak, muscarinic activity (guinea pig ileum assay: molar potency 1.27×10^{-4}, ACh = 1, activity lost in the presence of hyoscine but not affected by hexamethonium.[149] The N^aO^e, N^eO^a, and N^eO^e analogues were all inactive. The muscarinic properties of *trans*-2-trimethylammoniumcyclohexyl acetate (**74**a) are lost when a *t*-butyl group is included in the molecule and this result may be due to a detrimental effect of the extra substituent on the affinity of the molecule for the receptor. An additional factor, however, may be the fact that the energy barrier for the inversion of **74**b is substantially greater than that of **74**a; hence significant populations of antiplanar N/O conformations may only be

available in the disubstituted derivative. Criticism of the inverted form of **74a** as the active conformation have been made on the grounds of the free-energy difference (ΔG°) between diaxial and diequatorial conformers probably being greater than the energy released on ligand–receptor binding.[132] However, if the free energy of binding of substrates and inhibitors to AChE (~4.2 kcal/mol)[141] be taken as a realistic figure for the muscarinic receptor, ΔG° for chair conformers of **74a** may well approach this value if the diaxial form is deformed in the same manner as the N^aO^a *t*-butyl analogue **95**. Precedent for the uptake of a ligand in an unfavored conformation is provided by the case of *N*-acetylgluosamine residues, which appear to be distorted from chair to half-chair conformations on binding to the active site of lysozyme.[142] Synthetic and stereochemical aspects of the *t*-butylcyclohexanes mirrored those of the decalins.[137]

Cannon's group chose the cyclopropane ring as the smallest system capable of conferring conformational rigidity on an ACh analogue and succeeded in obtaining an isomer that had a high muscarinic potency.[143,144] This was (+)-*trans*-2-acetoxycyclopropyltrimethylammonium iodide (ACTM, **96**), which equaled or surpassed ACh itself in two test systems; (−)-ACTM was several hundred times weaker than the (+)-form while the racemic *cis*-isomer **97** was virtually inactive



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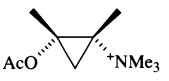


97

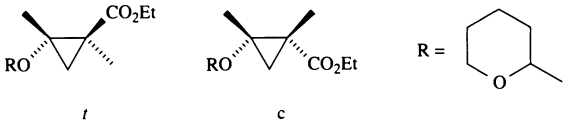
(Table 8.15). All isomers were feeble as nicotinic agonists on the frog rectus preparation. X-ray diffraction analysis of (+)-*trans*-ACTM established the N-C-C-O torsion angle as 137° in the crystalline state[73] and this angle (within the anticlinal range of 120° ± 30°) is probably close to that of the solute conformation because of the rigidity of the molecule. The (+)-isomer had a 1*S*,2*S*-configuration, hence the arrangement of substituents about C-2 in **96** is the same as that about related asymmetric centers of the potent muscarinic agents (+)-β-methyl ACh[17,19] and (+)-muscarine.[40]

Chemistry. The copper-catalyzed reaction between ethyl diazoacetate and 2-vinyltetrahydropyran led to a mixture of the cyclopropane isomers **98** (the tetrahydropyran group protects the cyclic structure from ring opening) which were

TABLE 8.15.
Relative Potencies of 2-Acetoxycyclopropyltrimethylammonium Iodides[143, 144]

Compound	Dog blood pressure[b]	GPI[c]	Frog rectus
ACh	1.0	1.0	1.0
(+)-*trans*-ACTM[a]	4.70	1.13	0.013
(−)-*trans*-ACTM	0.023	0.0022	0.0028
rac-cis-ACTM	—	0.0001	0.0042

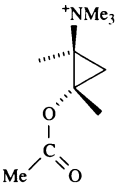
[a] Relative values of hydrolysis by AChE: (+)-*trans* 96, (−)-*trans* 59, ACh 100.[144]
[b] Depressor effects of antipodes of ACTM blocked by atropine sulfate (2 mg/kg).[144]
[c] Action of (+)- and (−)-ACTM blocked by atropine sulfate but not by hexamethonium.[143]



t c

98

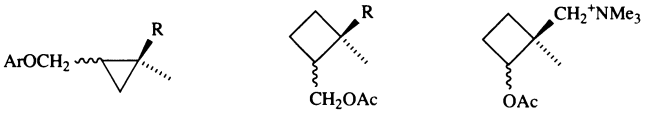
separated by fractional distillation.[145] The major isomer was converted to 2-acetoxycyclopropyltrimethylammonium iodide (ACTM) by a sequence involving the Hofmann hypohalite reaction ($CO_2Et \rightarrow CONH_2 \rightarrow NH_2$), and the racemic base so produced resolved with (−)-tartaric acid. The pharmacologically potent (+)-isomer was shown to be the *trans*-form **99** and to have the 1*S*,2*S* absolute



99

configuration by X-ray diffraction analysis.[73] The minor isomer, *cis*-**98**, did not yield pure products in the Hofmann sequence and this route to *cis*-ACTM was abandoned. No alternative procedure has yet been described although pharmacological data on both *cis*- and *trans*-ACTM are reported.[143,144] The cyclobutyl and additional cyclopropyl derivatives later reported[146] were all derived from the appropriate *trans*-1,2-dicarboxylic acids or *cis*-1,2-dicarboxylic anhydrides.

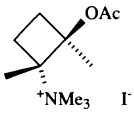
The cyclopropyl and cyclobutyl derivatives (**100** – **102**) have also been



100 **101** **102**

R = $^+NMe_3$ or $CH_2N^+Me_3$

examined.[146] These are analogues of acetyl γ-homocholine or 4-acetoxybutyltrimethylammonium[147] and provide no useful data relevant to the active conformation of ACh. All isomers had feeble muscarinic effects but *cis*- and *trans*-**100** (R = NMe_3) had appreciable nicotinic activities (*cis* 1/18, *trans* 1/8 that of ACh, frog rectus preparation, responses blocked by curare). The cyclobutane **103** related

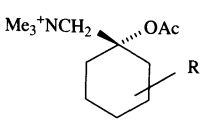


103

to ACTM was reported in 1975,[148] prepared from *trans*-2-carbomethoxycyclobutanecarbonyl chloride by a multistep procedure. Although its steric disposition

of functionalities is close to that of the cyclopropyl derivative its muscarinic potency fell far below that of the latter compound (relative potencies, ACh = 1; *rac*-**96** 1.01, *rac*-**103** 0.02, GPI; *rac*-**96** 0.93, *rac*-**103** 0.09, dog blood pressure). Cyclopentane analogues have ben described in a published dissertation[123]; feeble spasmogenic properties are claimed for both *cis*- and *trans*-2-trimethylammoniumcyclopentyl acetate (**72**) with the latter (near anticlinal $^{+}$N/O conformer) the more potent and of higher intrinsic activity (rat ileum pD_2: *cis* 2.6, *trans* 3.4, carbachol 6.7).

Analogues of the β,β-dimethyl derivative of ACh (page 236) have also been examined in which the β-methyls form part of a six-membered ring (**104**, R = H, 2-Me, 3-Me, and 4-Me).[149] Of this set, only the *r*-1-acetoxy-*c*-3-methyl derivative (**105**) possessed muscarinic activity on guinea pig ileum (molar potency $\sim \times 10^{-5}$,



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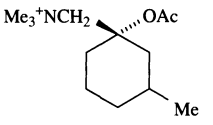
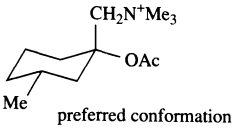
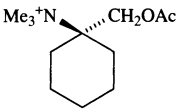




105

ACh = 1, effects abolished by hyoscine but not by hexamethonium) and the (+)-form was twice as active as the racemic mixture.[133] The weak, although significant, activity of **105** is in contrast to the complete inactivity of the 3-desmethyl derivative (**104**, R = H) and suggest that an equatorial OCOMe function is pharmacologically advantageous in these derivatives [OCOMe is axial in the preferred conformation of **104** (R = H) but equatorial in that of **105**]. The role of the methyl substituent could then be that of a conformationally holding group so placed that it does not impede binding to a receptor as may, for example, methyl in equatorial OCOMe isomers of the corresponding 2- and 4-substituted derivatives (both inactive). It is argued that the low potencies or inactivity of β,β-dimethyl ACh and the cyclohexyl derivatives **104** may be due, in part at least, to difficulty in attaining an antiplanar $^{+}$N/O conformation. The populations of such species should be greater in the α,α-cyclohexyl analogue (**106**) on account of the much lower steric demands of CH_2OCOMe as compared with $^{+}NMe_3$, and it is of interest that **106** showed significant activity (molar potency 1.1×10^{-4}, ACh = 1) in the ileum test while **104** (R = H) was inactive.[133]

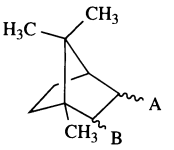


106

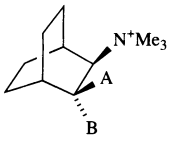
Interpretation of these results in terms of demands of the muscarinic receptor must be made cautiously, however, because there is evidence that **105** has an indirect mode of action.

The chemistry of diastereoisomeric aminobornanes **107** related to ACh has been extensively reported,[150,151] these derivatives providing NCCO torsion angles in the approximate range 0° to 120°. None of the 3-amino isomers **107** caused

guinea pig ileum to contract at a bath concentration of 100 μM, or affected contractions produced by ACh; they did antagonize nicotine, however, and were shown to possess weak ganglion blocking activity which had only a small dependence on stereochemistry.[152] In another report the compounds antagonized ACh on both guinea pig ileum and frog rectus preparations with *cis*- more effective than *trans*-isomers (Smail, private communication). *Cis*-derivatives (**107**a) also proved more effective than *trans*-forms as inhibitors of AChE but orders of activity were low.[153] The bicyclo[2,2,2]octane system has also been employed as a rigid support for ACh functionalities.[154,155] On rabbit ileum, the anticlinal form (**108**b) had significant muscarinic activity (370 times less than ACh on a molar basis, blocked by atropine but not by hexamethonium, no evidence for indirect action) while the synplanar analogue (**108**a) had no activity up to 10^{-3}M; eel AChE hydrolyzed **108**b about 40 times as fast as **108**a.[154]



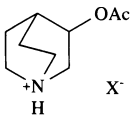
(a) A = OCOMe, B = $^{+}NMe_3$
(b) A = $^{+}NMe_3$, B = OCOMe
107



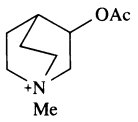
(a) A = OCOMe, B = H
(b) A = H, B = OCOMe
108

8.9. Derivatives of Quinuclidine and Related Bicyclic Agonists

The conformationally restrained analogue of ACh based on 3-quinuclidinol (**109**, aceclidine, HCl, or salicylate) is of special interest on account of its relatively high potency and rigid *anticlinal* disposition of charged nitrogen and oxygen functions. It presents a puzzle, however, in that the corresponding methiodide **110** is a



109
acelidine



110

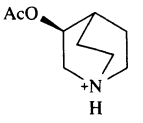
much weaker muscarinic agonist than the protonated tertiary amine. Mashkovsky reported the methiodide to be weaker by a factor of 200.[156] In fact, other exceptions to be superiority of quaternary ($^{+}NMe_3$) over protonated amines ($^{+}NHMe_2$) are known but most relate to piperidines where intramolecular interactions between carbonyl oxygen and ^{+}NH (which may restrict the conformational options of acyclic analogues) cannot occur.[157–159]

Several comparative studies of antipodes of aceclidine and its methiodide have been made. In earlier papers the optical purity of the levo isomer was low, but in subsequent reports antipodes of comparable purity were employed. Data of Lambrecht[160] are shown in Table 8.16 which reveal:

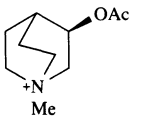
TABLE 8.16.
Muscarinic Activities of ACh Analogues Based on 3-Quinuclidinol[160]

	pD_2[a]	i.a.	EPMR	*R*/*S* ratio
HCl *rac*-**111**	6.85	1.03	14	8.6
R-(−)[c]	6.32 (5.02)[b]	0.98	48	(11.5)
S-(+)	7.25 (6.10)	1.04	5.6	
MeI *rac*-**112**	4.68	0.95	2,100	0.11
R-(−)[c]	4.92 (3.97)	1.01	1,200	(0.39)
S-(+)	3.96 (3.56)	0.58	11,000	
ACh	8.00	1.00	1	

[a] GPI assay.
[b] Values in parentheses refer to GP atrium (negative effects on force of contraction in the presence of hexamethonium and di-isopropyl fluorophosphate).[119]
[c] Better substrates for AChE than their corresponding *S*-antipodes.[161]



S-(+)-**111**



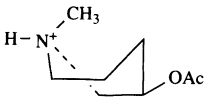
R-(−)-**112**

(a) the near tenfold difference in potency between the antipodal hydrochlorides with *S* the eutomeric form (i.e., a relatively modest difference),
(b) the pronounced fall in potency following quaternization (*rac*-HCl ~ 230 × *rac*-Me I),
(c) the *reversal* of stereoselectivity seen after quaternization with the *R*- exceeding the *S*-form in potency (ileum 9×, atrium 2.6×).

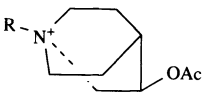
Similar results were reported by Barlow and Casy[162] who first drew attention to the differing receptor stereoselectivities for protonated bases and methiodides, not detected by Weinstein *et al.*[163] In yet another study Ringdahl *et al.*[164] reported an *S*:*R* potency ratio of 14 (6 in the presence of an anticholinesterase showing part of the antipodal potency difference to be due to susceptibility of the *R*-isomer to attack by AChE). In binding experiments (rat brain stem, displacement of [^{3}H] QNB) the affinity of *S*-(+)-aceclidine was 35 times and twice that of the levo antipode measured at high and low affinity sites, respectively (differentiation of dual sites was not possible in the case of the more weakly bound levo ligand).[164]

Stereochemistry. X-ray diffraction analyses of both the acetate methiodide[165] and benzilate HBr derived from (−)-3-quinuclidinol[166] have established the *R*-configuration of this series. Most recently X-ray analysis of campharate esters has involved use of an internal chiral standard.[167] Hence the eutomer of the *t*-amine (aceclidine) is the *S*-(+)-isomer **111** while that of the methiodide is the *R*-(−)-isomer **112**. In the crystalline (−)-methiodide the NCCO torsion angle is 108° (anticlinal), which must be maintained in the solute state because of the rigid nature of the molecule. The moderate potency of the *S*-(+)-HCl (it has more than a tenth of the activity of ACh from the data of Table 8.16) thus provides good

evidence that ACh and its congeners bind to the receptor in an approximately anticlinal conformation (cf. conclusions drawn from previous work, especially on *trans*-ACTM, page 267). The active conformation of *S*-3-acetoxy-*N*-methylpiperidine (eutomer) may thus be deduced to be the boat form **113**, since it corresponds exactly with the absolute geometry of *S*-3-acetoxyquinuclidinol **114**; the high energy of conformer **113** may be the reason for the very low level of activity of this piperidine derivative (see page 278).[119] Of additional significance is the steric correlation of eutomers of 3-acetoxyquinuclidinol and β-methyl ACh, revealed most directly when the acyclic compound adopts an anticlinal N/O conformation (see **115** and **116**). In contrast the more potent *R*-(-)-methiodide **112** correlates with the distomeric form (*R*) of β-MeACh.



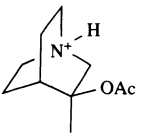
S-**113**



S-**114**

(after Mutschler and Lambrecht)[119]

anticlinal



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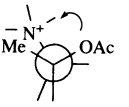
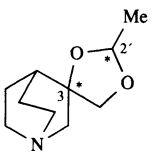
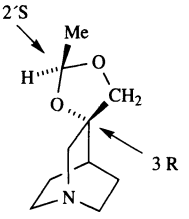


116

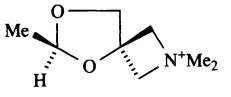
Saunders *et al.*[167] examined the four diastereoisomers of AF30 (**117**), a hybrid of aceclidine and the dioxolanes of Fourneau (page 240). This work was part of a search for agents of potential value in treating defects in central cholinergic transmission, as seen in patients with senile dementia of Alzheimer's type. In binding and pharmacological tests the 3*R*,2′*S*-isomer **117**a proved the most potent followed by the 3*R*,2′*R*-isomer (Table 8.17). Thus receptor stereoselectivities toward the dioxolane series (3*R*-eutomer) were followed rather than those toward esters of 3-quinuclidinol. The stereochemistry of **117**a (3*R*,2′*S*) followed from X-ray analysis.



117 AF30



117 a



118

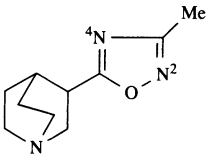
The earlier reported compound **118**,[168] also a spiro analogue of the dioxolanes, had one-tenth of the potency of ACh. In later MSD work, analogues of **117** with a 1,2,4-oxadiazole ring were found to be of notably high affinity and efficacy at central muscarinic receptors, such as **119**.[171] These studies were followed

TABLE 8.17.
Binding and Pharmacological Assays of Some Isomeric 2′-Methylspiro[1-azabicyclo 2.2.2]octane-3,4′-[1,3]dioxolanes and Standards[167]

	Heart (M-2) [^{3}H]NMS[a] K_{app} (μM)[b]	Cortex (M-1) [^{3}H]pirenzepine K_{app} (μM)	Ganglion pED_{50}(FE)[c]	GPI pED_{50}	GP atria pED_{50}
Carbachol	1.9	45	—	6.99 (1.0)	6.68 (1.0)
Muscarine (*rac*)	2.3	28	6.77 (1.1)	—	—
3*R*,2′*S*-**117**	2.2	2.7	5.25 (0.8)	5.66 (0.8)	4.83 (0.8)
3*R*,2′*R*-**117**	8.5	4.1	4.56 (6.7)	4.70 (0.7)	~ 4.0 (0.2)

[a] *N*-methylscopolamine.
[b] Apparent affinity constant.
[c] Functional efficacy compared to standards.

by examination of related azabicyclo ligands based on 1-azanorbornane (**120**) and isoquinuclidine (**121**), both classes providing pairs of geometrical isomers.



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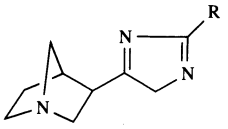
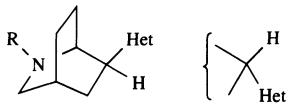


(a) R = Me; (b) R = NH_2
exo- and *endo*-
120



(a) R = H; (b) R = Me
anti- and *syn*-
121

Binding experiments employing [^{3}H]oxotremorine-M (OXO-M) and [^{3}H]-*N*-methylscopolamine (NMS) provided data on uptake at the high- and low-affinity state of cerebral cortex muscarinic receptors. The log of the NMS/OXO-M ratio has been shown to be predictive of the ability of the ligand to stimulate cortical inositol–phospholipid (PI) hydrolysis, i.e., to give a measure of intrinsic activity.[170]

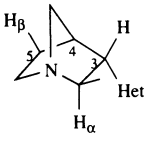
TABLE 8.18.
Binding Data for Azabicyclic Oxadiazoles and Standard Ligands[a] [169]

Compound (*rac*)	Stereochemistry	Binding data K_{app} (μM) [^{3}H]NMS[b]	[^{3}H]OXO-M[c]	Ratio[d]
119 (quinuclidine)[e]	—	0.44	0.0009	490
120a	*exo*	0.10	0.00009	1100
	endo	3.6	0.0021	1700
120b	*exo*	0.031	< 0.000043	> 1000
	endo	1.6	0.0015	1060
121a	*anti*	1.1	0.0032	340
	syn	5.2	0.025	210
121b	*anti*	8.9	0.3	30
	syn	29.0	0.95	31
Carbachol		22	0.0049	4500

[a] Displacement of tritiated ligands from rat cortical homogenates.
[b] *N*-Methylscopolamine (low-affinity sites).
[c] Oxotremorine-M (high-affinity sites); radioligand made by treating oxotremorine with [^{3}H]MeI.[23]
[d] Full agonists such as carbachol have ratios greater than 800; values 10–200 and 200–800 are characteristic of weak and partial agonists, respectively.
[e] Saunders *et al.*[171]

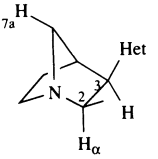
Results of the quinuclidine **119** and some of its analogues are shown in Table 8.18. The azanorbornates **120**a, congenors of **119** of slightly reduced bulk, both exceeded the parent in efficacy as judged by the NMS: OXO-M ratio values with the *exo*-isomer having, in addition, greater affinties. Further work on this series and speculations on features governing receptor affinity have been reported by Orlek *et al.*[203] The same pattern of binding parameters was found for the amino congeners **120**b. The isoquinclidines **121** were inferior to the quinuclidine **119** in the same tests—again *anti* (≡*exo* of **120**) stereochemistry was preferred.

Chemistry. Stereochemical assignments were based on ^{1}H/COSY-NMR spectra and by making use of four-bond couplings between transantiperiplanar protons: for example, 3-H shows a four-bond coupling of 1.9 Hz to 5 β-H in the *endo*-isomer, and one of 1 Hz to 7a-H in the *exo*-isomer.



endo

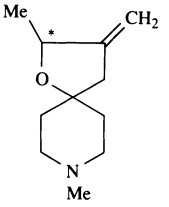
$^3J_{2\alpha,3}$ 4.2 Hz
$^3J_{2\beta,3}$ 11.3 Hz
$^3J_{3,4}$ 4.1 Hz
$^4J_{3,5\beta}$ 1.9 Hz



exo

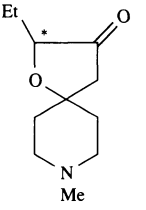
$^3J_{2\alpha,3}$ 8.6 Hz
$^3J_{2\beta,3}$ 5.5 Hz
$^4J_{3,7a}$ 1 Hz

A Japanese group examined some spiranes related to AF30 (**117**) based on *N*-methylpiperidine, and reported some antipodal comparisons for displacement of [^{3}H]pirenzepine (M_1 ligand) from rat frontal cortex sites.[172]



YM 796 (**121**a)

IC$_{50}$ mM vs. [^{3}H]pirenzepine
(+) 6.95
(−) 2.12



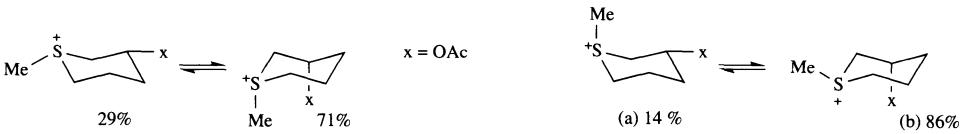
YM 954 (**121**b)

(+) 0.79
(−) 0.44 cf. carbachol 2.16

Levo isomers were the eutomers in this test and one in competition with [^{3}H]QNB for binding sites of cerebellum, a tissue devoid of M_1 receptor subsites and considered to contain the M_2 and/or M_3 variety.[173] Selectivities for M_1 sites were about 5 for eutomers and 10 for distomers. Details of chemistry have not been published.

8.10. Piperidine and Thiane Analogues

Up to this point work on conformationally restrained congeners of ACh points to a receptor bound species of the anticlinal to antiplanar kind with a negative (O→N) sign of torsion angle. In addition, results for aceclidine suggest receptor preference for an axially inclined N-methyl (in the sense of the piperidine boat moiety of the quinuclidine molecule with N-Me 1,3-*cis* to OCOMe). In the case of 3-acetoxy-*N*-methylpiperidine (protonated) such a conformation is the one of highest energy (**113**, see page 272). Mutschler and Lambrecht[119] examined the sulfur isosteres of 3-acetoxy-*N*-methylpiperidine for two reasons. First, since sulfur has a higher barrier to pyramidal inversion than nitrogen (~100 kJ/mol for S, 38 kJ/mol for N),[174,175] isolation of *cis*-**122** and *trans*-**123** 3-acetoxy-1-methylthianes is possible. Second, computations and other techniques show that populations of conformers having an axial methyl group attached to the onium (+S) center are much greater than in piperidine analogues (see references cited).[119] The *trans*-conformer **123**a (shown as the 1*R*,3*R* antipode) thus mimics the geometry of aceclidine most closely (boat forms of **122** and **123** have very high energies). Even though the equatorial S-Me conformer **123**b is the preferred *trans*-conformation, the low energy difference between axial and equatorial S-Me chairs (0.76 kJ/mol, cf. 11–14 kJ/mol for piperidines) makes reasonable the idea that the energy for inversion (**123** b→a) would be provided by that released when the ligand binds to the receptor.



cis-**122** 1*S*, 3*R* shown *trans*-**123** 1*R*, 3*R* shown

(axial preference of 3-OCOMe governed by electronic O:... $^+$S interaction)

Biological data for the thianium salts, shown in Table 8.19, substantiate predictions that 1*R*,3*R*-**123** should be the most potent isomer of this series. In fact this compound attained about one-tenth the potency of ACh in the GP atrium—its antipode and corresponding *rac-cis* analogue were less potent by several orders of magnitude. Binding parameters for *rac-cis*-**122** and (+)-*trans*-**123** correlated with their muscarinic potencies.

Contributions from boat conformations (as locked into the rigid aceclidine molecule) are considered insignificant because of the greater energy of such forms over corresponding. chairs. If boats were the receptor-bound species then eutomers should have the *S*-configuration—thus *S*-3-acetoxyl-*N*-methylpiperidine is five times more potent than the *R*-isomer at GP atrial sites (page 259). Mutschler and

TABLE 8.19.
Muscarinic Activities[119] and Binding Parameters[176] of Cyclic ACh Analogues Based on Thiacyclohexane

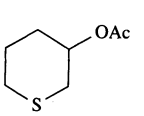
Compound	pD_2[a,b]	pK_1[c]	pK_2
rac-cis-**122**	3.56 (4.46)[d]	5.03	3.85
1*R*,3*R*-(+)-*trans*-**123**	6.50 (6.57)	7.27	5.03
1*S*,3*S*-(−)-*trans*-**123**	4.85	—	—
ACh	7.51	—	—

[a] Guinea-pig isolated left atrium, negative effects on force of contraction. Experiments performed in the presence of hexamethonium and di-isopropyl fluorophosphate.
[b] Similar results were found using GPI and rat blood pressure tests; in the GPI, EPMR values were (ACh = 1) *rac-cis*-**122** 1704, (+)-*trans*-**123** 13.3, (−)-*trans*-**123** 704.[177]
[c] pK_1 high-affinity, pK_2 low-affinity site, competition with [^{3}H](−)-quinuclidine benzilate, GPI tissue.
[d] GPI values.[176]

Lambrecht[119] point out that if one accepts that the active muscarinic conformation of ACh involves a transoid (anticlinal – antiplanar) NCCO orientation, then the neurotransmitter would attain less than 1% of its potential activity on the basis of an energy difference between *gauche*- and *trans*-conformers of about 13kJ/mol.[116] The fact that rigid ACh congeners having a transoid NCCO orientation and with potencies far above that of ACh have not yet been found may be a result of the adverse influence of the restraining atoms of these molecules on receptor affinity. It is probable, however, that conformational changes of ACh are in fact induced by the receptor itself (acting as a "conformerase") whereby raising the population of active species to a high level.

Remarkably, the *cis*-analogue **122** proved more potent than the *trans*-compound in a nicotinic assay (frog rectus abdominis muscle, pD_2 values in presence of phystostigmine: ACh 5.97, *rac-cis*-**122** 4.23, *rac-trans*-**123** 3.52).[178]

Chemistry. Treatment of 3-acetoxythiacyclohexane **124** with methyl iodide gave a 1:1 mixture of *cis*- and *trans*-*S*-methyl iodides **125** which were separated by



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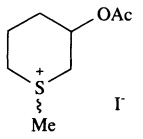
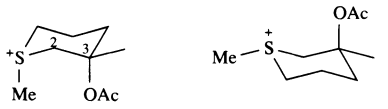


125
c-t mixture

fractional crystallization of appropriate salts.[178] ^{1}H-NMR spectroscopy (90 MHz) provided evidence of stereochemistry. Dimensions of the 3-H resonance were typical of equatorial protons and showed that the acetoxy substituent preferred an axial orientation in both the *cis* ($W_{1/2}$ ~12 Hz) and *trans* ($W_{1/2}$ ~10 Hz) isomers, while the greater 2-CH_2 ^2J values of the *cis* isomer (14.5 Hz, 12.5 Hz for *trans*) was evidence for an axial S-methyl in the *cis* and equatorial in the *trans* form (*c* and *t*-**126**).[179]



cis **126** *trans*

These assignments were later confirmed by X-ray analysis of the *rac-cis*- and (−)-*trans*-perchlorate[180] work, which also established the absolute configuration (1*S*,3*S*) of the latter salt (the *distomeric* antipode). Use of (+)- and (−)-3-hydroxy-thiacyclohexane (resolved with *S*-1-phenethylamine via the phthalic esters) in the synthesis (**124**→**125**) provided antipodes of the *trans*-series.[177]

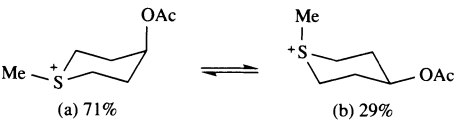
Lambrecht and colleagues[181] later extended this work to 4-acetoxy-piperidines and -thiacyclohexanes. These compounds are analogues of acetyl-γ-homocholine (**127**) which is about fifty times less potent than ACh as a muscarinic agonist.[182]

$Me_3N^+(CH_2)_3OAc$ X^-

127

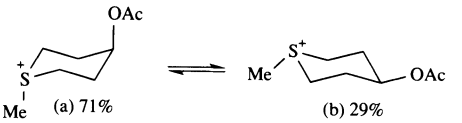
Synthetic and stereochemical methodology were similar to that used in the 3-acetoxy series.

In the solid state[183] *cis*-**128** had the axial *S*-methyl conformation (**128**b), while the eq-*S*-Me chair **128**a preponderated in solution (NMR evidence). The *trans*-isomer **129** as solute had the same preferred conformation as found for the solid



(a) 71% (b) 29%

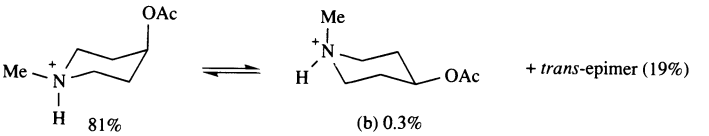
cis-**128**



(a) 71% (b) 29%

trans-**129**

(*ax*-*S*-Me). Energy differences between interconvertible chairs were low. The *cis*-axial 4-OCOMe chair was also favored for the 4-piperidine analogue **130**, but in this case its invertomer had a far higher energy content.

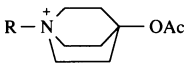


81% (b) 0.3% + *trans*-epimer (19%)

130

The thianes **128** and **129** proved weak partial agonists in a nicotinic assay (the piperidine **130** was inactive). However, all compounds were active in muscarinic tests with the *cis*-thiane **128** of notable potency, exceeding ACh (Table 8.20). Binding constants (pK_1) correlated with the pharmacological results. The authors argue that *cis*-**128** stands out as a potent muscarinic agonist because it is the only compound of the group that can provide a high population of the *ax*-1-Me, *eq*-4-OCOMe chair (**128b**) at the receptor. This arrangement is selected as the "muscarinic-essential" conformation (rather than the inverted form) because of the low potency of the nitrogen analogue **130** in which the *eq*-1-Me, *ax*-4-OCOMe chair preponderates and where inversion to the *ax*-4-OCOMe chair carries a high energy penalty. The problem remains of correlating active conformations of thiacyclic analogues of ACh (**122** and **123**) and homo ACh which, although sharing axial N-Me geometry, differ in the spatial separation of their oxygen and onium centers.

The 4-acetoxy analogue of aceclidine (**131**, R=H) and its methiodide (**131** R=Me) had very low potencies in muscarinic assays[184]—not surprising perhaps because the quinuclidine nucleus cannot in this case mimic conformation **128b**.



131

Most cholinergic agonists related to oxotremorine and discussed in Chapter 9 (page 314).

8.11. Metabolic Inactivation of ACh and AChE Inhibitors

Most stereochemical interest in factors affecting the *formation*, *storage* and *release*, and metabolism of endogenous ACh centers on acetylcholinesterase (AChE) and other cholinesterases (a general review can be found in Ref. 185). Chiral molecules designed as cholinergic ligands have often been examined as substrates and/or inhibitors of AChE, and information in this regard has been included in the preceding text. A review and interpretation of stereochemical data available up to the late 1960s has been published by Beckett.[14] His analysis of

TABLE 8.20.
Muscarinic Activities and Binding Parameters of Some 4-Acetoxy-piperidine and -thiacyclohexanes[174, 176]

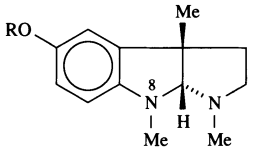
	EPMR GPI[a]	EPMR GP atria	EPMR rat blood pressure	pK_1[b]	pD_2 (GPI)
ACh	1.00	1.93	1.56	—	—
4-Acetoxy piperidine (**130**) HCl	93	352	116	5.84	5.65
cis-thiane-**128**	0.95	1.00	1.00	7.09	7.59
trans-thiane-**129**	23.3	91	60	5.94	6.20

[a] Evidence of direct action obtained by measuring degree of inhibition caused by morphine.
[b] See footnote *c* of Table 8.19.

data relating to interaction of the enzyme with isomeric substrates (α- and β-methyl ACh) and inhibitors (muscarines) led to a proposed model of the active site of AChE. The model was later extended when data on ligands based on bornane and bicyclo[2,2,2]octane became available.[186]

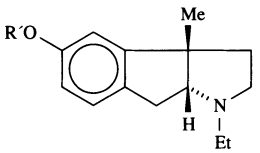
Belleau and Lacasse[187] measured the inhibition constants for AChE of cholinergic ligands of the dioxolane series: relative affinities of 4*R*-(+)-**20** (cholinergic eutomer) and the 4*S*-(−)-distomer were 11 and 0.67, respectively (ACh = 1). They used these data to deduce the mechanism of interaction of ACh and 4*R*-**20** with the enzyme, particularly in regard to the role of the ester methyl of ACh and 2-Me of **20**. Their proposals were elaborated in subsequent review.[188]

Physostigmine (eserine), the classical inhibitor of AChE isolated from Calabar beans, has the configuration **132** as established by an early application of the NOE phenonemon[189], its absolute geometry was solved by chemical methods.[190] In the first issue of a new journal (*Bioorganic and Medicinal Chemistry Letters*) the 8-deaza analogue **133** has been described.[191] Of the two antipodes isolated, the



R = CONHMe

132



R′ = $CONHC_6H_{13}$

133

levo isomer related in configuration to the natural alkaloid proved 12-fold more effective than the dextro form as an inhibitor of AChE (IC_{50} nM values were: levo 20, dextro 233, physostigmine 128). The stereochemistry was established by X-ray analysis of a resolved intermediate.

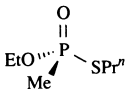
Many *organophosphates* have anti-AChE activities and certain of them are chiral as a result of the tetrahedral nature of their central phosphorus atom. They are important as pesticides and hence fall outside the scope of this book. A few examples will be mentioned, however, and the reader is directed to a chapter of a recent monograph on the stereoselectivity of pesticides for details.[192] In a series of antipodes of general structure **134**, high r_a (ratio of the rate constants for inhibition

134

Y	Z	X	k (M^{-1} min^{-1})	r_a	Ref.
EtO	Me	$S(CH_2)_2NPr^i$	4×10^7	150	193
Pr^iO	Me	$OC_6H_4$4-NO_2	1×10^6	370	192
Pr^iO	Me	F	1×10^7	4,200	192, 194[a]
$Bu^tCH(Me)O$	Me	F	2×10^8	17,500	196, 197
			3×10^7	2,700	
EtCH(Me)O	Et	$S(CH_2)_2NMe_2$	9×10^6	1,400	198
			3×10^6	1,600	

[a] Sarin, absolute configuration Ref. 195.

of AChE) values, i.e., those above 100, were only found for compounds of which the eutomer was of high potency ($k > 10^6$ M^{-1} min^{-1}), a relationship which mirrors Pfeiffer's rule in pharmacology (chiral organophophates form one of the examples of eudismic analysis presented by Ariëns and his colleagues; see Chapter 9, p. 304). The stereoselectivities appear little dependent on the nature of the leaving group X but more so on differences between groups Y and Z, such as bulkiness and hydrophobicity. The absolute configurations of a series of chiral organophosphates have been established by X-ray analysis and chemical correlations, and correspondence among the more active enantiomers of related series observed.[192] In compounds of type **135** and **136**, eutomers were of *S*- and *R*-configuration, respectively.



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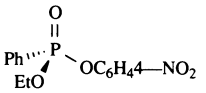

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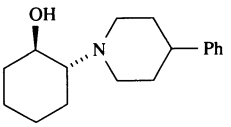
8.12. Storage and Release of ACh (see Ref. 205 for 1992 report involving antipodes of BM-5)

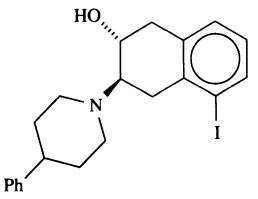
Recently *trans*-2-(4-phenylpiperidino)-cyclohexanol (**137**, vesamicol) has been shown to act as a noncompetitive ACh inhibitor of the storage of ACh by nerve terminal synaptic vesicles—a process which takes place by an active transport mechanism.[199] Vesamicol was later resolved[200] and a 25-fold potency difference found between the antipodes with the levo 1*R*,2*R*-isomer being the eutomeric form (configuration by X-ray analysis of the distomer as its di-*p*-toluoyl-D-tartrate salt).



137

The *cis*-racemic mixture was far less effective than the *trans*-mixture in preventing the uptake of [^{3}H]-ACh into Torpedo californica electric organ synaptic vesicles (IC_{50} nM: *trans* 40, *cis* 3000).

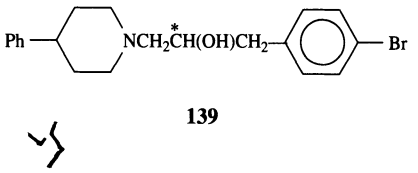
Studies of radioactive uptake in mouse brain suggest that (−)-IBVM ($^{125}I/^{123}I$ labeled levobenzovesamicol **138**) may be of value as an imaging agent for assessing



138 IBVM

neuronal damage in Alzheimer's disease.[201] After tail vein administration of levo $^{(125)}$I-**138**, radioactivity (%dose/g) accumulation in mouse brain was 2.56 (cerebral cortex), 6.25 (striatum), and 0.23 (cerebellum); corresponding values after the dextro antipode were 0.12, 0.26, and 0.06, respectively.

Among a variety of analogues, the levo enantiomer of **139** proved as effective as *rac*-vesamicol in preventing the uptake of [^{3}H]-ACh into synaptic vesicles (IC_{50} nM: levo **139** 36, dextro 328, *rac*-vesamicol 34).[202]



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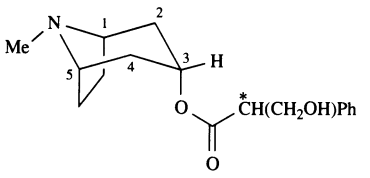
9

Muscarinic Antagonists

9.1. Introduction: Tropane Alkaloids

This chapter describes chiral agents which antagonize the action of cholinergic agonists at muscarinic (M) receptors. In much of the work prior to 1980, subclasses of M receptors were not recognized. Evidence for distinct varieties of M receptors is now strong, however (Chap. 8, Ref. 3), and, indeed, derives chiefly from the discovery of selective antagonists. The *TiPS* January 1991 supplement lists selective antagonists for three M-subtypes. Papers published during the last decade reflect this development.

Ligands which block cholinergic receptors usually include a functionality of greater bulk than acetyl of ACh composed of hydrophobic and sometimes H-bonding (donor) elements, separated by a variable number of atoms from the basic center of the molecule. When both basic and hydrophobic features are chiral, the configuration of the latter has the greater influence on receptor affinity. Atropine, the classical antagonist of the muscarinic actions of ACh, and the first example to be discussed in this chapter, possesses a bulky acyloxy unit of this kind. Its potency comparison with the related alkaloid hyoscyamine, reported by Cushny in 1904,[1] provided one of the first pharmacological examples of stereoselectivity. Both alkaloids are obtained from Solanaceous plants such as belladonna and henbane: atropine is the tropyl ester of racemic tropic acid (probably an artefact of isolation) while hyoscyamine is derived from tropine and *S*-(−)-tropic acid (**1**). Following



atropine *RS*-mixture
hyoscyamine *S*-antipode

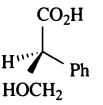
1

Cushny, a variety of comparisons of atropine and hyoscyamine have shown that most of the activity of the racemic product resides in the levo isomer. Long *et al.*[2] compared (−)-hyoscyamine sulfate and the antipodal (+)-camphor-10-sulfonate

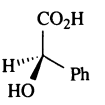
TABLE 9.1.
Affinity Constant (log *K*) Values of Tropane Derivatives for ACh Receptors of the Guinea-Pig Ileum[8]

Hyoscyamine derivatives (1)	log K^a	Eudismic ratio[b]
RS-Sulfate	9.007	330
S-HBr	9.38	
R-HBr	6.86	
RS-MeI[c]	9.45	87
S-MeI	9.66	
R-MeI	7.73	
RS-EtI	8.24	54
S-EtI	8.79	
R-EtI	7.06	
S-Pr^{n}I	7.50	17
R-Pr^{n}I	6.26	
S-Bu^{n}I	7.09	16
R-Bu^{n}I	5.89	
Hyoscine derivatives[e]		
S-HBr	9.36	62
R-HBr	7.57	
S-MeI	9.70	12
R-MeI	8.62	
S-EtI	8.60	28
R-EtI	7.15	
S-Pr^{n}I	8.27	32
R-Pr^{n}I	6.77	
S-Bu^{n}I	7.16	
Homatropine derivatives[f]		
S-sulfate	6.99	3[d]
R-sulfate	7.44	
S-MeI	7.28	4
R-MeI	7.86	
S-EtI	6.77	1.5
R-EtI	6.97	

[a] log *K* values measured at 37° C in the presence of hexamethonium (2.75×10^{-4} M). Contractions of GPI induced by carbachol.
[b] Eutomers: *S* for tropyl esters, *R* for esters of mandelic acid (homatropine).



S-tropic acid



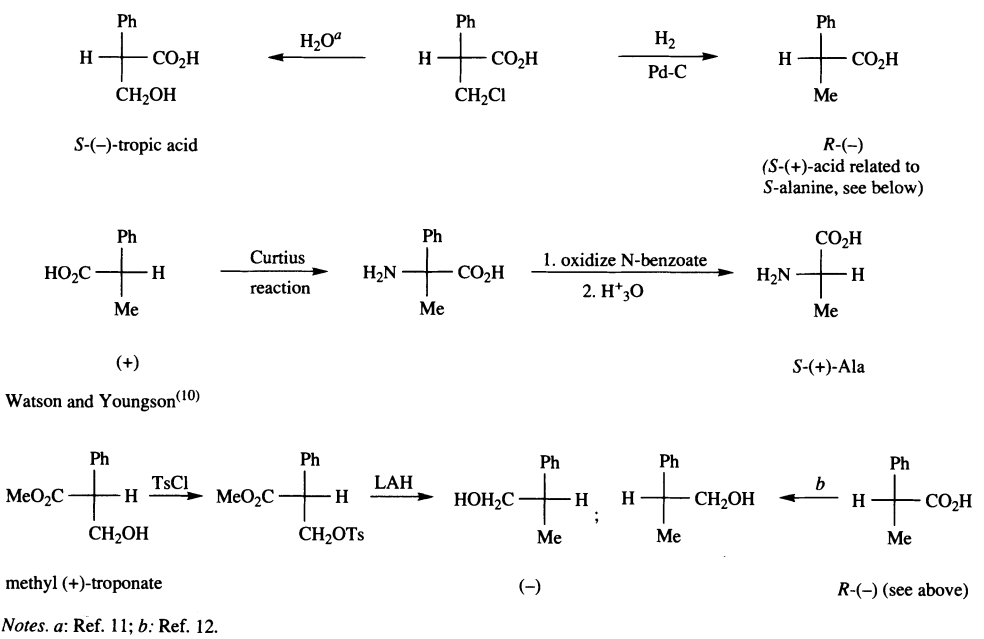
R-mandelic acid

[c] Quaternary salts prepared by adding alkyl halide to base in solvent and crystallizing the product which separated; in the case of nonsymmetrical salts, the product with the larger N-substituent axial was assumed to preponderate (see page 293).
[d] Low values may be due to loss of optical purity during the esterification process (prolonged heating with HCl).
[e] Structure **1** with an epoxide function attached to the bimethylene bridge.
[f] Esters of tropine and mandelic acid (see *b* above).

salt and found the latter to be virtually inactive while Bovet and Bovet-Nitti[3] stated that (−)-hyoscyamine was 10–100 times more active than the dextro form. Similarly (−)-hyoscine, an atropine analogue in which the bimethylene bridge carries an epoxide function, is twice as active as the racemic form (quoted by Barlow, 1964).[4] Antipodes of tropyl α-methyltropate also differ sharply in potency [(−) 40 × (+), GPI vs. ACh].[5–7] Some 1973 data are shown in Table 9.1 based on GPI experiments carried out under equilibrium conditions.[8] The highest eudismic ratio was observed for hyoscyamine sulfate (330). The *S*-methiodide had twice the affinity of the *S*-sulfate, but the methiodide eudismic ratio was lower than that of the sulfates. For other quaternary salts both eudismic ratios and affinities decreased with increasing size of the NR substituent. Similar results were found for hyoscines except in regard to the relative eudismic ratios of quaternary salts. Affinity differences between antipodes of homatropine were notably low (as found in general for basic esters of mandelic acid, see page 295) but could be due to loss of optical purity during preparation (optical purity was based solely on optical rotational measurements).

The absolute configurations of the chiral acid moiety of esters so far mentioned have all been established, except that of α-methyltropic acid (the levo acid is assumed to be of *S*-configuration since its sign of rotation is the same as that of *S*-tropic acid).[6] Fodor's chemical correlation of *R*-(−)-2-phenylpropanoic acid and levo tropic acid (hence establishing the latter's *S*-configuration) is shown in Scheme 9.1,[9] which includes a sequence due to Watson and Youngson.[10]

The absolute stereochemistry of mandelic acid is described in Chapter 4 (page 75).



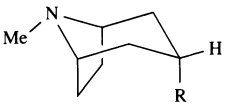
Notes. a: Ref. 11; *b*: Ref. 12.

Scheme 9.1. The configuration of antipodes of tropic acid (Fodor and Csepreghy).[9]

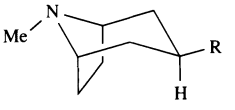
9.2. Orientation of the Acyloxy Function in Relation to the Basic Nitrogen of Tropane and Piperidyl Anticholinergics

The relative orientations of the 3-hydroxy and basic center of tropine and its stereoisomer pseudotropine have been known for many years (page 292).

From one early report on tropyl pseudotropine (β-analogue of atropine, *cis*-N/3-OCOR)[13] and a 1953 study of tropyl and pseudotropyl esters of benzoic acid[14] it may be concluded that a *trans*-orientation is optimal for activity in atropine and related compounds. Subsequent work supported this view, but the potency ratio of α/β pairs was found to be highly dependent on the nature of the acyloxy function (Table 9.2). Hunt and Robinson[15] found that atropine (α-**2**a) exceeded the spasmolytic potency of its 3β-analogue by factors of about 1700 (pA_2) and 832 (pA_{10}). Corresponding esters of 3-granatol (**2**, bimethylene replaced by trimethylene bridge) differed similarly with the α-isomer 600–700 times more potent than the β-ester. Earlier it was reported that α-3-(2,2-diphenyl-2-hydroxyethyl)-tropane (**2**b) was equipotent with, while β-(*2*b) was 1000 times less potent than, atropine in inhibiting thonium iodide-induced spasm of rabbit ileum.[17]



α-**2**



β-**2**

(a) R = $OCOCH(CH_2OH)Ph$; (b) R = $CH_2C(OH)Ph_2$; (c) R = $OCOC(OH)Ph_2$
(all racemic mixtures)

Similar comparisons of benzilates of 3-tropanol (α- and β- **2**c) revealed much reduced potency differences between isomers: the α-forms were superior but were

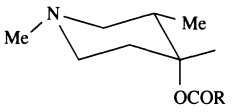
TABLE 9.2.
Antagonism of ACh-Induced Contractions of Guinea-Pig Ileum[15, 16]

3-Tropanol ester[a] 3-C-configuration	Acyloxy group	Form	pA_2	pA_{10}
α (tropine)[b]	*rac*-tropate	H_2SO_4	9.79	8.70
β (pseudotropine)	*rac*-tropate	HBr	6.55	5.78
α	benzilate	HCl	8.81	
β	benzilate	HCl	8.51	
α	benzilate	MeI	8.93	
β	benzilate	MeI	8.50	
4-Piperidyl benzilates				
(**3**a) *cis*		HCl	8.45	
(**4**a) *trans*		HCl	8.01	
(**3**a) *cis*		MeI	8.38	
(**4**a) *trans*		MeI	7.49	

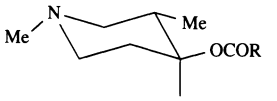
[a] Relative potencies of benzoates: α 0.01, β 0.003, atropine 1.0.
The α-benzoate methiodide was twice as active as tetraethylammonium (TEA) in blocking ganglionic sites of the cat nictitating membrane (β-ester < 0.2).[14]

[b] This compound (atropine) had a pA_2 value of 8.91 in tests of Ref. 16.

only 2–3 times more active than the β-isomers (Table 9.2). The isomeric pairs of benzilates (HCl or MeI salts) had similar mydriatic activities in mice and were all about one-quarter as active as atropine. Diastereoisomeric benzilate and diphenylacetate esters of 1,3-dimethyl-4-piperidinol were also examined.[16] The *cis*-esters **3** are the steric analogues of 3-α-tropanol esters (axial OCOR function) while *trans*-isomers **4** are related to β-tropanol derivatives (equatorial OCOR); the conformations shown are preferred as established from ^{1}H-NMR evidence.[18] The *cis*-benzilates (**3**a) were about twice (HCl) and 5 times (MeI) as active as the corresponding *trans*-esters **4**a (Table 9.2). Diphenylacetates of these 4-piperidinols were



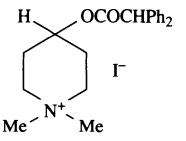
cis-**3**



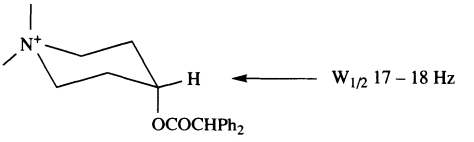
trans-**4**

(a) R = $C(OH)Ph_2$
(b) R = $CHPh_2$

4–5 times weaker than the benzilates; the *cis*-HCl (**3**b) was twice as active as the *trans*-analogue while the isomeric methiodides were equiactive. Lambrecht and others[19,20] reported that the diphenylacetate methosalt **5** was a selective antagonist of M_1-receptors in rat and GP ilea (affinity for the atrial M_2-receptor was less by a factor of 10–20). The selectivity of compound **5**, known as 4-DAMP, was first reported by Barlow *et al.*[21] (preference for smooth muscle) and has been confirmed by binding studies (low affinity in the myocardium, high in cerebral cortex and lacrimal gland).[22,23] The width of the 4-H ^{1}H-NMR resonance of **5** is typical of an equatorial proton, hence the axial OCOR chair **6** is the preferred conformation

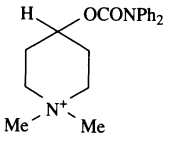


4-DAMP
pA_2 8.22 (1/5 × atropine, GPI)[16]
5



6

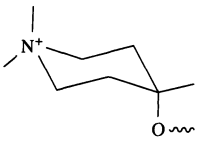
of this compound (see also Casy and Jeffery, 1972).[18] However, in the solid state 4-DAMP methodide adopts a chair conformation with an equatorial 4-acyloxyl substituent.[24] Crystals of the diphenylcarbamoyl analogue (**7**) contain two conformers, one with the ester group equatorial and the other with the ester group axial to the piperidine chair.[25] The carbamate **7** (log *K* 5.8) has less than one-thousandth of the affinity of 4-DAMP and is not selective.[26] Both phenyl groups



7

of 4-DAMP must contribute to affinity (cf. 4-phenylacetoxy analogue, log K 6.2, 4-DAMP 9.0, ileum),[27] while their presence in the carbamate trigonal unit is clearly unsuitable for receptor uptake.

All these data point to a preference for the arrangement **8** rather than **9** for the blockade of muscarinic receptors in intestinal muscle and other peripheral sites. This preference may be a functional group-separation factor, since the distance between charged nitrogen and the ether oxygen of the OCOR group is less in **8** by



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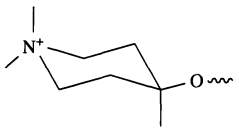
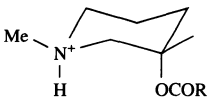


9

about 70 pm.[28] Both 1-methyl-4-piperidyl benzilate (pA_2 8.96) and its 3-piperidyl analogue (pA_2 9.10) (hydrochlorides) are potent spasmolytics[16,29] and it is probable that the ester substituent is axial in their receptor-bound conformations. The conformational free-energy difference between the chair forms of the 4-piperidyl hydrochloride is small[28] and the same is true for 3-piperidyl conformers; there is ^{1}H-NMR evidence for significant populations ($\sim$50% in $CDCl_3$; signals due to epimers are resolved) of the axial 3-acyloxy conformer **10** in solutions of 1-methyl-3-piperidyl ester hydrochlorides.[18] Replacement of $^+$N-H by $^+$N-Me in **10** would force the 3-substituent into the pharmacologically less favorable equatorial orientation **11** in order to avoid destabilizing 1,3-nonbonded interactions. It is of interest,



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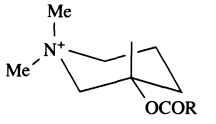


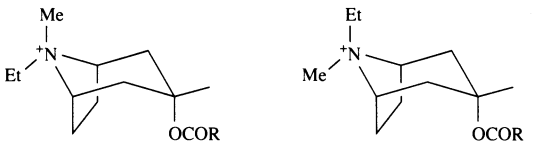
11

in this respect, that the methiodide of 1-methyl-3-piperidyl benzilate is less than one-half as active as the hydrochloride while the same two salts of the 4-piperidyl benzilate are equipotent (absence of 1,3-diaxial interactions in the latter derivatives.[16] (See, however, work on esters of 2-tropanol, page 294)

Chemistry. The stereochemistry of esters of tropine and pseudotropine is well known.[30] Several X-ray crystallographic studies have confirmed the relative orientations of nitrogen and acyloxy functions and shown that the piperidine ring of the bicyclic unit adopts a chair conformation in the solid state.[24,31–33] None of these solid state studies included assignment of absolute configuration by anomalous dispersion. The narrow dimensions of the 3-H and 1,5-H NMR signals of tropane[34] and its esters[18] show that the spin–spin couplings of these protons cannot involve large ^3J values (as would arise in boat forms)—evidence that chair conformations are also preferred for such tropanes in the solute state. In a later, high-frequency study (270 MHz) Feeney *et al.*[35] resolved the ^3J(2α-H/2β-3-H) couplings which had values of <2 and 4.4 Hz for atropine and 1.0 and 3.8 Hz for hyoscine cations in D_2O (nuclei in symmetrical positions in the tropane ring show nonequivalence,

because they experience different shielding contributions from the aromatic substituent). ^{13}C solid state findings of preferred equatorial N-Me in atropine and axial N-Me in hyoscine salts (eq—N-Me unfavored in hyoscine because of proximity to epoxide oxygen atom) have been confirmed by CP-MAS ^{13}C NMR (N-Me chemical shifts eq: 39.56 ppm, atropine, ax: 33.19, hyoscine) and also for cations in the solute state by solution NMR.[36]

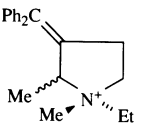
Quaternization of atropine base with ethyl iodide gives a mixture of equatorial N-ethyl and axial N-ethyl salts (**12**). In tests on guine-pig ileum the affinity of the axial N-Et isomer (85:15 ax/eq mixture) was about 10 times that of the eq N-Et isomer. Hence replacement of eq N-Me of atropine methiodide



R = $CH(CH_2OH)Ph$

12

(affinity 1.5 × ax N-Me derivative **12**) by ethyl reduces the affinity of the methiodide to a greater degree than does the same substitution of axial N-Me[36]; see also page 297. Structural assignments about nitrogen were based on ^1H-NMR evidence. Some N-ethyl–N-methyl isomers **13** related to diphemanil also differed in antiacetylcholine activity; the *cis*-Me/Et isomer was 1.95 and the *trans* 0.25 times as potent as atropine in the Magnus procedure.[38]



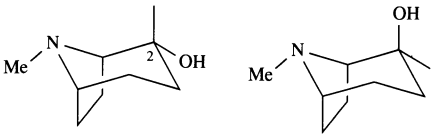
13

Barlow *et al.*[8] compared the GPI affinities of antipodal hyoscyamine hydrobromides with a series of corresponding quaternary salts. In the *S* (eutomeric) series affinity rankings were MeI > HBr > EtI > PrI > BuI. Eudismic ratios fell with increasing size of the onium group (HBr 316, MeI 87, EtI 54, PrI 17, BuI 16), hence it is apparent that binding of the eutomer is more disturbed than that of the distomer when steric demands around nitrogen increase. The same phenomenon was observed in other series of anticholinergic agents (page 296).

Barlow and Burston[39] measured the difference in free energy of binding ($\Delta G°$) of antipodal hyoscyamines by carrying out measurements at two temperatures (30°C and 37°C); the value fo -2.7 kcal/mol obtained is of a magnitude consistent with a hydrogen-bonding interaction of the $-CH_2OH$ substituent that is restricted, presumably, to the levo eutomer.

Anticholinergic esters of 2-tropanol, analogues of 1-methyl-3-piperidyl benzilate **10** (p. 292), also display a marked dependence of potency on stereo-

chemistry.[40] The α-antipode **14** and β-antipode **15** are available from cocaine,[41,42] and absolute configurations follow from the known geometry of this alkaloid.[43]

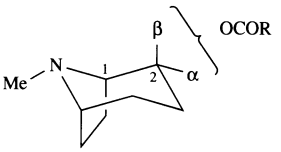


14 (α) **15** (β)
2*R*-antipodes shown

Factors governing the potency of esters in peripheral (mydriasis) and central (antitremorine) tests of anticholinergic activity (Table 9.3) were as follows:

1. The C-2 configuration: esters derived from *R*-(+)-2α-**14** were more potent than those from *S*-(−)-2α-**14** (see Table 9.3 items 1/2, 3/4),
2. the configuration of the glycollic acid chiral center: levo acids provided the eutomers in all cases (items 7/8, 3/5, 4/6),
3. in the case of (−)-2β, (+)-2α epimeric pairs, the (+)-2α ester had the higher potency (items 1/9) — in the case of a related antipodal pair [(+)-2β, (−)-2α] the β- exceeded the α-member in potency by a small factor (items 2/10). Results for the (−)-2β, (+)-2α pair do not support proposals regarding the receptor-bond conformation of the 3-piperidyl ester **10** (page 292).

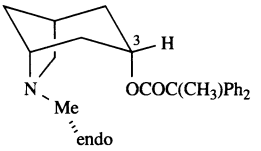
TABLE 9.3.
Anticholinergic Activities of Some Glycollic Esters of 2-Tropanol in Mice[40]



Item	2-Tropanol[a] geometry	R	Mydriasis ED_{50} (mg/kg sc)	Antitremorine ND[b] (mg/kg sc)
1.	*R*-(+)-2α	$C(OH)Ph_2$	0.05	0.03
2.	*S*-(−)-2α	$C(OH)Ph_2$	0.52	—
3.	*R*-(+)-2α	(−)-$C(OH)PhC_5H_9$	0.016	0.03
4.	*S*-(−)-2α	(−)-$C(OH)PhC_5H_9$	0.05	—
5.	*R*-(−)-2α	(+)-$C(OH)PhC_5H_9$	0.76	0.35
6.	*S*-(−)-2α	(+)-$C(OH)PhC_5H_9$	6.1	—
7.	*R*-(+)-2α	(−)-$C(OH)PhC_6H_{11}$	0.07	0.03
8.	*S*-(+)-2α	(+)-$C(OH)PhC_6H_{11}$	1.1	0.77
9.	*R*-(−)-2β	$C(OH)Ph_2$	1.4	1.75
10.	*S*-(+)−2β	$C(OH)Ph_2$	0.33	—
11.	Atropine		0.061	2.2

[a] Configuration at C-1 given.
[b] Normalizing dose.

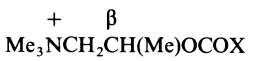
Azaprophen **16**, a 6-aza analogue of atropine, was 50 times more potent than the natural alkaloid vs. ACh in guinea-pig ileum and 1000 times vs. carbachol-induced release of α-amylase from pancreatic acini cells.[44] The α-stereochemistry at C-3 was established from the ^{1}H-NMR features of the 3-H resonance (5.35 ppm narrow m, $W_{1/2}$ 11.8 Hz). In addition, azaprophen displaced [^{3}H]*N*-methylhyoscine from binding sites in human IMR-30 neuroblastoma cells 27 times more effectively than atropine. Modeling studies showed that azaprofen does not fit the distance geometry pharmacophore constraints normally accepted for the ACh receptor. There is X-ray crystallographic and NMR evidence that azaprophen HCl prefers an *endo* N-methyl conformation.[45]



16

9.3. Choline Derivatives

A variety of cholinergic agonists provide muscarinic antagonists when suitably modified, and studies of agonists and antagonists based on the same molecular framework provide evidence about whether such ligands occupy common receptors.[46] Esters derived from choline and β-methylcholine and aromatic acids of the type encountered in tropyl and piperidyl spasmolytics (see above) form the first group of examples. It was found that while the cholinergic properties of β-methyl ACh (**17**, X = Me) depend greatly on the configuration of the β-center, enantiomers of analogues with antagonist properties show only small differences in potency with the *R*-form the more active [see **17**, X = Ph_2CH and $Ph_2C(OH)$].[47]



17

X	Affinity ratio (*S*/*R*)*
Me	320
Ph_2CH	0.2
$Ph_2C(OH)$	0.8

* *vs.* furtrethonium, rat jejunum.

However, in the case of choline esters with atropine-like properties that have a chiral center in the acyloxy moiety, marked degrees of stereoselectivity are again observed (**18**, and legend) dependent on the nature of the acyloxy moiety. Barlow *et al.*[8] has also provided evidence on this point by measuring the GPI affinity constants of choline esters of mandelic, cyclohexylphenylacetic, α-methyltropic, and hexahydrobenzilic acids; eudismic ratios were high for esters of the last two acids

and low for the first two. The evident stereochemical dominance of the acid rather than the choline portion of the molecule is further emphasized by results obtained with diastereoisomeric analogues. In α-methyltropate esters of β-methyl choline, isomers derived from the levo acid were far more potent anticholinergics than the dextro enantiomers; it is also clear from the results (see **19**) that the β-methylcholine configuration has a much smaller influence on activity.[47] Results of the same kind were obtained for esters of α-methylcholine with the chiral acid, hexahydrobenzilic acid.[48] Both *R*- (acid), *R*- (choline), and *RS*-diastereoisomeric methiodides had log affinity constants near 10. In these examples the α-methyl substituent elevated the affinity of the corresponding choline ester 2.5-fold (log *K* 9.66)—in agonists the same group depresses affinity (see p. 231). The small affinity ratio (5) found for the potent spasmolytic esters of choline and phenyl-2-thienylglycollic acid (**20**) was attributed to the likelihood of phenyl and its isostere 2-thienyl being poorly differentiated at the receptor.[47]

$Me_3N^+CH_2CH_2OCOX$

18

X	Affinity ratio (−)/(+)
$Ph(Me)C(CH_2OH)$	300[a], (115)[b]
$Ph(C_6H_{11})CH$	3[b]
$Ph(C_6H_{11})C(OH)$	100[a], 246[b], 191[c]

[a] Ref. 47. [b] Ref. 8. [c] Ref. 48.

$Me_3\overset{+}{N}CH_2\overset{*}{C}H(Me)OCO\overset{*}{C}(Me)Ph(CH_2OH)$

19

Configuration		
Parent acid	Parent choline	Relative activity* (atropine = 100)
(−)	*R*	0.25 (125)
(−)	*S*	1.3 (30)
(+)	*R*	0.008 (1.3)
(+)	*S*	0.013 (0.8)

* Values in parentheses refer to esters of hexahydrobenzilic acid, $Ph(C_6H_{11})C(OH)CO_2H$.[47]

$Me_3\overset{+}{N}CH_2CH_2OCO\overset{*}{C}(OH)Ph.(2\text{-}C_4H_3S)$ (−)/(+) = 5

20

The influence of the size of the onium group on the eudismic ratio is also illustrated by data on esters of 2-dimethylaminoethanol.[8] Thus among the hexahydrobenzilates (**21**), eudismic ratios fell sharply as $^+NMe_3$ was progressively

increased to $^{+}NEt_3$—due in two cases to a *rise* in the affinity of the distomer, results at variance with data on hyoscyamine salts (p. 293).

$$Ph(C_6H_{11})(OH)\overset{*}{C}CO_2CH_2CH_2X$$

21

X	Eudismic ratio	log *K* (GPI)	
		R	*S*
$^{+}NMe_2H$	78	8.87	6.98
$^{+}NMe_3$[a]	246	9.87	7.26
$^{+}NMe_2Et$	144	10.04	7.88
$^{+}NEt_2Me$	71	10.00	8.15
$^{+}NEt_3$	41	9.60	7.99

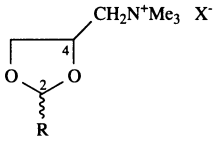
[a] Lower limit of optical purity 99.36%.

9.4. Dioxolane and 1,3-Oxathiolane Derivatives

The next group concerns analogues of the potent 1,3-dioxolane agonist **22** (R = Me) (page 240).[49,50] Potencies of the four isomeric forms of **22** (R = Me) as ACh agonists in the guinea-pig ileum test follow the order:

cis-2*S*,4*R*	*trans*-2*R*,4*R*	*trans*-2*S*,4*S*	*cis*-2*R*,4*S*
(500)	(50)	(25)	(8.5)

(activities relative to ACh = 100 are given in parentheses beneath each isomer).[51] Hence the geometry of both chiral centers influences activity, but that of C-4 (linked to CH_2N^{+}) is of greater weight. If the 2-methyl group of **22** (R = Me) is



22

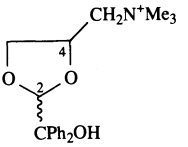
equivalent to acetyl methyl of ACh (or β-methylACh), replacement of hydrogens at this carbon center by two bulky substituents and a hydroxyl group should lead to

TABLE 9.4.
Affinity Constants (log *K*) for the Dioxolane Isomers (24) from Tests on Guinea-Pig Ileum[50]

Configuration benzylic center	4*R*,2*S* (*cis*)	4*S*,2*S* (*trans*)	4*R*,2*R* (*trans*)	4*S*,2*R* (*cis*)
R	11.09, 11.08 (8.98, 8.89)[a]	9.37, 9.33 (8.36, 8.41)	7.60, 7.53 (7.35, 7.40, 7.25)	7.28, 7.41 (7.07, 7.06)
S	not tested (< 6.5)	not tested (< 7)	6.77, 6.69 (6.24, 6.28)	6.65, 6.52 (6.56)

[a] Values in parentheses refer to the 4-CH_2NMe_2 HCl salts.

an antagonist. Such was in fact the case for the analogues **23** which had affinity constants remarkably close to that of the ACh analogue, benziloyl choline (log K 8.5).[52] The *cis- and trans*-isomers **23** differed little in activity, while the *cis*-agonist

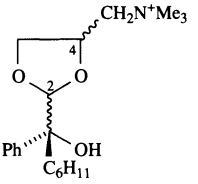


23

	log K (GPI)
rac-cis	8.5 (6.7)[a]
rac-trans	8.35 (6.65)

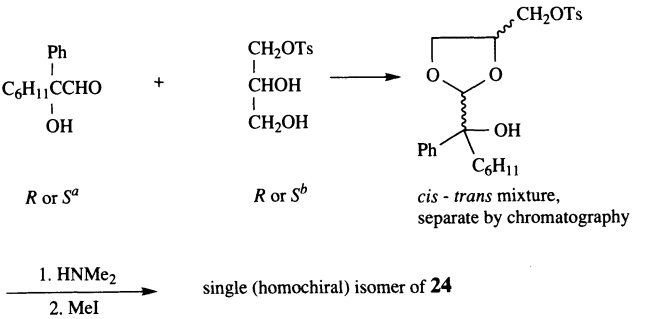
[a] Values of NMe_2·HCl salts.

22 (R = Me) is five times as active as the *trans*-form when both are tested as racemic mixtures.[53] Work on analogues with a chiral C-2 substituent followed; all 8 isomers of the 2-(1-cyclohexyl-1-hydroxy-1-phenyl)methyldioxolane **24** (Table 9.4)



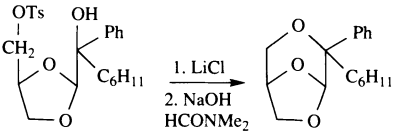
24

were isolated and examined. Reactions employed are summarized in Scheme 9.2; *cis*-dioxolanes were distinguished from the *trans*-forms by their ready conversion into bicyclo[3.2.1]octanes in the sequence **25** to **26**. Affinity constant rankings for the isomers **24** in the guinea-pig ileum test are shown in Table 9.4. From these data



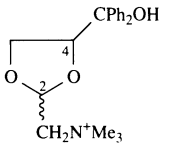
[a] See Scheme 9.3 for source.
[b] *R* from D-mannitol (Ref. 53 of Chapter 8); *S* (Ref. 48 of Chapter 8).

Scheme 9.2. Synthesis of set of 8 dioxolane stereoisomers **24**.[50]



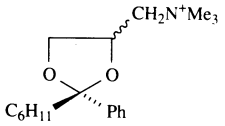
25 **26**

it follows that the configurational priorities for activity are in the order: (1) an *R*-benzylic center, (2) a 2*S*-center (adjacent to the benzylic carbon), and (3) a 4*R*-center (adjacent to CH_2N^+). The steric arrangement of the benzylic center is thus of paramount importance while, unlike the situation in the agonists (**22**, R = Me), the C-2 configuration is of greater importance than that of C-4. Evidence that the dioxolane ring contributes to the affinity of the molecule at the receptor and does not act merely as a "spacer" between the C-2 and C-4 substituents was provided by the significantly lower potency of the 2-$CH_2{}^+NMe_3$ derivatives **27**



27

compared with the 4-$CH_2\overset{+}{N}Me_3$ isomers (tertiary amine salts in the two series differed little in activity—all had log *K* values near 6.5).



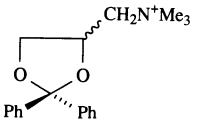
28

	cis-2*S*,4*R*	*trans*-2*S*,4*S*	*cis*-2*R*,4*S*	*trans*-2*R*,4*R*
log *K*	8.73	8.56	8.34	8.24
pA_2	8.26	8.03	7.83	8.73

guinea-pig ileum, *cis* and *trans* refer to Ph and $C\overset{+}{N}_2NMe_3$

In another series of anticholinergic dioxolanes, both 2-H and 2-methyl of **22** (R = Me) were replaced by bulky groups.[49,54] The correlation with benzilates of choline, etc. is not as close here, but some potent atropine-like agents resulted; the data on stereoisomers supplemented conclusions about steric influences already outlined. Thus results with the 2-cyclohexyl-2-phenyl derivatives **28** reveal the C-2 configuration has the major influence (2*S* for highest activity) but activity differences between the two weaker members of the group are too small to draw stereochemical conclusions. The C-4 geometry certainly has little influence in the 2,2-diphenyl enantiomers **29** as judged from pA_2 values [GPI, *RS* 7.66, (−) 7.55,

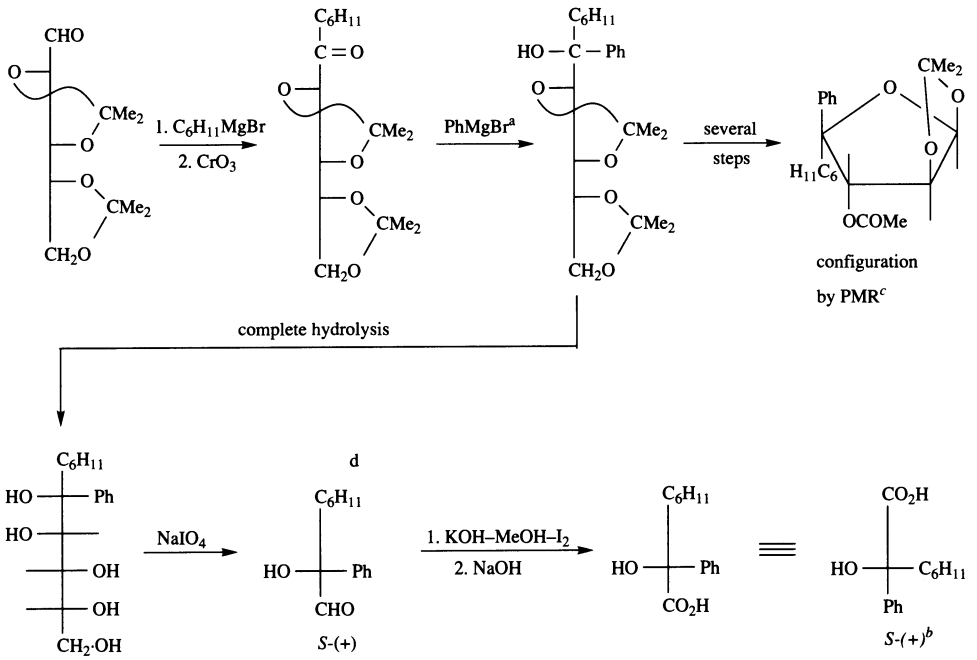
(+) 7.62].[54] Anticholinergic activity fell sharply when one of the 2-phenyl groups of **29** was absent and the *rac-cis-* and *trans*-forms were almost equipotent with log *K* values about 4.5.



29

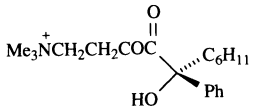
In this series the synthesis of antipodal pairs of known configuration involved use of *R-* and *S*-tosyloxyglycerol, as employed in the work on related agonists (page 242). The relative configurations of 2 and 4 substituents were based on NMR analyses[55] (see also Scheme 9.3).

For maximum activity an *R*-configurated benzylic center is required for the choline ester **30**[48] and an *S-* for the dioxolane **31**, but if 0-1 of the latter is regarded as equivalent to OH of **30**[49] it can be seen that the groups Ph, C_6H_{11}, and oxygen have the same arrangement about the benzylic carbon in both eutomers. Another



[a] Addition of the Grignard reagent is highly selective and the trace of minor isomer is readily removed.
[b] The *R*-(–) acid is made by reversing the sequence of the two Grignard reactions.
[c] Key signals: CMe_2 δ 0.44, 1.07; COMe δ 2.16. In isomer CMe_2 signal is lower field (δ 1.31, 1.61) and COMe higher field (δ 1.75) since acetate methyl is now shielded by the aromatic group while the isopropylidene methyls are not.
[d] Used to make enantiomeric forms of the dioxolane of Scheme 9.2.

Scheme 9.3. Synthesis of *R-* and *S*-hexahydrobenzilic acid from arabinose[56] (after Casy Ref. 11 of Chapter 8).



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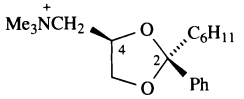
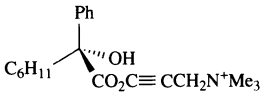


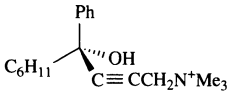
31

anticholinergic agent containing a -CPh(C_6H_{11})OH chiral center is the acetylene derivative **31a**, where again the eutomer has an *R*-benzylic center.[56] These *t*-amino acetylenes are unusual in being significantly more potent than corresponding methiodides. In contrast, enantiomers of the related derivatives **32** showed no configurational dependence.



31a

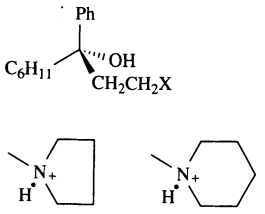
	log *K*
RS	8.96 (8.10 for Me I)
R	9.18 (8.34 for Me I)



32

	log *K*	
	HCl	Me I
RS	7.32	8.11
S	7.30	8.11
R	7.26	7.98

Large potency differences have been found between antipodal forms of the derivatives **33** (see later) which also contain a chiral cyclohexylphenylcarbinol unit of known configuration.



33

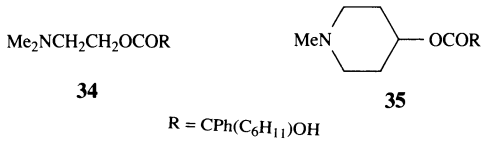
Hexahydrobenzilates feature prominently in the work of the Porton group since the *R* and *S* acids could be obtained in high states of optical purity from sugar precursors (Scheme 9.3). The data of Table 9.5 refer to esters of choline **34**

TABLE 9.5.
***R*:*S* Enantiomeric Potency Ratios for Some Hexahydrobenzilate Esters[48]**

Compound[a]	Form	*In vitro* test: Affinity constant GPI	*In vivo* tests: Mydriasis in mice	*In vivo* tests: Antagonism of oxo-T in mice[b]
34 (choline)	HCl	100	123	> 100 (> 22)
34 (choline)	MeI	200	38	147
35 (4-piperidinol)	HCl	272	20	43 (38)
35 (4-piperidinol)	MeI	100	2.3	17.6

[a] *R*-Eutomer in all cases; amino alcohol in parentheses.
[b] Oxo-T:oxotremorine; salivation (tremor); drugs injected ip 15 min before injection into tail vein of 100 μg/kg oxotremorine. ED_{50} for block of salivation and of tremors determined.

and 1-methyl-4-piperidinol **35**. Both *in vitro* and *in vivo* differences in potency ratios for **34** HCl were similar, from which it was concluded that potency differences were chiefly due to receptor events, and that the anti-ACh receptors in guinea-pig ileum, mouse eye, and mouse salivary gland are essentially identical (cf. Barlow *et al.*).[58] Potency ratio agreement was less satisfactory for methiodides of **34** where, in the case of the 4-piperidyl esters **35**, the *in vivo* ratios were much lower than the *in vitro* values.



34 **35**

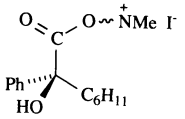
R = $CPh(C_6H_{11})OH$

The divergence between *in vivo* and *in vitro* ratios becomes greater the higher the affinity constant of the *R*-configurated ester: R-**34** HCl (log *K* 9.06); *R*-**34** Me I (9.66). *R*-**35** HCl (10.92); *R*-**35** MeI (11.08). Furthermore, although the *in vitro* potency of *R*-**35** Me I is greater than that of *R*-**34** Me I, the *in vivo* activities of the two salts are equivalent. From scuh comparisons it was proposed that there is a minimum dosage below which maximum anticholinergic effects cannot be obtained *in vivo*, and that the potencies of *R*-**34** and *R*-**35** methiodides approach this limit. The reason for a minimum dosage lies most probably in losses inherent in the transport of the drug to its site of action. With exceptionally potent agents such as **35** (piperidyl) the advantages of a high affinity for the receptor are offset by the need to administer enough drug to allow for its pharmacokinetic profile. The time–activity features of potent anticholinergics were also discussed by this group.[59]

The authors concluded that cental muscarinic receptors blocked by antiacetylcholine drugs are essentially the same as corresponding peripheral receptors (eye, salivary gland of mouse, and guinea-pig ileum); compare, however, the results of binding experiments carried out subsequently (pages 306 and 309).

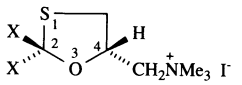
Although antimuscarinic receptors clearly differentiate between the *R*- and *S*-hexahydrobenzilate moiety, it is of interest that the less active *S*-isomer of **35**

(piperidyl) methiodide (log K 9.08) has an affinity close to that of atropine (pA_2 8.4–9.0).[8,16,60,61] Hence even the binding contribution of two of the three substituents attached to the benzylic carbon of the ester (**35a**) must add significantly to the affinity of the molecule for its receptor.



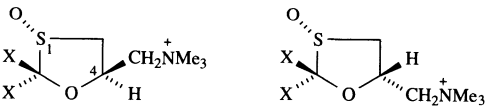
35a

Cholinergic antagonists based on the 1,3-*oxathiolane* nucleus have also been reported and stereochemical comparisons made with related 5-ring agonists and antagonists.[62] The racemic sulfide **36** was about 100-fold less potent than atropine



36
(X = C_6H_{11}, also for **37** and **38**)

in blocking guinea-pig ileal and heart and rat bladder muscarinic receptors and its antipodes were equiactive (chiral center too far removed from the hydrophobic feature at C-2; cf. low antipodal affinity ratios of diphenylacetyl and benzilyl esters of β-methylcholine, page 295). Modest degrees of stereoselectivity were observed when a chiral center was created at position 1 by sulfoxidation. Of the four isomeric sulfoxides, the two eutomers had a 1*R*-configuration in accord with the stereospecificities of related agonists (page 246) (see **37**).[63] Eudismic ratios recorded for the *trans* (1-S-O, 4-CH_2N^+) antipodes on guinea-pig heart were 17 (force) and 18 (rate), i.e., about twice those found at ileal and bladder sites.



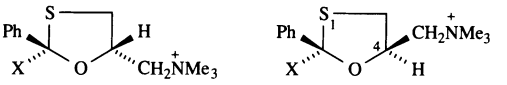
37 (eutomers shown)

	pA_2 (GPI)	ER[a]		pA_2 (GPI)	ER
1*R*,4*R*	6.69	8	1*R*,4*S*	6.32	3
1*S*,4*S*	5.81		1*S*,4*R*	5.92	

[a] ER = eudismic ratio vs. carbachol; applies also to **38** data.

Potency levels of the order of atropine were achieved when one of the 2-cyclohexyl substituents of **36** was replaced by phenyl[64] but eudismic ratios remained low in spite of the presence of a chiral center within the hydrophobic substituent at C-2 (see **38**). The order of potency of the four stereoisomers on the guinea-pig ileum (2*R*,4*R* > 2*R*,4*S* > 2*S*,4*R* ≫ 2*S*,4*S*) is essentially that found by Brimblecombe[49] and Triggle[54] for corresponding 1,3-dioxolanes. The authors

argue that the prevalence of chirality at C-2 over that at C-4 indicates that, for antagonists, hydrophobic interactions are more important that electrostatic interactions and that the reverse is true for agonists. Others contest that the differing influences of chirality among the two types of ligand point to their interacting at different receptors.



38

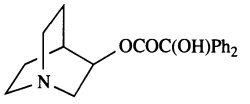
	pA_2 (GPI)	ER		pA_2 (GPI)	ER
2*R*,4*S*	8.57	5	2*R*,4*R*	8.79	9
2S,4*R*	7.86		2*S*,4*S*	7.86	

A eudismic analysis of 1,3-oxathiolane-based agonists and antagonists has recently been presented by Lehmann.[65] Conclusions reached were: (1) stereoselectivity increases with affinity for all enantiomers and epimers investigated, and (2) the contributions to the overall eudismic index of individual epimeric inversions, although not constant, are additive and allow it to be calculated. The greater dependence of ligand affinity on the chirality of centers 1 and 2 compared with that of center 4 (as seen from plots of epimeric eudismic indexes vs. the negative log of eutomer dissociation constant) was explained by the conformational rigidity of the first two and the greater conformational freedom of the substituent at center 4.

Chemical methodology followed that employed for related agonists (page 245) and included determination of the absolute configuration of (+)-*cis*-eutomer by X-ray analysis of its corresponding (+)-di-*p*-toluoyltartaric acid salt.

9.5. Quinuclidines

Analogues of the ACh agonist 3-acetoxyquinuclidine (aceclidine) form the next group of examples. The benzilate **39** (QNB) is well known as a potent M-AChR antagonist. In tests on antipodal forms (obtained from *R*- and *S*-3-quinuclidinol resolved with 4-chlorotartranilic acids), Lambrecht[66] observed the potency order *R*-Me I > *S*-Me I > *S*-HCl with methiodide eudismic ratios of 9.6 (GPI vs. ACh) and 6.5 (GP atrium vs. carbachol). Receptor affinities were high for both



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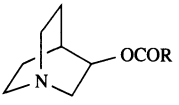
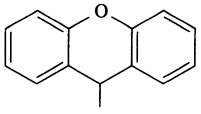


(a) R = $C(OH)PhC_6H_{11}$;

(b) R =  (xanthate);

(c) R = $C(OH)(CH_3)Ph$ (atrolactate)

40

quaternary salts (GPI pA_2: *R* 9.73, *S* 8.75). In related agonists (page 270) the *R*-methiodide is likewise the eutomer but with a much lower affinity than the corresponding base HCl. In binding studies,[67] relatively low eudismic ratios were observed (comparable to those of the functional tests) for both QNB (**39**) and QNB methiodide antipodes (Fig. 9.1). N-Methylation of QNB decreased the affinity of the *R*-antipode but raised that of the *S*-form; as a result the methiodides bound with an even lower eudismic ratio than the protonated bases at all receptor subtypes studied. The authors suggest that the $^+$NH center of *R*-QNB is very close to the anionic site of the receptor so that a very strong ionic bond is formed—so strong, in fact, as to produce a high energy barrier to dissociation (in functional tests *R*-QNB behaved as an alkylating mustard and its affinity constant could not be measured).

When *S*-QNB recognizes muscarinic receptors, its $^+$NH group is further removed from the anionic site due to steric hindrance. Thus its affinity is lower than that of the *R*-antipode but room is available for accommodation of an N-methyl substituent which raises the affinity of the methiodide ligand above that of the protonated base. Kinetic constants for QNB and its methiodide at cardiac M_2 sites support these ideas: K_{off} (min^{-1}) values were: *R*-QNB 0.0092, *S*-QNB 0.60, *R*-QNB MeI 0.11, *S*-QNB MeI 0.25.[67] Radiolabeled analogues of *R*-QNB have been reported.[137]

Inch *et al.*[59] reported the *RR*-isomer of (**40**a) and its methiodide to be the most potent muscarinic antagonists of an extensive group (vs. carbachol GPI: log *K* 11.67, 11.28 MeI; mydriasis × atropine: 3.11, 3.37 MeI).

An American group[68] have compared the actions of esters of *R*- and *S*-3-quinuclidinol formed from two achiral (benzilic acid and xanthene-9-carboxylate) and one chiral acid (atrolactic) (**40**a–c and Table 9.6). The object of the work was

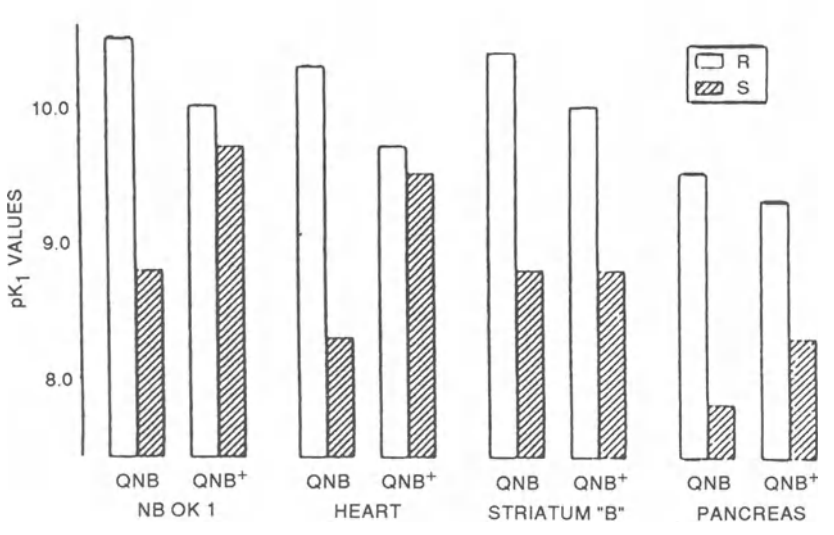


FIGURE 9.1. Comparison of apparent pK_i values of *R*- and *S*-QNB and of *R*- and *S*-QNB methiodide (QNB^+), obtained after 4 h incubation with [^{3}H]NMS at 25° C, in NB–OK1 neuroblastoma cell line (M_1 sites), heart (M_2 sites), striatum "B" (M_3 sites), or pancreas (M_3 sites) homogenates (after Waelbroeck *et al.*).[67]

TABLE 9.6.
Receptor Binding and Functional Studies of Optical Isomers of Some Quinuclidinol Derivatives[68]

	Binding studies: K_b (nM)			Functional studies: K_i (nM)		
Compound[a]	[^{3}H]pirenzepine (M_1) bovine striatum	[^{3}H]QNB (M_2) rat myocardium	M_2/M_1	PI[b] turnover rat cortex (M_1)	Inhibition of AC[c] rat heart (M_2)	M_2/M_1
R-**40**a	0.19	0.23	1.2	8.4	2.2	0.3
S-**40**a	0.57	5.83	10.2	15.4	123	8.0
R-**40**b	0.2	0.39	1.9	0.9	6.9	7.7
S-**40**b	5.16	74	14	27	1660	61
Ra,*Rb*-**40**c	0.29	6.9	23.8	0.8	153	190
Ra,*Sb*-**40**c	2.9	37	13	4.3	1460	340
Sa,*Rb*-**40**c	19	934	49	11.3	26,700	2362
Sa,*Sb*-**40**c	156	1372	8.8	87.5	13,700	157
Atropine	0.2	0.7	3.5	0.5	7.5	12
Pirenzepine	3.6	377	105	8.1	18,300	2316

[a] *Ra* (*Sa*) refers to 3-quinuclidinol, and *Rb* (*Sb*) to benzylic chiral center of the atrolactate.
[b] Phosphatidyl inositol.
[c] Adenylate cyclase.

to detect selectivities for ACh-M subsites as judged from binding and functional studies. Binding affinities at M_1 sites were established by measuring the abilities of test compounds to displace [^{3}H]pirenzepine (page 319) from bovine striatal membranes (an M_1-rich tissue); M_2-affinities were obtained using rat myocardial membranes (M_2-rich) with nonselective [^{3}H]QNB as the radioligand. Functional tests were based on phosphatidyl inositol (PI) turnover and inhibition of adenylate cyclase as measures of M_1 and M_2 blockade, respectively. In these procedures the ability of antagonists to reverse carbachol-stimulated PI hydrolysis[69] and oxotremorine-induced inhibition of adenylate cyclase[70,71] were measured employing the radiolabeled agents myo-2-[^{3}H]inositol and [^{3}H]cAMP, respectively (M_1-agonists stimulate PI hydrolysis while M_2-agonists depress cAMP levels). Findings were as follows:

1. In all cases esters formed from *R*-3-quinuclidinol exceeded the affinity of *S*-esters for M_1 and M_2 sites. Selectivity was low. The eudismic ratio for the benzilates was low (3) and moderate for the xanthates (26) at M_1 sites.
2. All the atrolactates showed selectivity for M_1 sites (M_2/M_1 8.8–23.8) and affinities were governed by the benzylic followed by the quinuclidinol configuration. The *RR*/*SS* eudismic ratio was high at both M_1 (538) and M_2 (199) sites, while *RR*/*RS* ratios were modest (M_1 10, M_2 5.4).

Similar steric relationships were observed in the functional tests. As in the binding results, the SaRb diastereoisomer (which ranked third in potency among the four) displayed the greatest selectivity for M_1 sites. The esters were prepared by transesterification of *R*- and *S*-3-quinuclidinol with the appropriate ethyl esters. The optical purity of ethyl esters of *R*- and *S*-atrolactic acid was established by chiral HPLC on a Pirkle column (see Eliel[72] for evidence of absolute configuration).

9.6. Procyclidine, Benzhexol, and Miscellaneous Anticholinergics

A large number of chiral muscarinic antagonists with molecules composed of a bulkyl benzylic function linked by a short chain to nitrogen and which have no agonist counterpart have been designed. Some compounds of the type have found clinical use as spasmolytics and mydratics (*Martindale* **29**, pages 527, 542). The two compounds procyclidine **33**a and benzhexol (trihexylphenidyl) **33**b contain a chiral cyclohexylphenyl carbinol unit related to that of the hexahydrobenzilates already discussed. Optically active forms of these compounds obtained by resolution with (+)-tartaric acid[73] had eudismic ratios of 49 for the pyrrolidine and 9.8 for the piperidine derivatives (levo isomers were more potent). Barlow,[74] using the same samples, confirmed that the **33**a pair had the higher ratio but found differences to be more extreme (375 for **33**a, 5.5 for **33**b). From a relationship between the degree of resolution and observed stereospecific index (S^*, eudismic ratio) previously derived (Fig. 9.2)[75] a value of $S^* = 375$ shows that the pyrrolidino pair must be almost optically pure. The low value found for the piperidine derivatives proved to

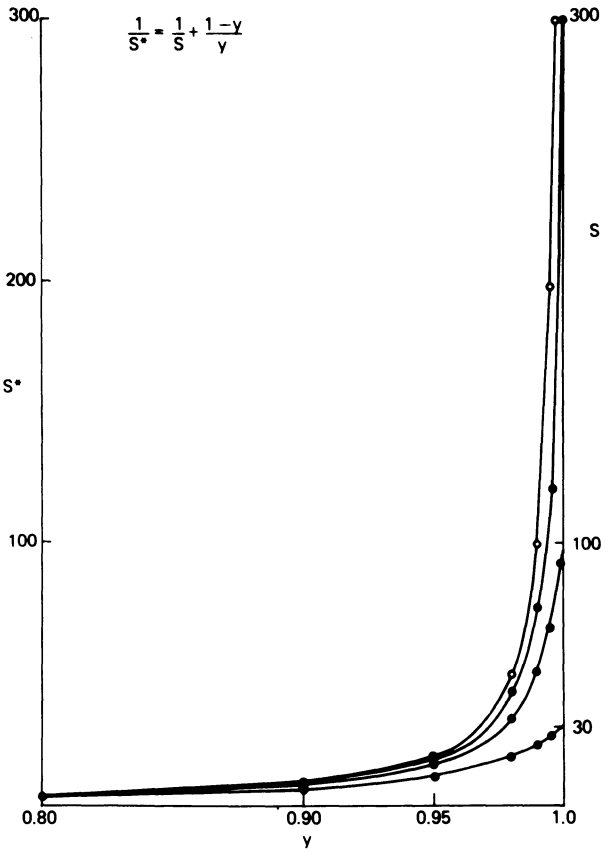
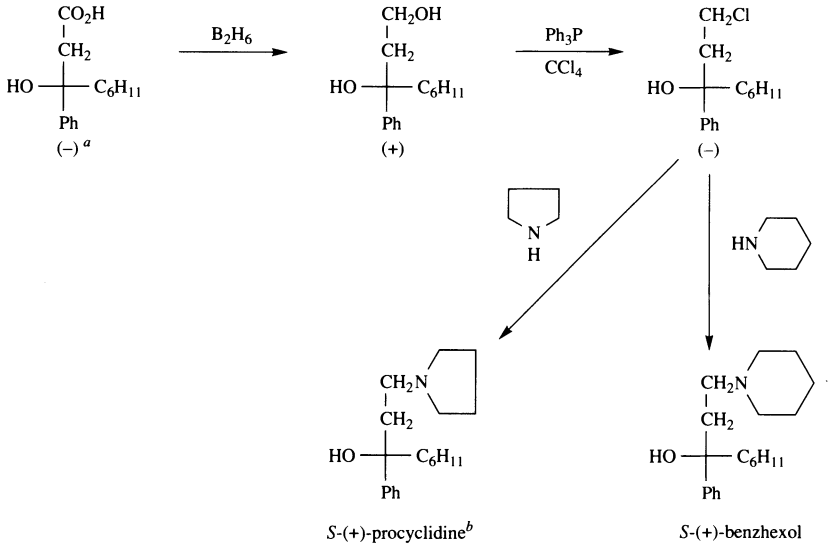


FIGURE 9.2. The effect of the degree of resolution (y) on the observed stereospecific index (S^*). S^* is plotted against y for true values of the stereospecific index of 30, 100, 300, and infinity (open circles). Values have been calculated on the assumption that both enantiomers are resolved to the same extent (y) (after Barlow *et al.*).[75]



[a] *RS*-acid resolved with 1-phenylethylamine.
[b] *R*-configuration of the levo isomer established by X-ray crystallograhy.

Scheme 9.4. Configurational correlation of dextro antipodes of procyclidine and benzhexol. [77]

result from incomplete resolution, since examination of antipodes of benzhexol resolved with *N*-benzoyl-D-threonine[2] gave a eudismic ratio of over 1000. Recent data of Tacke *et al.*[76] relate to antipodes of procyclidine HCl and its methiodide (tricyclamol iodide). Eudismic ratios of 385 and 34 were recorded for the HCls at guinea-pig ileal and atrial sites respectively (*vs* carbachol), while corresponding values for the methiodides were 89 and 48. The more potent levo isomers (*R*-configuration, see below) showed 10-fold (HCl) and 3-fold (Me I) preferences for ileal sites.

The absolute configurations of antipodes of **33**a and **33**b are now known. That of levo procyclidine is *R* from X-ray crystallography carried out on the methiodide,[76] while the sequence of Scheme 9.4 correlates the dextro antipodes of **33**a and **33**b.[77]

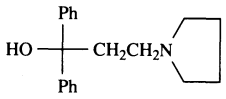
TABLE 9.7.
Affinities of Antipodes of Benzhexol HCl and MeI at M_1, and $M_{2\alpha}$, $M_{2\beta}$ Muscarinic Sites[79]

	pA_2 value		
	RVD (M_1)[a]	GPA ($M_{2\alpha}$)[b]	GPI ($M_{2\beta}$)
Pirenzepine	8.24	6.82	6.88
R-**33**b HCl	10.11	8.15	8.68
S-**33**b HCl	6.88	6.32	6.03
R-**33**b MeI	10.61	8.96	9.21
S-**33**b MeI	7.79	7.05	7.06

[a] Rabbit vas deferens.
[b] Guinea-pig atrium.

Reports that *rac*-benzhexol was a M_1-selective antagonist[78] prompted Lambrecht *et al.*[79] to examine the affinities of antipodes **33b** at M_1 (field-stimulated rabbit vas deferens), $M_{2\alpha}$ (GP atrium), and $M_{2\beta}$ (GPI) sites. The results of Table 9.7 confirm M_1-selectivity, especially notable for the *R*-eutomers ($M_1 > M_{2\alpha} \geqslant M_{2\beta}$) which were greater than that of pirenzepine. Methiodides exceeded the affinities of hydrochlonde salts, and eudismic ratios at M_1 sites were 660 for the former and 1700 for the latter salts.

To understand the forces responsible for the discrimination of procyclidine antipodes by muscarinic receptors, binding data on the diphenyl (**41**, pyrrinol) and dicyclohexyl (**42**, hexahydroprocyclidine) analogues were acquired (Table 9.8).[67]



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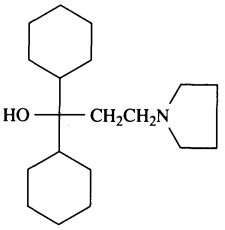

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The affinity of *R*-procyclidine was reduced about tenfold when its cyclohexyl substituent was replaced by phenyl, and *vice versa*. Both changes take place when *R*-procyclidine is converted (formally) to its *S*-antipode, and the pK_1 and pA_2 values recorded ($\sim$100-fold drop over those of *R*-**33a**) reflect the additive effect of the two changes. Hence it may be concluded *S*-procyclidine maintains the 3-point attachment of the corresponding eutomer in spite of the fact that interactions involving the hydrocarbon groups must be weaker than those of the *R*-ligand–receptor complex. Structures of Fig. 9.3 illustrate this point. Similar studies have been made in regard to hexahydrodifenidol (**45**, see later).[133]

Sila-analogues of procyclidine (HCl and MeI **43**) had somewhat higher affinities for guinea-pig atrial and ileal sites than the parents.[80] Racemic **43**, prepared in five steps from dichlorophenylvinylsilane, was resolved with tartaric

TABLE 9.8.
Potency of Procyclidine Enantiomers, Pyrrinol, and Hexahydroprocyclidine at M_3 Receptors[67]

Antagonist[a]	pK_i values for rat striatal "B" M_3 sites	pA_2 values for GPI M_3 sites
R-Procyclidine	8.1	8.04
Pyrrinol (**41**)	7.2 (0.9)	6.9 (1.13)
Hexahydroprocyclidine (**42**)	7.0 (1.1)	6.37 (1.67)
S-Procyclidine	6.0	5.46
Expected eudismic index[b]	(2.0)	(2.80)

[a] Measured at striatal M_3 receptors by binding studies, and at ileal M_3 receptors by functional studies. Values in parentheses are the differences between the drug pK_i or pA_2 values and the *R*-procyclidine pK_i or pA_2 values.
[b] Corresponds to the sum of the values in parentheses.

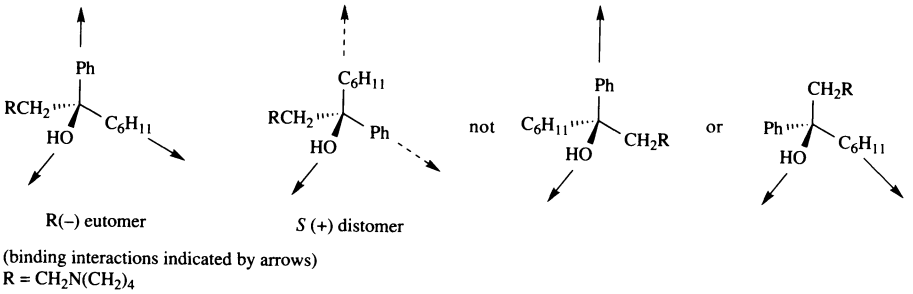
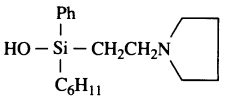


FIGURE 9.3. 3-Point attachment of the chiral moiety of procyclidine antipodes.

acid as usual[81] and the absolute configuration of the levo isomer shown to be *R* by X-ray crystallography.[82] Affinity data shown in Table 9.9 reveal the preference (13–2.1-fold range) of all isomers for ileal over atrial sites. Of special note, however, are the eudismic ratios—these are markedly lower than values found for the procyclidines. Lambrecht and Mutschler[84] argue this to be a consequence of the chiral center's OH forming a stronger H-bond with a receptor acceptor site when attached to silicon than when linked to carbon (acidity Si-OH > C-OH)—a fact which may also account for the greater affinities of the sila derivatives. In *R*-isomers (eutomers) a three-point attachment to the receptor is postulated as for procyclidine. In distomers only a two-point link is possible—however, because of the greater strength of the H-bonding interaction, this association together with that of either of the two carbon rings is sufficient to produce reasonable levels of affinity with the result that binding differences between eutomers and distomers fall (Fig. 9.4).



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Antipodes of the sila-analogues **43** are optically stable in the solid state but slowly racemize in aqueous solution.[85] This factor may contribute to the lower values of the eudismic ratio, although the fact that the value for methiodides (which racemize faster than HCls) is higher than the HCl values counts against this possibility.

TABLE 9.9.
pA_2 Values of Sila-Analogues of Antipodal Procyclidines (43)[83]

Form	Configuration	pA_2 GP ileum	pA_2 GP atrium	Eudismic ratio[a]
HCl	*R*	8.26	7.15	4 (2)
HCl	*S*	7.65	6.90	
MeI	*R*	8.72	8.37	21 (23)
MeI	*S*	7.36	7.04	

[a] Ileum (atrium).

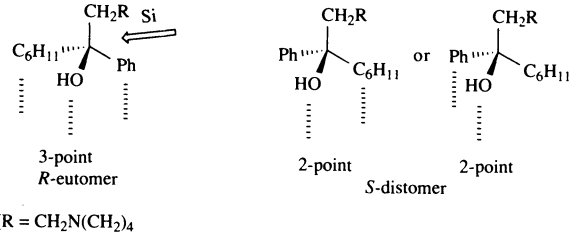
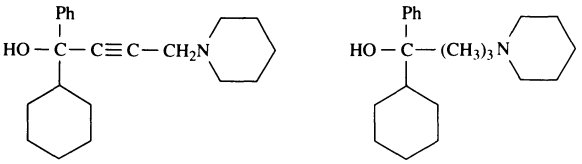


FIGURE 9.4. Different modes of binding of antipodes of sila-procyclidine.[84]

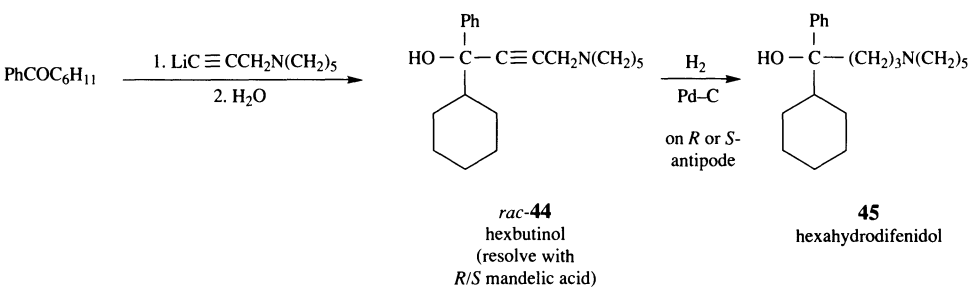
This work has been extended to analogues of benzhexol in which nitrogen and the quarternary carbon are linked by a three-carbon chain. Antipodes of the alkane (**45**, hexahydrodifenidol) and alkyne (**44**, hexbutinol) were obtained as shown in Scheme 9.5.[86] The absolute configuration of one of the isomers of **44** (*S*) was established by X-ray crystallography of its *R*-mandelate. The optical purities of the hexbutinol isomers (99.7%) were established by calorimetry (ofus/°C *R* 126.5, *S* 126.5, *RS* 134.4). Pharmacological data are presented in Table 9.10 in relation to three receptor subtypes. In all cases the affinities of the *R*-antipodes exceeded those of the *S*-forms. Eutomers of **44** and **45** showed a preference for M_1 and M_3 sites



44 **45**

over the M_2 atrial species. Both antipodes of *p*-fluorohexbutinol, however, showed a specificity for M_3 (ileal) sites. Without exception the ranking of eudismic ratios was $M_1 > M_3 > M_2$, an observation which may have value for subtype classification (e.g., for *R*- and *S*-hexahydrosiladifenidol: M_1 550, M_3 191, M_2 17). Hexahydrosiladifenidol and *p*-fluorohexahydrosiladifenidol are listed as M_3-selective antagonists in the *TiPS* January 1991 receptor supplement.

In a paper describing the SAR of a variety of hexahydrodifenidol



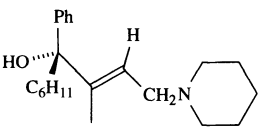
Scheme 9.5 Synthesis of hexbutinol and hexahydrodifenidol.[86]

TABLE 9.10.
pA_2 Values for Antipodes of Hexahydrodifenidol and Its Relatives at M_1, M_2, and M_3 Muscarinic Receptors[87,88]

	Rabbit vas deferens (M_1)[a]	Guinea-pig atria (M_2)[b]	Guinea-pig ileum (M_3)[b]	Affinity ranking
Hexahydrodifenidol				
R-**45**	8.71	7.03	8.35	$M_1 > M_3 > M_2$
S-**45**	5.97	5.80	6.07	
Hexbutinol				
R-**44**	8.78 (9.43)[c]	7.77 (8.62)	8.78 (8.85)	$M_1 = M_3 > M_2$
S-**44**	6.75 (7.83)	6.84 (7.40)	7.14 (7.26)	
p-Fluorohexbutinol				
R	8.08	6.97	8.50	$M_3 \geqslant M_1 > M_2$
S	6.55	6.75	7.57	$M_3 > M_2 \cong M_1$

[a] Contractions elicited electrically.
[b] Vs. arecaidine propargyl ester as agonist.
[c] Methiodide data in parentheses.

analogues,[87] the alkene **46** and its *cis*-isomer were reported (no chemistry). The affinities of the *rac-trans*-isomer **46** for M_1, M_2, and M_3 subsites were enhanced over those of the *rac*-parent **45**. The 50-fold (M_1), 10-fold (M_2), and 4-fold (M_3) lower affinities of the corresponding *cis*-isomer provide evidence that the active conformation of hexahydrodifenidol is the fully extended form.[88]



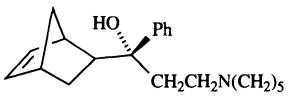
trans
46

pA_2 values[a]
M_1 8.8 (7.8)
M_2 7.5 (6.7)
M_3 8.1 (8.0)

[a] Values for *rac*-**45** are given in parentheses

Biperiden **47** and benzhexol are close analogues and their antipodes display the same dependence of ligand affinity and selectivity on stereochemistry. Thus the eutomer of biperiden has an *R*-configurated benzylic center; pA_2 data (alongside **47**) demonstrates the affinity order $M_1 > M_{2\beta} > M_{2\alpha}$ for (+)-biperiden.[89] Racemic biperiden was resolved via its *R*- and *S*-mandelates, and absolute configurations established by X-ray crystallography.[90]

The hexahydrobenzilic fragment appears in a number of anticholinergic agents in clinical use, such as oxyphenonium **48** and oxyphencyclimine **49** (*Martindale* **29**, page 540). Barlow *et al.*[8] included the former compound in their extensive com-

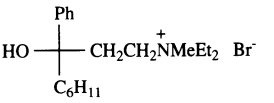


47
(+)-*exo*-*S*, benzylic-*R*

	pA_2values[a]	
M_1 (RVD)	$M_{2\beta}$ (GPI)	$M_{2\alpha}$ (RLA)
(+) 9.15	8.29	7.24
(–) 6.36	5.91	5.59
pirenzepine 7.64	6.98	6.46

[a]RVD, rat vas deferens; RLA, rat left atrium

parisons of antipodal pairs and reported GPI log *K* values: *RS* 9.78 (9.37); *R* 10.0 (9.65); *S* 8.15 (7.26) (values for corresponding $^{+}NMe_3$ compounds in parentheses). Antipodes of oxyphencyclimine **49** were prepared by treating *R*- and *S*- hexahydrobenzilic acids with 2-chloromethyl-1-methyl-1,4,5,6-tetrahydropyrimidine.[91] The *R*-isomer was 38.5, and the *S*-form 1100 times *less* effective than atropine in displacing [^{3}H]-QNB from bovine cortex binding sites.[92]



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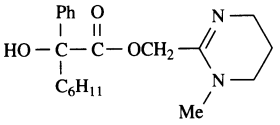
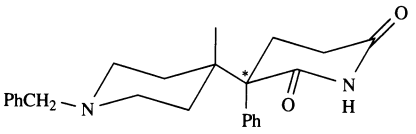


49

Benzetimide **50**, an anticholinergic agent about as potent as atropine,[93] also contains a chiral benzylic carbon atom and its dextro isomer (dexetimide) is over 1000 times as active as the levo form from pA_{10} values (GPI).[94] Dexetimide has a configuration (*S*, from X-ray study)[95] that is compatible with that of hexahydrobenzilates (*R*-eutomers) since all may present the same sequence of aromatic, hydrogen bonding donor, and C....N^+ features to the receptor (see **51**).



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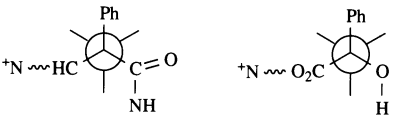
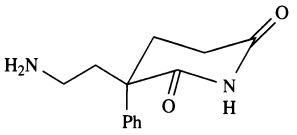


51

The binding of *R*- and *S*-[^{3}H] benzetimide to subcellular fractions of rat brain is stereospecific (*S*-isomer displaced by atropine but not by *R*-benzetimide or tubocurarine) and the use of dexetimide for the localization of muscarinic receptor has been advocated.[96,97] Guinea-pig ileum pA_2 values reported by Inch[59] were 9.95 for benzetimide and 10.25 for dexetimide (other tests were also carried out). The binding affinities of dexetimide and levetimide have also been determined vs. the [^{3}H] *cis*-dioxolane **22** (R = Me)[98] when a 10^4-fold difference was found. The affinities of dexetimide for M_1, M_2, and M_3 receptor subtypes were comparable, and in all cases eudismic ratios were high.[67] Thus dexetimide, although of high potency, is a nonselective muscarinic antagonist.

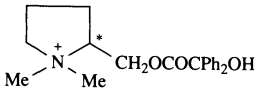
The antiparkinsonian agent phenglutarimide **52** is closely related to benzetimide and in both compounds eutomers have the *S*-configuration. Receptor selectivity for *S*-phenylglutarimide is $M_1 > M_{2\beta} > M_{2\alpha}$, i.e., the same as established for eutomers of benzhexol and biperiden (pages 309 and 312). At M_1 sites (rat vas



52

deferens) (+)-**52** (pA_2 8.53) had 6000 times the affinity of the levo isomer (pA_2 4.75).[99] Absolute stereochemistries were based on CD spectral correlations.[100]

Information on antipodes of the following chiral anticholinergics, marketed as racemic mixtures, is sparse or lacking: glycopyrronium (**53**, poldine), tropicamide **54** and empronium **55**. A stereospecific synthesis of *S*-(−)- and *R*-(+)-empronium from *S*-lactic acid has been described.[101] Antipodal emproniums differed little in their affinities for M_2 (GP atria, pA_2 *R* 7.73, *S* 7.53) and M_3 (GPI, pA_2 *R* 7.63, *S* 7.71) sites, as anticipated from the fact that the chiral center is well removed from the diarylcarbon feature of the molecule (Lambrecht, private communication, 1990).



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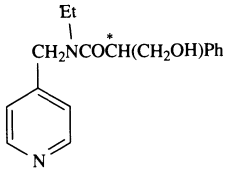

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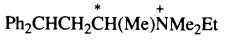
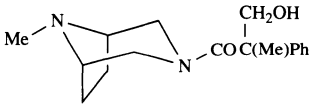


55

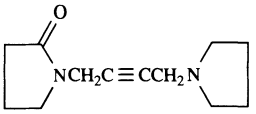
Antipodal α-methyltropates of tropine (page 295) and the amido-3,8-diaza analogue **56** show comparable differences in their affinities for GPI sites.[102,103] In both series the eutomer derives from the levo acid (configuration unknown—probably *S*, see page 289) and the eudismic ratio for esters of **56** exceeds 1000. Methylation reduces the affinity of the (−)-ester but raises that of the (+)-distomer to give a eudismic ratio of 46.



56

9.7. Chiral Oxotremorine Derivatives

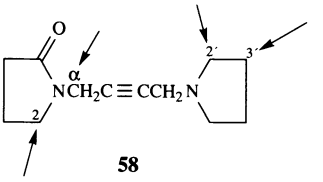
During the past 20 years Dahlbom and Ringdahl and co-workers have conducted extensive studies of chiral analogues of oxotremorine and its relatives.[104,105] Oxotremorine **57**, the active metabolite of tremorine (1,4-dipyrrolidino-2-butyne), produces tremor and other symptoms of Parkinson's disease together with intense parasympathetic stimulation. It lacks the usual quaternary ammonium head of cholinergic agonists and hence gains ready access to the CNS. Its actions together with those of structural variants with antagonistic actions are generally discussed in



57

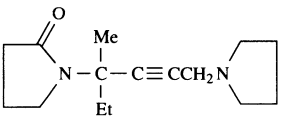
terms of interactions with muscarinic receptors. There is evidence, however, that the stimulation of choline acetyl transferase may be its mechanism of action—in doses sufficient to produce tremor and rigidity tremorine causes an increase of up to 50% in the ACh content of brain.[106]

Many chiral analogues of oxotremorine and the corresponding succinimide have now been reported, produced by methylation of achiral centers. Remarkably, most analogues behave as cholinergic antagonists, capable of blocking oxotremorine-induced tremor, causing mydriasis, and antagonizing ACh (or carbachol) in guinea-pig ileum. Of the sites of methylation (alkylation) investigated, depicted in **58**, antipodal potency ratios were greatest when substitution was at the α-CH_2 site followed by that at C-2′ of the pyrrolidine ring and C-2 of the pyrrolidone, and configurational relationships were found among eutomers of appropriate structure.



58

Data for some α-alkylated derivatives are shown (Table 9.11). Relative to atropine, these derivatives were more effective in blocking oxotremorine than acting as mydriatics or spasmolytics. The α,α-disubstituted derivative **59** was far less effective than either the mono-methyl or mono-ethyl analogues. Again the *R*-antipode was the eutomer (dose to block oxotremorine *R* 28, *S* 56 μmol/kg), a fact which shows that methyl is better tolerated in the adverse orientation (**60**) than ethyl. Similar relationships were found for corresponding succinimides of lower potencies (Table 9.11).



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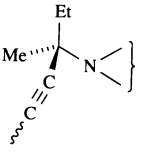
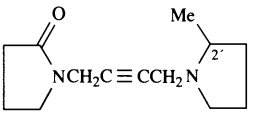


60

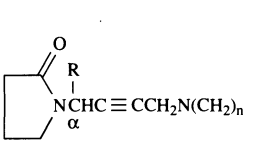
In 2-methyl pyrrolidino analogues the *S*-antipode proved to be the eutomer (see **61**) while 3-methyl derivatives displayed only minor stereoselectivites.[109] When isomers of derivatives with chiral centers at both the α-carbon and 2-position of the pyrrolidine ring were compared, a satisfying additivity of stereochemical preference

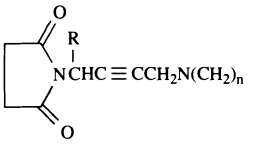


61

dose for blockade of oxotremorine (μmol/kg): *rac* 5.0; *R*-(−)56; *S*-(+) 2.6

TABLE 9.11.
Pharmacological Data for Some Chiral α-Alkylated Derivatives of Oxotremorine and Corresponding Succinimides[107, 108]

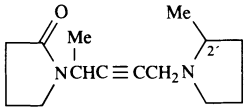




Isomer	R	*n*	Blockade of oxotremorine[a]	Mydriasis[b]	GPI vs. ACh[c]	Blockade of oxotremorine	Mydriasis	GPI vs. ACh
rac	Me	4	0.51	0.15	6.5	0.76	0.1	6.0
R-(+)			0.26	0.3	6.9	0.48	0.15	7.0
S-(−)			v. weak	0.003	v. weak	28	0.004	v. weak
rac	Me	6	1.3	0.08	6.6	—	—	
R-(+)			0.65	0.12	6.8	—	—	
S-(−)			v. weak	v. weak	4.7	—	—	
rac	Et	4	1.2	0.16	—	3.0	0.07	
R-(+)			0.52	0.23	—	1.2	0.15	
S-(−)			27	0.006	—	30	0.001	
rac	Pr^n	4	6.2	0.12	—	13	0.1	
R-(+)			3.5	0.2	—	8.2	0.13	
S-(−)			51	0.02	—	68	0.007	
Atropine			2.8 (2.3)[d]	1.0	—			

[a] In vivo dose (μmol/kg) required in mice to produce oxotremorine blockade.
[b] Dose of test compound required to double the pupil size relative to the control.
[c] pA_2.
[d] Value quoted in Ref. 108.

was found.[108] Data for the dimethylated set **62** are shown; only the α-*R*-members had significant potencies as mydriatics (0.24–0.21 × atropine).

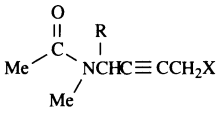


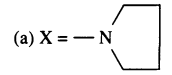
62

dose for blockade of oxotremorine (μmol/kg)

R(α)*S*(2′)	0.1	
SR	20	eudismic ratio 200
RR	0.5	
SS	12	eudismic ratio 24

In an attempt to obtain chiral analogues of oxotremorine which retained agonist activity, the *N*-methylacetamides **63** were investigated[111]—these represent



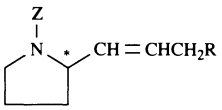


(b) X = —NMe_2

63

ring-opened analogues of the parent compound. The compound **63**a (R = H) is about half as active a tremorogenic agent as oxotremorine.[112] Methylated derivatives **63** (R = Me) provided a set of agonists, partial agonists, and antagonists, and in all cases *R*-antipodes were the more potent. The *R*-agonist **63**b (R = Me) was 24 times less active than oxotremorine as a tremorogenic agent (*S*-inactive) and induced GPI contractions 4.5 times less effectively than carbachol (*S*, 70 times less active); the methiodide was somewhat more potent at GPI sites and had no central effects. The pyrrolidine **63**a (R = Me) behaved as a tremorolytic (potency, μmol/kg: *R* 0.35, *S* 10) and a partial agonist at GPI sites (pD_2 *R* 6.63, *S* 5.51) (see Ref. 112a for most recent study).

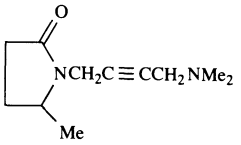
The racemic mixture behaved as an antagonist at presynaptic sites as evident from its elevation of [^{3}H]ACh release evoked by potassium from rat hippocampal sites (AChE inhibited by eserine).[113] Chiral analogues of **63**b (R = Me) prepared from *R*- and *S*-proline in which methyl attached to the chiral center and that to nitrogen were linked by methylene (**64**), had weaker muscarinic properties than the parent in a variety of muscarinic assays.[114] *R*-Enantiomers of acetamides (**64**, Z = COMe) had 5–10-fold greater affinities than *S*-forms in these tests. Lower degrees of stereoselectivity were found for carbamates (**64**, $Z = CO_2Bu^t$).



64

N-Acetyl-pyrrolidino, -piperidino, and -azacycloheptano analogues of **63**a (BM5) have been reported.[134] In all cases *R*-isomers had higher affinities for ileal and cortical muscarinic receptor vs. [^{3}H]NMS than their antipodes, and the piperidine derivative was more effective than *R*-**63**a in this regard (see Ref. 135 for synthetic methods).

Agonist properties were also encountered in the dimethylamino derivative **64a**.[115] This compound, along with its methiodide, approached the potency of carbachol in the GPI (*R*-eutomer). In central tests, however, *R*-**64a** behaved as a tremorolytic, one-third as effective a atropine. The base **64a** lacked action at



64a

EPMR (GPI, carbachol = 1)	
rac	8.8
R	4.6 (1.2)[a]
S	74 (20)

[a] Methiodide value.

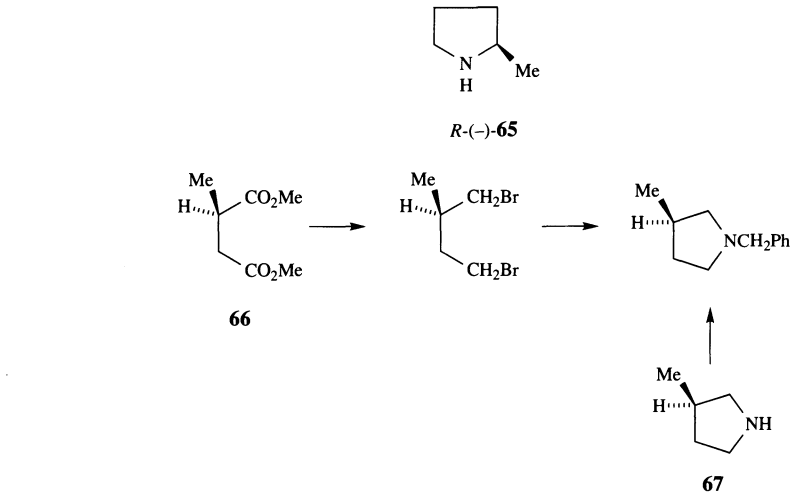
nicotinic sites (frog rectus abdominis) but the methiodide had a significant effect (~0.1 × that of carbachol, nonstereospecific). The derivative *R*-**64a** give evidence of having agonistic as well as antagonistic actions at central sites in that it caused profound hypthermia in mice which is characteristic of muscarinic stimulation—this effect was reversed by atropine. The related pyrrolidine [**64a**, NMe_2 replaced

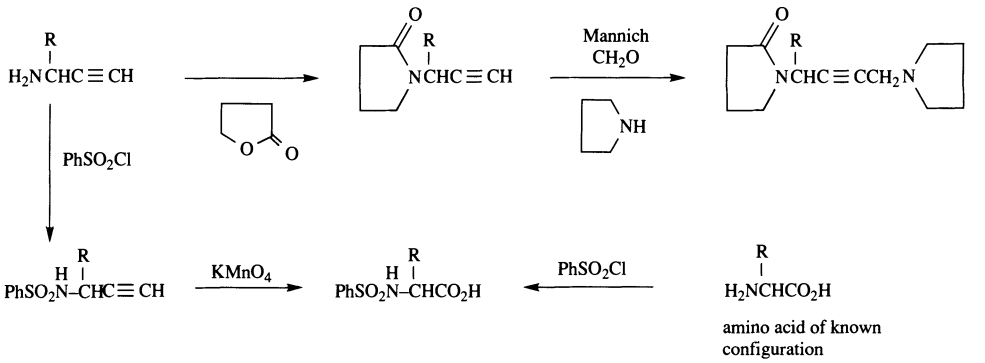
by $N(CH_2)_4$] followed the usual pattern of activity found among methylated oxotremorines: it behaved as an antagonists at GPI sites [affinity constant K_B μmol/l) *R* 0.051, *S* 0.98; atropine 0.00085], was a central tremorolytic (vs. oxotremorine), and had mydriatic activity (*R*-eutomer in all cases). The authors considered that the stereostructure activity relationship among this series gave evidence that all members (agonists and antagonists) bind to the muscarinic receptor in a similar manner.

Agonist and antagonist conformations of acetylenic imidazoles and other relatives of oxotremorine have been proposed by Moon *et al.*[136]

Chemistry. The α-alkylated derivatives **58** were obtained in homochiral form by use of *R*- and *S*-1-alkyl-2-propynylamine in the sequence of Scheme 9.6.[116,117] In the case of antipodes of 1-ethyl-1-methyl-2-propynylamine, oxidation of the (+)-benzoyl derivative led to the benzoyl derivative of *S*-(+)-isovaline.[118]

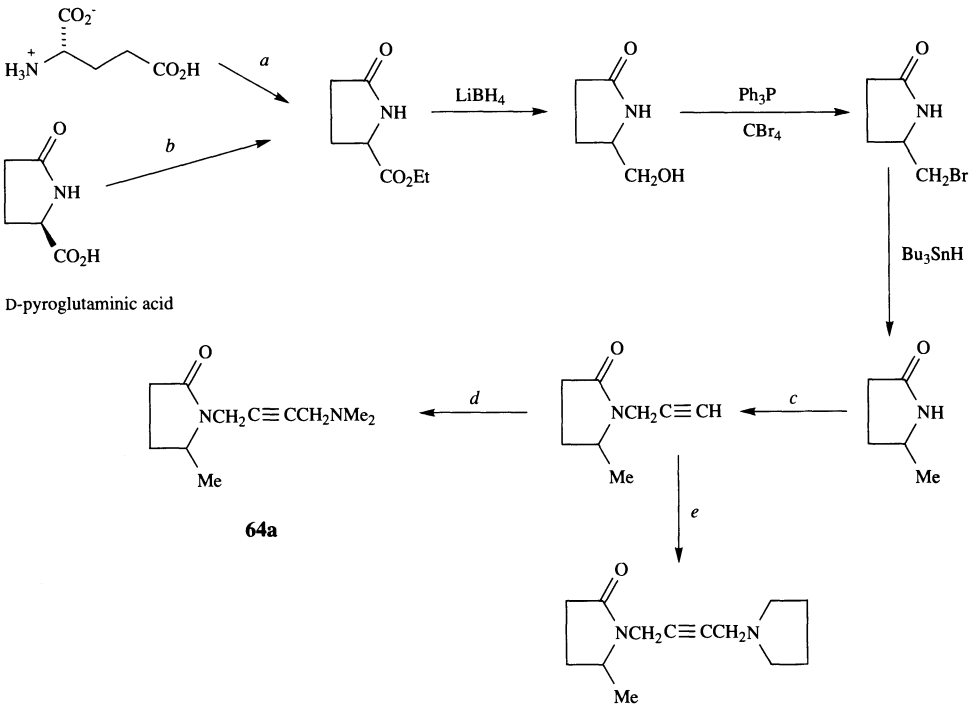
Derivatives methylated in the pyrrolidine ring were obtained by use of antipodal 2- and 3-methylpyrrolidines in the Mannich stage of the synthesis (Scheme 9.6). The levo 2-methyl isomer **65** has the *R*-configuration, assigned through chemical correlation to *S*-proline,[119] while the sequence **66**→**67** provides





[a] Use succinic anhydride for the succinimide series.

Scheme 9.6. Synthesis of α-alkylated derivatives of oxotremorine in homochiral form.[116,117]

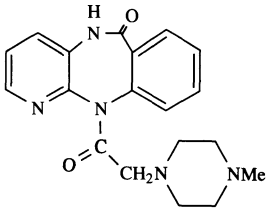


Scheme 9.7. Synthesis of antipodal oxotremorine derivatives with a methylated pyrrolidine ring. [115]

evidence for the configuration of the 3-methyl pyrrolidines.[120] Antipodal *N*-methylacetamides **63** were obtained by use of chiral 1-methyl-2-propynylamines (see above) in the method described for racemates.[121] Finally, *R*-derivatives with methyl positioned in the 2-pyrrolidone fragment were synthesized from *S*-glutamic acid; *R*-pyroglutamic acid was the precursor of the *S*-series (Scheme 9.7).[115]

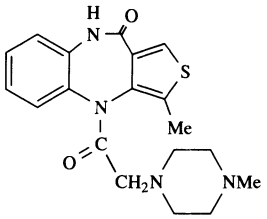
9.8. Chiral Analogues of Pirenzepine

In the search for ligands selective for subtypes of the muscarinic receptor, the benzodiazepine-6-one **68** (pirenzepine) has achieved notoriety as an agent with high affinity for M_1 and low affinity for M_2 sites. Thus pirenzepine binds with about



68

30-fold higher affinity to muscarinic receptors in sympathetic ganglia than to the receptors in the smooth muscle of stomach wall.[122] Pharmacologically it behaves as an antagonist and is used clinically to cause a reduction in the secretion of gastric acid in patients with ulcers (*Martindale* **29**, page 1103). The compound exists in solution as a mixture of conformers which arise as a result of hindered rotation about the exocyclic amide bond; this equilibrium has been studied by ^{1}H NMR and conformer ratios of 1:1.76 and 1:2.37 reported at 2 °C and 30 °C, respectively.[132] Its solid state structure has been established by X-ray crystallography.[110] Its analogue telenzepine (**69**, Tz) exhibits a selectivity in functional tests that is comparable to that shown by pirenzepine, but it is at least 10 times more potent than



69

the original agent.[123] Study of the binding kinetics of [^{3}H]Tz gave evidence that **69** existed as a mixture of enantiomers which are resolvable. Thus in experiments where a low concentration of [^{3}H]Tz was incubated with increasing concentrations of cortical membranes, only 50% of the radioligand was found to be capable of binding to receptors of high affinity. Dissymmetry arises as a result of the nonplanarity of the tricyclic ring system, and is another example of atroisomerism (Fig. 9.5). The energy barrier for racemization is high (35 kcal/mol)—in contrast, that of pirenzepine is 18–20 kcal/mol, a value too low to permit resolution at room temperature. Resolution of Tz was initially accomplished on a picomole scale by using muscarinic receptors to selectivity bind the active form of [^{3}H]Tz. Larger-scale resolutions by conventional methods have now been achieved [fractional crystallization of maleate salts of the isopinocampheyloxymethyl derivative (V. Figala and B. Kohl, private communication)]. The subsite selectivity of the (+)-eutomer was considerably greater than the (−)-distomer, with consequent variation in the eudismic ratio (Table 9.12).

Schudt *et al.*[125] obtained similar results for the binding of enantiomers of Tz to guinea-pig brain, heart, and salivary glands. Affinity constants, determined from

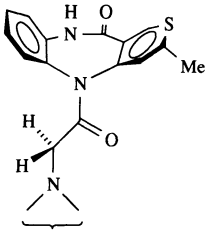


FIGURE 9.5. Representation of one of the antipodes of Tz 2HCl shown in the form of its major conformer. Inversion of the tricyclic ring system (producing the antipode of opposite configuration) is resisted by generation of nonbonded interactions between thienyl-methyl and the N-substituent.

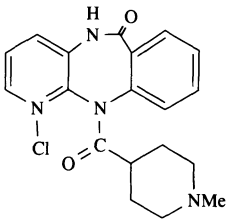
TABLE 9.12.
Affinity Constants for the Binding of (+)- and (−)-Tz (69) to Muscarinic Receptors from Different Tissues of the Rat[a] (124)

	(+)-Tz	(−)-Tz	Stereoselectivity
Cerebral cortex			
high-affinity sites	9.48 (9.17)[b]	6.79 (6.63)	510 (400)
low-affinity sites	8.23	6.03	160
Heart	7.77 (7.87)	5.89 (6.14)	75 (55)
Lacrimal gland	8.39 (8.33)	6.20 (6.10)	160 (180)

[a] Competition against [^{3}H]pirenzepine and from two-site analysis of data from experiments against tritiated PrBCh or NMS (PrBCh: *N*,*N*-dimethyl-*N*-propyl-2-aminoethyl benzilate, NMS: *N*-methylscopolamine).
[b] Values in parentheses refer to guinea-pig tissues labeled with [^{3}H]Tz (cortex) and [^{3}H]NMS (heart and salivary gland).(125)

functional assays, are shown in Table 9.13; they are in general accord with the binding parameters of Table 9.12. Half-times for the start ($t_{1/2}$ on) and end ($t_{1/2}$ off) of blockade of M_1-receptors of rabbit vas deferens exhibited an inverse kinetic pattern [(+)-Tz 23 and 174 min, respectively, (−)-Tz 3.0 and 0.38 min], a result possibly linked to the extremely slow dissociation of the (+)-isomer from the receptor sites.(126)

The chloro analogue of pirenzepine with the piperazine replaced by a piperidine ring (**70**, UH-AH 37) displays a similar subsite receptor preference



70 UH-AH37

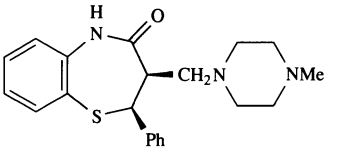
profile to that of the parent compound [pK_1 values 8.05 (cortical M_1), 7.05 (cardiac M_2), 7.46 (glandular M_3)] but with a tenfold higher affinity for ileal than atrial

TABLE 9.13.
Affinity Constants (pA_2) for the Action of Enantiomers of Telenzepine on Muscarinic Receptors in Rabbit Vas Deferens and Rat Left Atrium(125)

Agent	Rabbit vas deferens (RVD) inhibitory receptors	Excitatory receptors	Rat left atrium	Selectivity ratio inhibRVD/atrium
rac-Tz	8.86	7.51	7.32	28
(+)-Tz	9.12	7.64	7.67	7
(−)-Tz	6.98	6.14	7.32	35
Pirenzepine	7.79	6.23	6.34	28
Atropine	9.46	9.05	8.92	3
Eudismic ratio for Tz	140	30	35	

muscarinic receptors.[127] The barrier to racemization is evidently raised above that of pirenzepine by these structural changes since UH-AH37 has been resolved—the dextro isomer is 50–100 times more potent than the levo form in muscarinic tests.[128]

Finally, enantiomers of the compound **71** (levo isomer, BTM-1086, dextro BTM-1031) which retains many of the features of pirenzepine have been examined



71

by binding and functional tests.[129] The inhibition of gastric acid secretion by the levo isomer has been attributed to a high selectivity for M_1-receptors.[130] The same isomer displayed high affinities for rabbit vas deferens (M_1), guinea-pig atrium (M_2), and ileum (M_3) sites ($M_1 > M_3 > M_2$) and bound to guinea-pig cotex (M_1), heart (M_2), and salivary gland (M_3) in the order $M_1 > M_3 > M_2$. The affinities of the dextro isomer for these sites was very low and, in consequence, some remarkably high eudismic ratios were recorded, such as 5754 in rabbit vas deferens and 6918 in salivary gland. Magnitudes of ^3J coupling between 2-H and 3-H protons provided evidence of *cis*-geometry.[131]

No investigations of absolute configuration have been reported for this group of anticholinergics.

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10

Ligands of Nicotinic Cholinergic Receptors (nAChR) Including Neuromuscular Blocking Agents

10.1. Introduction

Chiral cholinergic ligands with actions characteristic of nicotine rather than muscarine (e.g., effects blocked by hexamethonium but not by atropine) have already been encountered in preceding sections of Chapter 8. These include α-methylacetylcholine and muscarone, and a more general account of such agents is now presented. Interpretations of their stereostructure–activity relationships is made difficult by evidence that nicotinic agents often function indirectly by promoting the presynaptic release of ACh,[1–3] a feature of the action of certain muscarinic agents as well.[4] Thus nicotine itself has been classified as having indirect cholinergic action at guinea-pig ileum sites.[1] Tissue incubated with hemicholinium (which inhibits the synthesis of ACh[5] for 15 min) showed a 70–100% reduction in its response to the spasmogenic action of nicotine. Pretreat-

TABLE 10.1.
Affinity Constants of Cholinergic Agents for Rat Brain Thalamus Sites[a] Labeled by [^{3}H]ACh and [^{3}H]-(−)-Nicotine[6]

	K_i (nM)	
	vs. [^{3}H]ACh	vs. [^{3}H]-(−)-nicotine
(−)-Nicotine	5.5	4.2
(+)-Nicotine	282.0	148.0
ACh	13.9	11.7
Carbachol	93.4	81.2
Cytisine	3.8	2.0

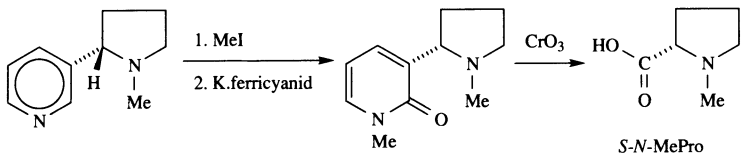
[a] Rat brain, muscarinic sites blocked by atropine sulfate.

ment with procaine and morphine (agents which block ACh release) similarly attenuated the actions of the tobacco alkaloid. Likewise, there are many reports of nicotine facilitating the release of ACh in the CNS.[1b,3] On the other hand, and in support of a direct component of action, [^{3}H]-(−)-nicotine has been shown to label the same recognition sites as [^{3}H]ACh in rat brain (thalamus) and with greater affinity [K_d nM) nicotine 3.5; ACh 8.6].[6]

Cholinergic drugs competed for sites labeled by the two ligands with similar affinities—the same experiments demonstrated the much reduced affinity of unnatural dextro nicotine (Table 10.1). It is clearly desirable that tests which differentiate direct and indirect cholinergic mechanism be carried out prior to structural comparisons between nicotinic agents and ACh and other directly acting agents. It is regrettable that pharmacological evidence of this kind is often absent from reports on nicotinic agonists.

10.2. Nicotine

Nicotine, the prototype agonist of nicotinic cholinergic receptors (nAChR), occurs naturally as the (−)-*S*-antipode **1**; its configuration was established by oxidation in a two-step procedure to *S*-*N*-methylproline.[7,8] Several pharmacological



comparisons of *R*- and *S*-nicotine have been made. The unnatural *R*-(+)-antipode is obtained by resolution of racemic material derived by racemization of the natural alkaloid, or by total synthesis. Barlow and Hamilton,[9] using (+)-nicotine judged to be 97% optically pure on the basis of its specific rotation, found the levo to exceed the potency of the dextro isomer in most nicotinic tests and in varying degrees, e.g., cat tibialis × 3.1, frog rectus ~ × 10, rat blood pressure × 14, guinea-pig ileum × 42. Later work in which the presumed optically pure (+)-antipode was employed confirmed the greater potency of levo nicotine in producing lethality in mice (7.1), raising blood pressure in rats (17), and inducing contractions of GPI (53) (*S*:*R* protency ratios in parentheses).[10]

In a review of the comparative actions of nicotine antipodes[11] the authors point out the generally modest order of potency difference between isomers in a variety of tests (most pronounced in regard to contraction of guinea-pig ileum 40–50 ratio, and certain behavioral tests 50–100 ratio). Admittedly, nicotine is a small flexible molecule (antipodes can adopt conformations that are superimposable to a large degree) but many chiral molecules of this kind display high eudismic ratios in spite of their flexible nature, such as β-methylacetylcholine.

In antipodal activity comparisons carried out by Ikushima *et al.*,[12] *R*-(+)-nicotine was found to have the following potencies relative to the *S*-(−)-antipode

(1.0): 0.06 rat blood pressure (rise); 0.2 cat superior cervical ganglion (stimulation and blockade); 1.0 neuromuscular junction of rat diaphragm (blockade). The levo isomer caused release of NA at adrenergic nerve terminals of rabbit pulmonary artery, an effect blocked by the dextro form.

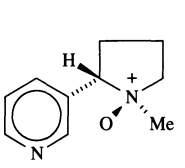
The high-affinity binding of [^{3}H]-(−)-nicotine to rat brain sites mentioned above has been confirmed by the work of Reavill *et al.*[13] IC_{50} (nM) values for displacement of 50% of the radioligand were: (−)-nicotine 9.7, (+)-nicotine 126, cytisine 1.8. Antipodes of nornicotinic (N-demethy) had similar low affinities (IC_{50} levo 135, dextro 118).

Natural nicotine had only 6 times the affinity of the dextro antipode for frog muscle end-plate sites [K_1 (μM) for inhibition of α-[I^{125}]bungarotoxin: (−)-nicotine 4.3, (+)28] cf. eudismic ratios 13 and 35 recorded at brain sites vs. [^{3}H]-(−)-nicotine.[13]

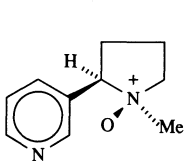
Stereoselectivities of the same order as found for end-plate sites were observed in regard to muscle contractures (rectus abdominis), depolarization, and peak magnitudes of end-plate current.[14] *R*- and *S*-Nicotine were equipotent as ion channel blockers.

The higher degrees of stereoselectivity exhibited by [^{3}H]-(−)-nicotine binding sites in mammalian brain, with preference for the levo antipode, may account for the high eudismic ratios found in the case of certain central tests of nicotinic activity.

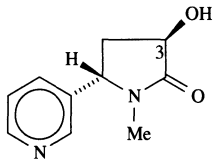
Pharmacokinetic factors, however, may well contribute to the often modest antipodal potency differences seen after *in vivo* test procedures. Thus in central tests using rats in which levo nicotine proved more effective than the dextro isomer by factors of 6 (spontaneous activity), 15 (Rotarod), and 30 (antinociception by rat tail-flick assay), brain levels of the eutomer exceeded those of the distomer after sc administration of equal amounts of drug (1 mg/kg), e.g., cortex 0.94 (−), 0.38 (+), ng/mg tissue.[15,16] Nicotine antipodes bound plasma proteins with equal facility but measurement of plasma levels of metabolites indicated that the (+)-distomer was biotransformed at a faster rate than the (−)-isomer. Later Cundy *et al.*[17] established that whereas *R*-(+)-nicotine was a substrate for *S*-adenosyl methionine-dependent guinea-pig lung N-methyltransferase (K_m 1.42×10^{-5} M), the *S*-(−)-isomer behaved as a competitive inhibitor (K_1 6.25×10^{-5} M) of the N-methylation of its antipode. The fact that N-methyl nicotine is detected in the urine of smokers indicates that significant racemization of natural nicotine occurs in the pyrrolysis event during smoking. The metabolism of nicotine by the route of oxidation of the pyrrolidino nitrogen provides an example of product stereoselectivity.[18,19] In several species *S*-nicotine was oxidized to the *cis*-N-oxide (**2**), while the *R*-form gave rise to the *trans*-isomer (**3**). 3-Hydroxycotinine (**4**), of specific stereochemistry, is another metabolic product of *S*-nicotine.[20]



2 *cis*-(2-H/N-O)



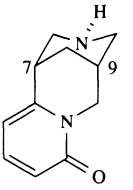
trans-**3**



4

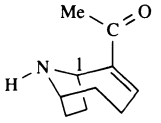
10.3. Cytisine and Other Agonists

The alkaloid cytisine (**5**), which occurs in seeds of the common Laburnum, shares many of the properties of nicotine and is generally more potent (up to 4-fold).[21] Structurally, the alkaloid is composed of a piperidine ring fused by 7,9 axial bonds to a 2-pyridone unit. The absolute configuration (7*R*,9*S*) shown in **5** was established by Okuda.[22] Cytisine's affinity for brain sites labeled by [^{3}H]-(−)-nicotine was 2–5-fold greater than that of (−)-nicotine itself (Table 10.1). Caulophylline, a related alkaloid, is the N-methyl analogue of **5**.



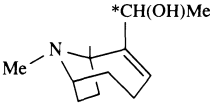
5 7*R*,9*S*

During the 1980s several studies of anatoxin-a (**6**), a novel nicotinic agonist of high potency, were reported. The dextro antipode is produce naturally by blue green algae and is responsible for the death of livestock and waterfowl by respiratory paralysis.[23] Chirospecific syntheses of (+)- and (−)-anatoxin from D- and L-glutamic acid, respectively, have been reported.[24,25] Original evidence of absolute configuration followed from an X-ray analysis of the N-acetyl derivative of **6**.[26] The preferred solute conformation of anatoxin-a (base in $CDCl_3$ and the



6 Anatoxin-a
(+)-1*R*-isomer shown
with *S-cis*-conformation

HCl in D_2O) is one with a twist-chair 7-membered ring and a *S-cis*-enone.[25] *Rac*-**6** had a similar potency to ACh and was tenfold more active than carbamoylcholine in causing contracture of skeletal muscle when AChE was blocked[27] and inhibited the binding of ACh and (+)-tubocurarine to nAChR[28]; relatively high concentrations were needed to displace quinuclidine benzilate from brain mAChR. In antipodal comparisons (+)-**6** proved 110 ×, and (−)-anatoxin-a 0.74 × carbamoyl choline in the frog rectus abdominis test, and the dextro isomer was judged 8 times more potent than ACh in this assay carried out with inhibition of AChE by DFP (diisopropylfluorophosphate).[29] In binding assays (+)-**6** was three times more



7 1*R* shown

TABLE 10.2.
Binding Parameters for *rac*-PHT (8/12) and Related Compounds vs. [^{3}H]-[−]-nicotine[32]

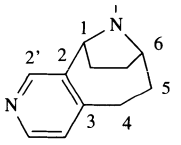
	Rat brain membranes		Torpedo membranes	
	IC_{50} M	Rel. potency	IC_{50} M	Rel. potency
rac-PHT	5×10^{-9}	1.0	1×10^{-9}	1.0
rac-Nicotine	7×10^{-9}	0.71	4×10^{-9}	0.25
rac-Nornicotine	8×10^{-8}	0.0062	1×10^{-7}	0.01
rac-Anatoxin-a	8×10^{-9}	0.62	1×10^{-9}	1.0
vs [^{3}H]-MCC[a]				
rac-PHT	1×10^{-8}	1.0	1×10^{-10}	1.0
rac-Nicotine	3×10^{-9}	3.3	1×10^{-9}	0.1
rac-Nornicotine	1×10^{-7}	0.1	3×10^{-7}	0.0003
rac-Anatoxin-a	1×10^{-8}	1.0	1×10^{-10}	1.0

[a] [^{3}H]-*N*-methylcarbamylcholine.

effective than ACh and 50 times superior to (−)-anatoxin-a in displacing [^{125}I]BGT (α-bungarotoxin) from Torpedo membranes. Ion-channel binding was also studied.

Both isomers of N-methylanatoxinol (**7**) had poor ability to inhibit the binding of [^{125}I]BGT or to open ACh channels, and failed to elicit contracture of frog rectus muscles.[30] However, blockade of the nAChR ion channel was demonstrated (*R* 4 × *S*).

We now consider pyridohomotropanes. The *rac*-pyridyl analogue (**8**) of anatoxin-a has been synthesized[31]; in this compound pyridyl nitrogen replaces carbonyl oxygen of *S-cis*-anatoxin-a (both serve as H-bond acceptor sites). In binding experiments[32] PHT equaled or surpassed the affinity of *rac*-anatoxin-a for



8 pyrido[3,4-b]homotropane(PHT); 1*R* shown

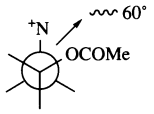
nicotinic sites of rat brain and *Torpedo* membranes; only in one case was a superior affinity recorded for *rac*-nicotine (Table 10.2). N-Methylation at secondary nitrogen or the 2′-carbon of the pyridyl ring caused drastic falls in the affinity of PHT (in contrast, the affinity of nornicotine fell below that of nicotine by factors of 11–333). In a functional test (iv injection of rat brain and observation of the characteristic prostration syndrome) the potency of *rac*-PHT equaled that of *rac*-nicotine and was twice that of *rac*-anatoxin-a. *Rac*-PHT has been resolved and the evaluation of antipodes is in progress (Kanne, private communication).

10.4. Stereo-SAR Analysis

Several attempts have been made to correlate nicotine and related agonists in terms of a "nicotine pharmacophore" and to provide reasons for differing degrees

of stereoselectivity observed and variable preferences for *N*-methyl quaternary salts over protonated N-bases.

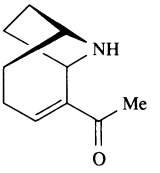
Beers and Reich,[33] like previous authors,[34,35] consider that dual receptor action (muscarinic/nicotinic) is due to interactions arising from two different conformations of functional groups of the transmitter molecule. Essential elements of their proposals, based on semirigid agonists, are a center of positive charge and a hydrogen bond acceptor (e.g., pyridyl nitrogen for nicotine, carbonyl oxygen for cytisine and ACh) disposed in a particular geometrical relationship. This idea was supported by a molecular modeling study of Sheridan *et al.*[36] According to Spivak's group[37,38] this formulation corresponds to a *gauche* disposition of the $^{+}NMe_3$ and OCOMe functions with a τ2 (O1-C5-C4-N) dihedral near 60° (**8a**, see Chap. 8, page 249). Studies of conformationally restrained analogues of ACh which



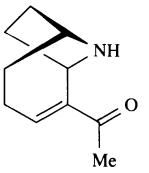
8a

favor an active ACh conformation with τ2 near 180° in regard to the muscarinic receptor have already been presented (Chap. 8, page 258). Recent NMR studies of ACh bound to the nicotinic acetylcholine receptor (purified from the electroplax organs of *Torpedo californica*) provide evidence for a bound conformation with a similar *trans*-orientation of the N/O functionalities (τ2 220°).[39] Conclusions about conformation were based on 2-D NOE proton data and energy calculations; allowance was made for the fact that the raw NMR data represented the average of free and bound states (R. W. Behling, private communication). Results for free ACh in D_2O agreed with X-ray crystallographic data and previous NMR investigations (Chapter 8, pages 250 and 252), and gave an acetylmethyl–cholinemethyl distance of ~5 Å. When bound, the same distance parameter was close to 3.3 Å. This bent conformation places the oxygens adjacent to one another and allows the methyl groups to form an uninterrupted hydrophobic surface over the rest of the molecule. By carrying out spectral measurements in the presence of α-bungarotoxin (which blocks nAChR sites) nonspecific contributions to binding could be measured and deducted from the total to give specific contributions. Effective interproton distances in ACh bound to lipid and nAChR did not differ significantly in evidence of the conformational similarly of the bound molecules. This work suggests, therefore, that if active conformations of ACh at muscarinic and nicotinic receptors differ, they do so in regard to torsion angles other than τ2.

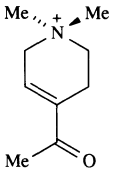
The speculations of Spivak and others (outlined above) preceded discovery of anatoxin-a, a nicotinic agonist that is unusual in displaying a high eudismic ratio (>100, see page 330) and which is therefore an important structure to include when modeling nicotinic activity. Koskinen and Rapoport[25] argued that the s-*cis* chair (**9**), which provides an N–O distance of 6.04 Å close to the 5.9 Å of Beers and Reich's pharmacophore, is the probable receptor-bound conformation. However, a superposition of N,O and carbonyl carbon of s-*cis*-**9**, (−)-cytisine, and isoarecholine methiodide (**11**, a potent nicotinic agonist, 50× carbachol at the frog neuromuscular junction)[38] produced a combined van der Waals volume of exten-



9
s-*cis*-chair

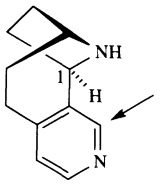


10
s-*trans*-chair



11
trans-favored

sive area. In contast, when the s-*trans*-chair **10** was used in the modeling, a smaller volume resulted which allowed rationalization of the enantioselectivity of anatoxin-a.[40] The structural modeling was performed by use of the interactive computer graphics program MIMIC and Chem-X. The eutomer of PHT predicted on this basis is the 1*R*-isomer **12**. 2-Methyl of the feebly potent 2-methyl PHT would



12 1*R*-PHT

correspond with methyl of the s-*cis*-anatoxin-a, further evidence that the s-*trans*-form is the active conformation. The Swedish model accounted for the relative low stereoselectivity of nicotine by virtue of the flexibility of its molecule in comparison with that of anatoxin-a.

10.5. Nicotinic Receptor Antagonists: Neuromuscular Blocking (NMB) Agents

The ability to stimulate receptors in the neuromuscular junction and autonomic ganglia is a nicotinic-like property of ACh. Agents which block the actions of the natural neurotransmitter at such sites are henced deemed to represent antagonists of the nicotinic acetylcholine receptor (nAChR). The stereochemical features of antagonists of this kind are now presented in a contribution made by Dr. George H. Dewar.

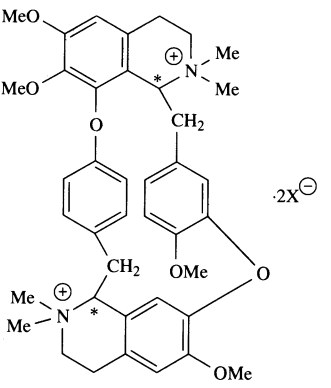
The term "neuromuscular blocking agent" applies to any substance capable of abolishing the response of voluntary muscles to nerve impulses by an action at the junction between the somatic motor nerve terminal and the muscle endplate. Such blockade produces voluntary muscle relaxation, which is required as an adjunct to anaesthesia in surgery. Although this general definition of neuromuscular blocking agents (NMB agents) encompasses many molecules acting by a variety of mechanisms, the clinically useful muscle relaxants are restricted to two classes. Both act at nicotinic acetylcholine receptors (nAChR) in the muscle end-plate, and they are referred to as (i) nondepolarizing NMB agents, and (ii) depolarizing NMB agents. Although a postjunctional mode of action is implied for both groups, a prejunctional component is evident in the former.[41,42]

A vast literature is available on the mechanisms of action and structure-action aspects of the two classes[43–47] and it is considered adequate here to outline only fundamental pharmacological and chemical points as a preliminary to addressing stereochemical detail.

10.5.1. Nondepolarizing Agents

The nondepolarizing NMB agents act by combining with the externally directed recognition sites of the end-plate cholinoceptors, the consequence of which is to deny access of the natural hormone acetylcholine. Contraction fails when the end-plate potential amplitude falls below that threshold required to trigger off a propagating muscle fiber action potential. This challenge to acetylcholine's action by such agents shows the characteristics of competitive antagonism, at least in small concentrations.[48] It follows that elevation of acetylcholine levels in the end-plate region, through use of anticholinesterase drugs, will reverse the neuromuscular blockade. This ready reversal of action of nondepolarizing agents explains their popularity in medicine—it provides for sophisticated, controlled, and safer anesthetic practice. Consequently, the vast majority of NMB agents used clinically are nondepolarizing.

Chemically, most nondepolarizing agents are complex structures containing two nitrogen atoms, suitably spaced, that are either both quaternary or, less often, *mono*-quaternary together with *mono*-amine (tertiary and protonated). In many cases the nitrogen atoms are part of a heterocyclic ring system. The semisynthetic and powerful paralyzing agent *N*,*O*, *O*-trimethyltubocurarine ("metocurine"; **13**) serves as a representative example. The nondepolarizing group of drugs provide an interesting, diverse range of structural types, rich in stereochemical detail.

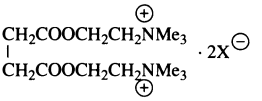


13 chiral centers marked

10.5.2. Depolarizing Agents

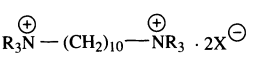
Blockade by depolarizing agents is accompanied by prolonged depolarization of the postjunctional membrane, which has similarities to the effect observed when the action of acetylcholine is prolonged by anticholinesterase drugs.[49] Paralysis is preceded by muscle fasciculation, which in human expresses itself as deep muscle

pain in postoperative circumstances. Additionally, raising local levels of acetylcholine through administration of anticholinesterase substances does not reverse such blockade and, indeed, may even enhance it. These disadvantages essentually preclude the use of depolarizing agents in medicine, with the exception of suxamethonium (succinylcholine; **14**) whose place is established due to a rapid onset of action and brief duration. The brevity of action is associated with rapid hydrolysis of the two acetylcholine-like fragments by pseudocholinesterase.[50,51]



14

The structural requirements for depolarizing NMB action are quite rigid—a *bis*-quaternary structure of appropriate internitrogen distance, of slender dimension (Bovet's "lepto-curares"[52]), and bearing small groups on nitrogen (specifically $\overset{+}{N}Me_3$). Any major departure from these structural features alters the character of blockade, from depolarizing to nondepolarizing. The prototype depolarizing agent is decamethonium (R = Me in **15**); the corresponding *bis*-triethyl analogue, decaethonium (**15**; R = Et), is a low-potency nondepolarizing agent. Quite apart



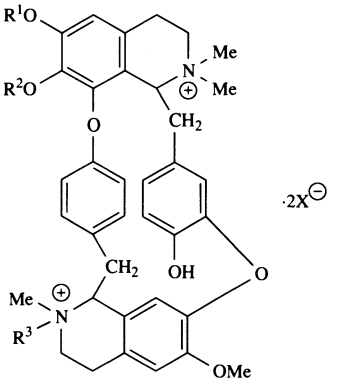
15

from their lack of clinical application, the depolarizing group of agents are not rich in stereochemical detail. The main reason for this is that the insertion of groups, and attendant branching, required to impart a chiral center is often sufficient to alter the nature of the block itself. A few examples will be cited after the section on nondepolarizing agents.

10.6. Stereochemistry in Nondepolarizing NMB Agents

10.6.1. Tubocurarine and Closely Related Substances

The prototype nondepolarizing NMB is the naturally occurring alkaloid tubocurarine, a *bis*-1-benzylisoquinoline obtained from the plant *Chondrodendron tomentosum*. The isolation and structure elucidation was undertaken by King in work dating from 1935[53] and he proposed the *bis*-quaternary structures (**16a**) for the alkaloid. His studies, together with more recent aspects of the chemistry of tubocurarine and its relatives, are presented in several volumes of *The Alkaloids* series.[54–56,144] Another important alkaloid called chondrocurarine was assigned the isomeric structure (**16b**) by the same worker. In a startling paper published some 30 years later,[57] following NMR, exchange, and degradative experimentation on the two compounds, it was revealed that chondrocurarine has, in fact, the structure (**16a**) originally assigned by King to tubocurarine, and the latter is the *mono*-quaternary (**16c**).

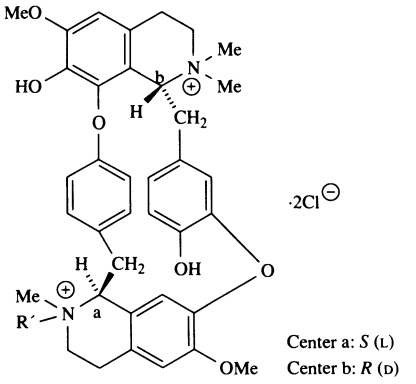


16

(a) $R^1 = R^3 = Me$, $R^2 = H$

(b) $R^2 = R^3 = Me$, $R^1 = H$

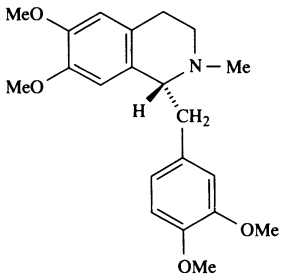
(c) $R^2 = R^3 = H$, $R^1 = Me$



16d

Center a: *S* (L)
Center b: *R* (D)

Active tubocurarine is one of the four possible stereoisomers, and is *dextrorotatory*. Degradation work on (+)-tubocurarine's precursor *bis*-tertiary amine, (+)-tubocurine [now known to be the same as (+)-chondrocurine], revealed that, relatively, the protons at the two asymmetric centers (see **16d**) are *trans*.[58] The absolute stereochemistry followed from work in which the degradation products secured by Bick and Clezy were related to *R*-(−)-laudanosine (**17**),



R-(−)-**17**

of known absolute structure.[59–61] An X-ray study on (+)-tubocurarine dibromide tetra-methanol solvate employing anomalous scattering confirms the absolute stereochemistry established by chemical methods.[145] (+)-Tubocurarine and (+)-chondrocurarine have the same absolute stereochemistry, and their full structures are, respectively, **16d**; R′=H and **16d**; R′=Me.

The Cahn–Ingold–Prelog system will be used to provide stereochemical detail in potency correlations, and the asymmetric centers are labeled a and b as shown in **16d**. The diastereoisomeric *bis*-quaternary salts bearing a *cis*-relationship of the protons at the asymmetric centers are called (+)- and (−)-curarine. Taking the a and b centers in turn, the stereochemical detail for the important alkaloids is as follows: (+)-tubocurarine, (+)-chondrocurarine, and (+)-*N*,*O*,*O*-trimethyltubocurarine (**13**) are (*S*) (*R*); (−)-tubocurarine, (*R*) (*S*); (+)-curarine, (*S*) (*S*); and

(−)-curarine, (*R*,*R*). The curarines have the *bis*-quaternary structure of chondrocurarine but alternative stereochemistry as shown.

The related compound (+)-isotubocurarine differs from (+)-tubocurarine in having its quaternary nitrogen next to the *S*-center (center a in **16d**) and the protonated nitrogen adjacent to the *R*-center (center b in **16d**). It is prepared by treating (+)-tubocurine in a large volume of acetone with only one mole equivalent of methyl iodide.[64] Separation of the required compound from unreacted (+)-tubocurine, (+)-tubocurarine, and (+)-chondrocurarine is effected by column chromatography using alumina (having first converted iodide to chloride by ion exchange) followed by crystallization of *mono*-quaternary hydrochloride salts.

Potency data for important quaternary alkaloids and derivatives, in various species, are presented in Table 10.3.

Despite considerable species variations, it is clear that masking of phenolic OH groups through methyl ether formation enhances potency (higher alkyl ethers are, invariably, detrimental to activity; see Marshall *et al.*[61]). The *bis*-quaternary (+)-chondrocurarine, having the structure originally assigned by King to (+)-tubocurarine, is approximately three times more potent than the parent, and (+)-chondrocurarine dimethyl ether ("metocurine"; **13**) is approximately nine times as potent as the parent.

What these results emphasize is the importance of an *S*-center adjacent to permanently charged nitrogen for high potency [cf. (+)-tubocurarine with isotubocurarine and metocurine; and (+)-curarine with (−)-curarine]. Dimensionally, this important (*S*)-laudanosinium unit (see also below) may be directly related to the potency-inducing (*R*)-*cis*-components of atracurium, a recently introduced NMB agent, and (*R*)-*cis*-norcoralydine methiodide, one of four stereoisomers employed as probes at the NM junction (see atracurium section, page 341).

TABLE 10.3.
Relative Potencies of (+)-Tubocurarine and Related Substances in Four Test Systems

Compound	Stereochemistry		Relative potency			
	Center a	Center b	Rabbit head drop assay[a]	Frog rectus muscle[b]	Hen gsn[b,c]	Rabbit atm[b,d]
(+)-Tubocurarine	*S*	*R*	100	100	100	100
(−)-Tubocurarine	*R*	*S*	slight[e]	—	—	—
(+)-Isotubocurarine[f]	*S*	*R*	—	—	—	—
(+)-Chondrocurarine	*S*	*R*	290	—	—	—
(+)-*N*,*O*,*O*-Trimethyltubocurarine[g]	*S*	*R*	870	58	204	933
(+)-Curarine	*S*	*S*	350 (63[h])	—	—	—
(−)-Curarine	*R*	*R*	130	32	30	117
(+)-*O*,*O*-Dimethylcurarine	*S*	*S*	— (133[h])	—	—	—
(−)-*O*,*O*-Dimethylcurarine	*R*	*R*	330	97	56	143

[a] From Wintersteiner, 1959.[62]
[b] From Marshall *et al.*, 1967.[61]
[c] gsn = gastrocnemius–sciatic nerve preparation.
[d] atm = anterior tibialis muscle.
[e] 1/30th–1/60th the activity of (+)-tubocurarine on rat diaphragm.[146]
[f] Potency of 231 relative to the parent (100) using the cat tongue–hypoglossal nerve preparation.[64]
[g] Also named as (+)-*O*,*O*-dimethylchondrocurarine.
[h] From Marsh, Sleeth, and Tucker, 1948.[63]

A solution conformation study of (+)-tubocurarine chloride by ^{1}H NMR (DMSO-d_6 solvent)[148] suggests that the bulky aromatic rings orientate perpendicular to the planes of the isoquinoline rings, and the molecule as a whole has considerable flexibility. The central trisubstituted aromatic ring does not rotate, even at elevated temperatures, due to *meta*-attachment to the isoquinoline ring bearing chiral center a. Solid state conformational data are available from X-ray analysis of appropriate salts. *N,O,O*-Trimethyltubocurarine diiodide (**13**; X = I) has an internitrogen distance of 10.7 Å and is an inflexible, tightly compact structure.[149] Tubocurarine dichloride pentahydrate assumes a folded conformation in the solid state and has an internitrogen distance of 8.97 Å. The associated water molecules form a two-dimensional hydrogen bonding network, and one of the chloride ions is hydrogen bonded to the parent cation.[150] (+)-Tubocurarine dibromide tetra-methanol solvate has an internitrogen distance of 10.66 Å, and is extensively hydrogen bonded through its phenolic and tertiary nitrogen groups with solvent and bromide ions.[145]

10.6.2. Synthetic, Symmetrical 1-Benzylisoquinolinium Agents

The incorrect assignment of the *bis*-quaternary structure **16**a to tubocurarine by King, although unfortunate for the author, nonetheless focused the attention of subsequent researchers on *bis*-quaternaries as potential NMB agents. Despite exceptions, including, of course, tubocurarine itself, it is *bis*-quarternary, and not *mono*-quaternary, structures that provide the bulk of the clinical agents and interesting examples.

The work of Taylor and colleagues on the agent laudexium (**18**) is a good example of drug design.[65–67] This symmetrical molecule unites the decamethylene chain of decamethonium (**15**; R = Me) with the laudanosinium moiety of *N,O,O*-trimethyltubocurarine ("metocurine"; see Fig. 10.1); this di-*O*-methylated compound is chosen because of its enhanced potency over tubocurarine and chondrocurarine (Table 10.3) and for reasons of synthetic accessibility. Laudexium has about half the potency of tubocurarine in man,[68] is less likely to cause histamine release, but

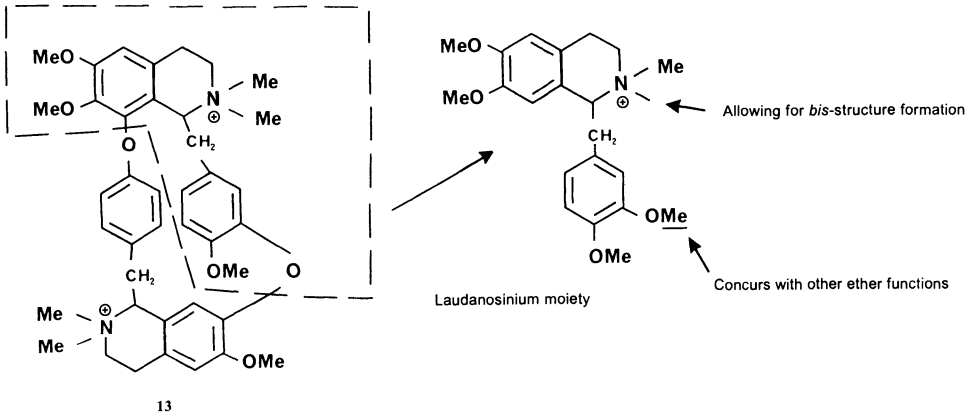
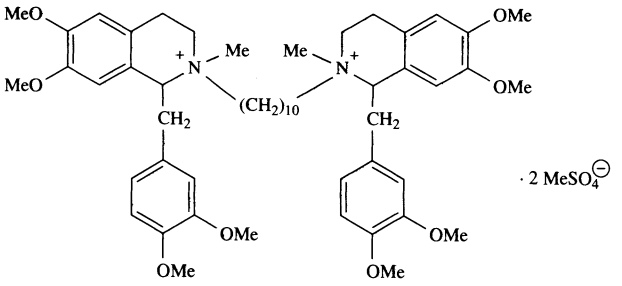


FIGURE 10.1. Fragmentation and simplification of metocurine (**13**) to highlight the laudanosinium moiety present. Incorporation of this moiety into accessible, symmetrical structures has encouraged the emergence of laudexium, atracurium, and related substances (see below).



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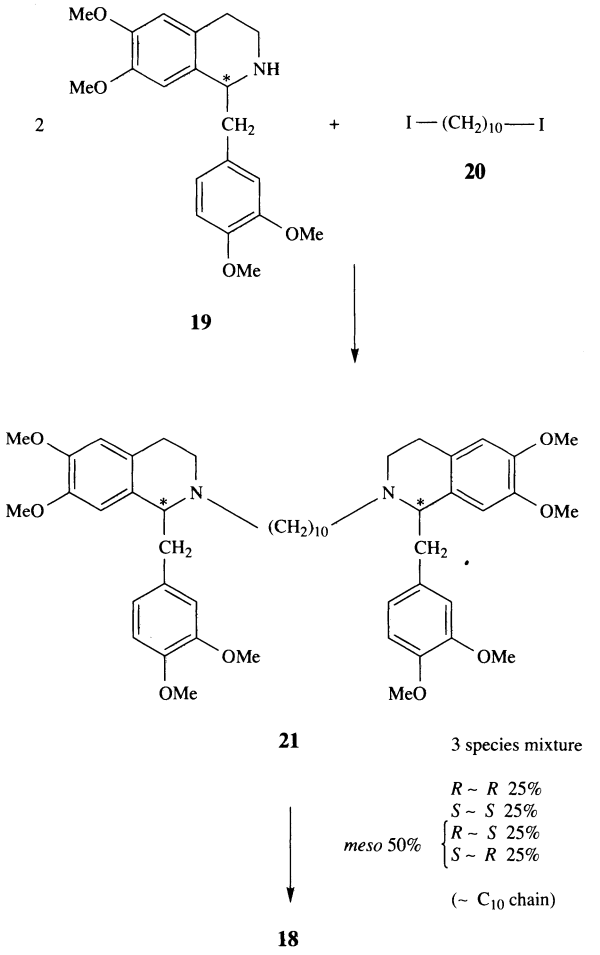
is longer acting. Laudexium saw only limited clinical application because of postoperative recurarization in a proportion of patients.

The material studied most, including that utilized in the clinic, was synthesized from (*R,S*)-tetrahydropapaverine (**19**) and decamethylene diiodide (**20**) to yield the precursor *bis*-amine **21**, followed by double quaternization to **18** (Scheme 10.1). The

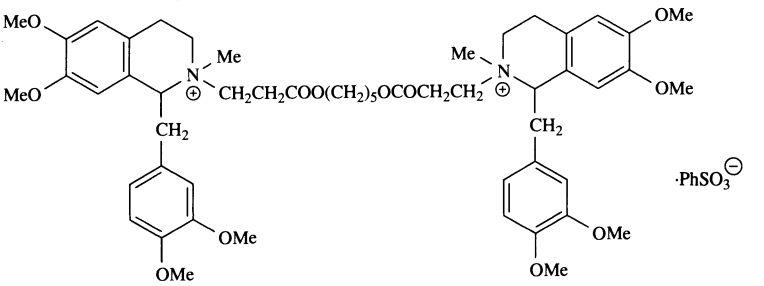


Scheme 10.1. Synthesis of laudexium mixture from racemic tetrahydropapaverine.

stereochemical complexity in **18** requires some elaboration. The intermediate *bis*-amine **21** has two chiral centers (denoted by an asterisk * in Scheme 10.1), but the natural symmetry reduces possible isomer numbers from four to three: (i) 25% (*R,R*), (ii) 25% (*S,S*), which together constitute a 50% racemate, and (iii) 50% *meso*. Since the quaternization process yields two new asymmetric centers (on each of the nitrogens), it follows that each of the three *bis*-amine components in **21** will yield four quaternary species each (i.e., a total of 12 quaternary isomers in **18**). However, considerations of symmetry again reduce the number of possible quaternary species in the (*R,R*) and (*S,S*) components from four to three, yielding a still formidable mixture of 10 entities.

The separate (*R,R*), (*S,S*), and (*R,S*; *meso*) quaternary mixtures described above have been synthesized and evaluated.[69] (The synthesis of *meso*-**21** in Scheme 10.1 requires two steps. Decamethylene diodide is reacted with excess of one of the optical isomers of **19** to yield an intermediate *mono*-amine *mono*-iodide; 1:1 molar interaction of this latter with the other antipode of **19** yields *meso*-**21**.

Relative molar potencies for (*R,R*), (*S,S*), and *meso*-quaternary mixtures of laudexium are presented in Table 10.4. This potency order, where the (*R,R*) mixture shows highest activity, with *meso*-mixture intermediate, is consistent with results on atracurium (**22**) isomers and, indeed, the 1,10-*bis*-paviniumdecane diiodides reported by Genenah *et al.*[93] Since isomer mixture quaternary composition is



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more highly defined in atracurium, a consideration of structure in relation to receptor association will follow the section on that drug.

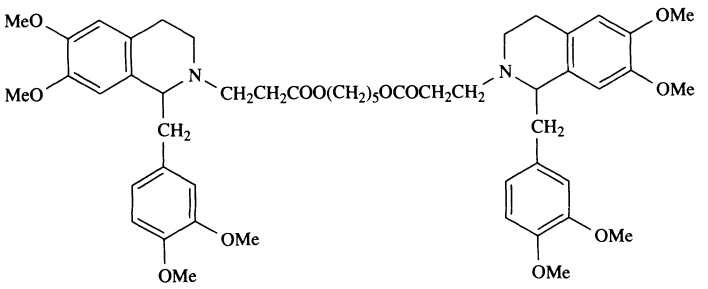
TABLE 10.4.
Relative Molar Potencies of Laudexium Isomers in the Chick (Isolated Biventer Cervicis Muscle)

Stereochemistry at position 1 of both isoquinolinium rings[a]	Potency[b,c]
(*R,R*)	275
(*R,S*)	140
(*S,S*)	98

[a] Nature of the quaternary mixtures not defined.
[b] Tubocurarine = 100.
[c] Laudexium (10 species mixture) = 245.

Atracurium (**22**; "Tracrium") was designed as a short-acting NMB agent in an extension of a study on simpler model compounds.[71–73] Its structure utilizes the laudanosinium units seen in laudexium, together with an internitrogen chain accommodating a double Hofmann elimination reaction at body pH.[47,73] Its pharmacological and clinical features have been reviewed.[74,75]

The precursor *bis*-tertiary amine **23** to atracurium has stereochemical features directly comparable to the corresponding precursor (**21**; Scheme 10.1) to laudexium (**18**). The (*R,R*), (*S,S*), and (*R,S*; *meso*) isomers of **23** have each been prepared and



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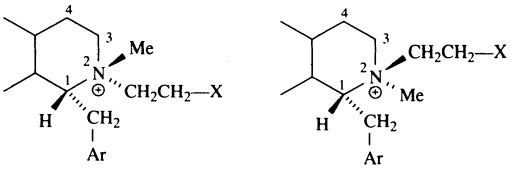
quaternized to mixtures containing three, three, and four species, respectively[70] (see also Table 10.5). The quaternization process favors attack by the alkylating agent on nitrogen at the side remote from the bulky 1-(3,4-dimethoxybenzyl) group (1-veratryl group). There is therefore a predominance of species in which the

TABLE 10.5.
Isomer Composition and Relative Molar Potency[a] of Atracurium Isomers

Product	Configuration of component isomers[b]				Isomer ratios (HPLC)	% *cis* and *trans* components[c]				Relative molar potency
	C_1	C_1-N_2	C_1	C_1-N_2		*R-cis*	*R-trans*	*S-cis*	*S-trans*	
(*R,R*)	*R*	*cis*	*R*	*cis*	10.7	76.5	23.5	0	0	231
	R	*cis*	*R*	*trans*	6.5					
	R	*trans*	*R*	*trans*	1.0					
(*R,S*)	*R*	*cis*	*S*	*cis*	10.3					
meso	*R*	*cis*	*S*	*trans*		38.2	11.8	38.2	11.8	133
	R	*trans*	*S*	*cis*	6.3					
	R	*trans*	*S*	*trans*	1.0					
(*S,S*)	*S*	*cis*	*S*	*cis*	10.6					
	S	*cis*	*S*	*trans*	6.6	0	0	76.5	23.5	89
	S	*trans*	*S*	*trans*	1.0					

[a] From studies in anesthetized cats, and relative to tubocurarine = 100.[70]
[b] *Cis* and *trans* refer to the relationship between C_1-Ar and $N_2-CH_2CH_2COO-X$ groups.
[c] The aromatic ring of the position 1-veratryl group exerts a shielding effect on the position-8 aromatic proton of the isoquinolinium system. This C-8 proton resonance moves to an upfield position of *ca* δ 5.7 and is well displaced from all other aromatic proton resonances. The C-8 proton resonates at different positions in the *cis* and *trans* entities: the larger resonance assigned to *cis*, at δ 5.9, and the smaller (*trans*) at δ 5.65 (ratio *cis*/*trans ca* 3.0). ^{13}C-NMR analysis also shows differences in the ^+N-Me carbon resonance of the two entities: *cis*, 47.4 ppm; *trans*, 49.2 ppm.[70]

C_1-veratryl and N_2-methyl are *trans* to one another (that is, the bulkyl C_1-veratryl and bulky N_2-ester chain are *cis* to one another).[70]

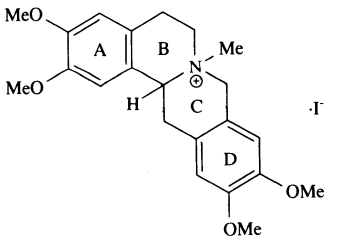


R-cis species *R-trans* species

Relationship of the two bulky groups on C_1 and N_2 to one another

The (*R*,*R*) isomer of **23**, for example, would yield a high proportion of *cis–cis*-entities in the quaternary mixture, a lesser proportion of *cis–trans*-entities, and only modest amounts of *tran–trans*-species. NMR and HPLC analysis agree closely in suggesting a *cis*:*trans* ratio of approximately 3:1. Table 10.5 lists the configuration of component isomers obtained on separately quaternizing (*R*,*R*), (*S*,*S*), and (*R*,*S*; *meso*) bases of **23**. Isomer ratios, full data on *cis*/*trans*-entity ratios, and relative molar potencies in anaesthetised cats are also presented. Thus, potency orders compare well with corresponding laudexium isomers (whose *compositions* are assumed to be similar to atracurium). Maximum potency resides in the (*R*,*R*) mixture, which contains *ca* 75% *cis*-entries.

The curarizing power associated with (*R*)-*cis*-stereochemistry in atracurium is consistent with observations among the four isomeric norcoralydine *mono*-quaternary methiodides **24** and, indeed, tubocurarine and related substances.[45,61,76,77] The



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norcoralydine methiodides have been used as stereochemical probes of the NM junction, although their potency is somewhat low in view of their *mono*-quaternary nature and lack of a second nitrogen. The relative molar potencies (tubocurarine = 100) are (*R*)-*cis*-norcoralydine methiodide (13.6), (*R*)-*trans*-norcoralydine methiodide (4,3), (*S*)-*cis*-norcoralydine methiodide (2.1), and (*S*)-*trans*-norcoralydine methiodide (3.2); (data from studies on chick isolated biventer cervicis muscle).[76]

(*R*)-*cis*-Norcoralydine methiodide may be depicted as **24a** (Fig. 10.2), and association with an anionic receptor site which is stereoselective for the quaternary center and its environs at the upper, β-face of the molecule, as shown, may be envisaged. Atracurium (*R*)-*cis*-entities may be similarly presented (Fig. 10.2) and it seems likely that (*S*)-laudanosine methiodide (**25**)[92] is conformationally identical with the tetrahydroisoquinolinium moiety comprising rings A and B of (*R*)-*cis*-norcoralydine (see Fig. 10.2). (*S*)-Laudanosine methiodide, although of low potency

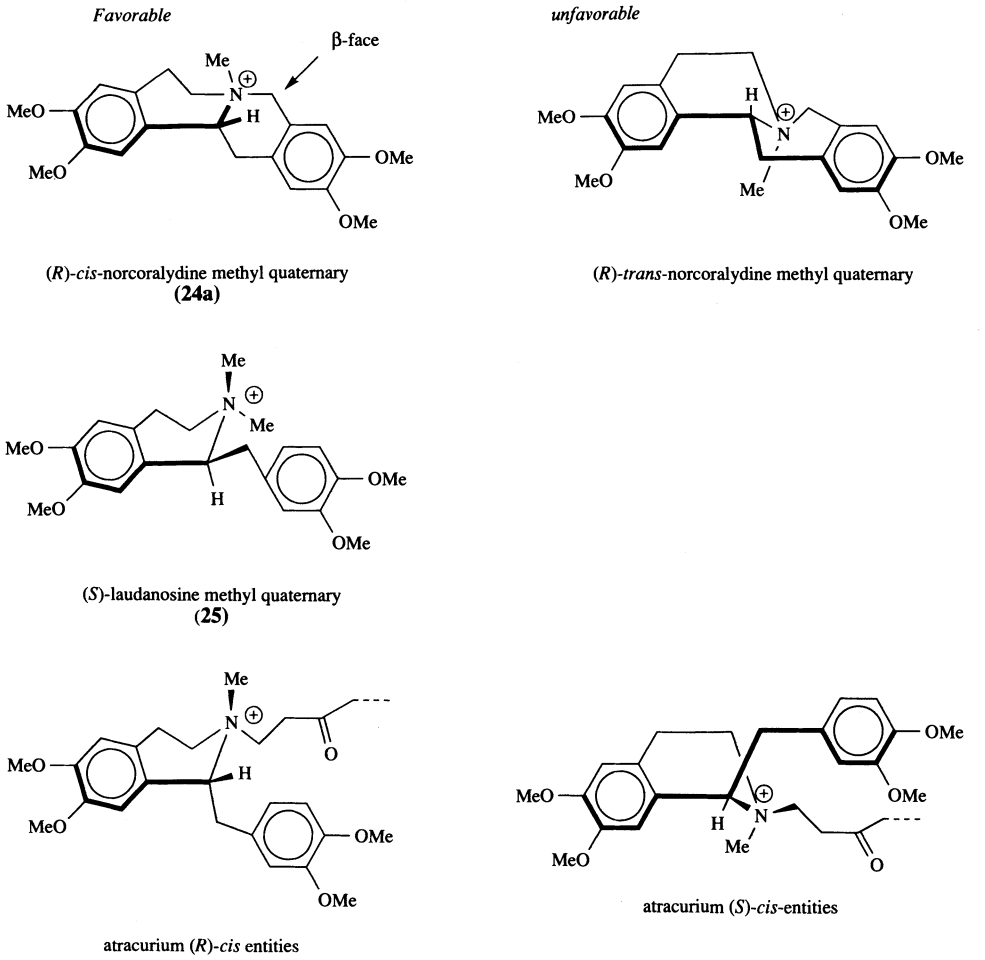


FIGURE 10.2. Configurational features of atracurium (*R*)- and (*S*)-*cis*-entities relative to norcoralydine methiodides.

due to its *mono*-quaternary nature and lack of a second nitrogen, is nevertheless more potent as an NMB agent than its antipode (5.7 vs. 1.3; tubocurarine = 100). These two isomers, respectively, function as models for the chemical and stereochemical environment applying at the (*S*) (a) center and (*R*) (b) center of tubocurarine and related substances (see page 336).

Potency variation among the tubocurarine family correlates well with data from the above *mono*-quaternary probes.[77] Thus, (+)-tubocurarine can only present its nonquaternary center a, of absolute stereochemistry (*S*), to the receptor in a like manner to favored (*R*)-*cis*-norcoralydine, whereas the quaternary center b corresponds to unfavored (*S*)-norcoralydine stereochemistry. In isotubocurarine[64] (Table 10.3), with inverted quaternary and protonated nitrogens, it is now the quaternary group that is associated with center a, with its favorable (*R*)-norcoralydine conformation, while center b, the nonquaternary one, is associated with unfavorable (*S*)-norcoralydine geometry. The higher potency of isotubocurarine (=230) over tubocurarine (=100) is consistent with this analysis.

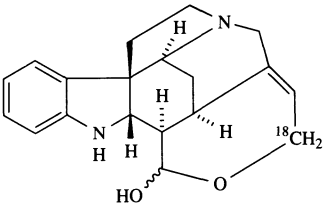
Additionally, while potency enhancement to 290 in the *bis*-quaternary chondrocurarine (**16d**; $R' = CH_3$), with identical (*R*,*S*) stereochemistry to isotubcurarine, is only marginal, the *bis*-quaternary (+)-curarine, with doubly favored (*S*,*S*) stereochemistry, has a potency of 350, while (−)-curarine, with doubly unfavored (*R*,*R*) stereochemistry, has a potency of 130 (all relative to tubocurarine = 100; the enhanced potency of (−)-curarine over tubocurarine, despite its unfavored stereochemistry, may be accounted for by its *bis*-quaternary status—cf. (+)-tubocurarine and chondrocurarine).

10.6.3. Alkaloids of Calabash Curare (Strychnos)

Most of the alkaloids of calabash curares are obtained from the barks of various *Strychnos* species, particularly *S. toxifera*. Of the forty or so complex alkaloids present, only about nine possess significant neuromuscular blocking activity and these are all *bis*-quaternary compounds. The remaining alkaloids are mainly *mono*-quaternaries or *mono*-amine protonated salts. This section will be confined to the *bis*-quaternary agents which have been grouped into three groups of three by Waser[78]:

1. The dihydrotoxiferine group: dihydrotoxiferine, C-alkaloid H, and toxiferine 1.
2. The curarine group: C-curarine, C-alkaloid G, and C-alkaloid E.
3. The calebassine group: C-calebassine, C-alkaloid F, and C-alkaloid A.

All of the compounds mentioned above actually have four nitrogen atoms present in their structure, two of which are indole-like or, in some cases, more correctly, aniline-like nitrogens of weakly basic character. The structure elucidation of toxiferine 1 and dihydrotoxiferine has its roots in the observation that the Wieland–Gumlich aldehyde (**26**; shows cyclic hemiacetal form with C18 OH),which is a degradation product from strychnine (**27**), is a *Strychnos* alkaloid.[79] The



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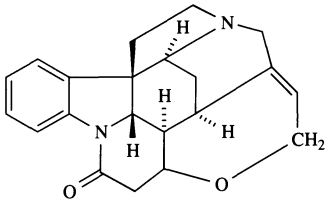

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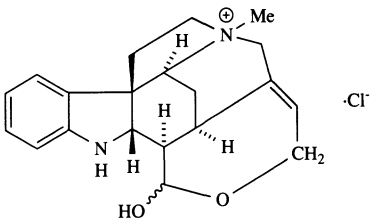

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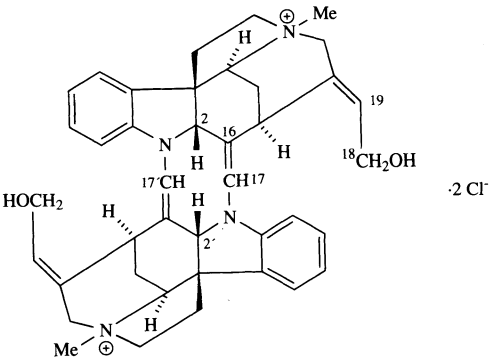
absolute stereochemistry of this aldehyde follows from the relative and absolute stereochemistry known for strychnine.[80] The corresponding methochloride of compound **26** is hemitoxiferine 1 (**28**) which undergoes a condensation reaction in acetic acid to form toxiferine 1 (**29**). Similarly, the related hemidihydrotoxiferine 1 (**30**) condenses to form dihydrotoxiferine (**31**). This condensation clearly unites two molecules by mutual attack of indole nitrogens at electron-deficient aldehyde carbonyls (generated in acidic media for **28**), followed by double dehydration. Chemical evidence,[81] supported by NMR data,[82,83] confirm the structures to be as shown, and the relative and absolute stereochemical detail follows from that known in **28** and **30**. Both toxiferine 1 and dihydrotoxiferine display strong levorotatory signs at 589 nm ($[\alpha]_D$ *ca*–600° in aqueous or aqueous ethanolic solutions). C-alkaloid H (**32**) is a "hybrid" molecule containing H at C18 and OH at C18′.



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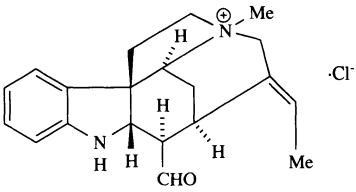

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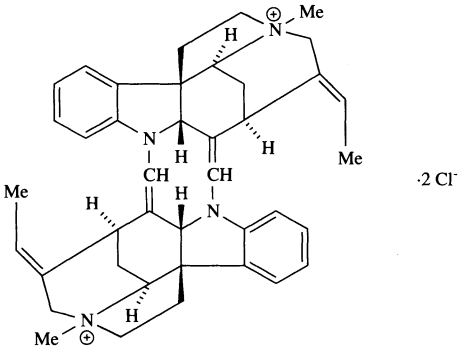

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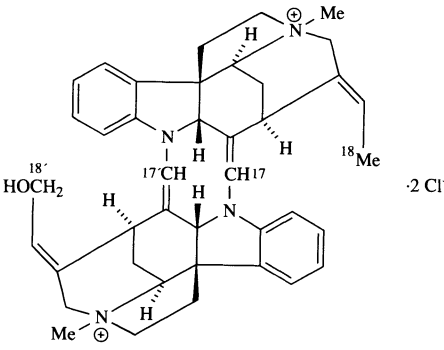

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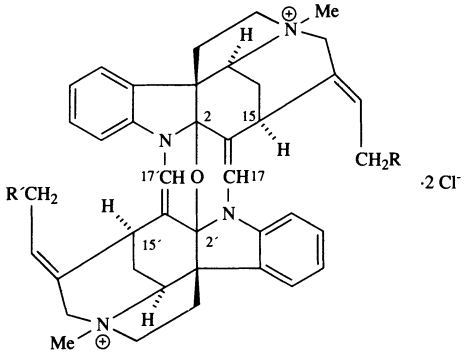
The three groups mentioned above are in fact interrelated, and it is possible to effect conversions in the laboratory. For example, toxiferine 1 (**29**) can be converted to its corresponding member in the curarine group (C-alkaloid E; **33**; R = R′ = OH) and its corresponding member in the calebassine group (C-alkaloid A; **34**; R = R′ = OH), in both cases by irradiation in the presence of molecular oxygen.[84]

The curarine group retains the structural features of the dihydrotoxiferine group but contains an additional ether function linking position 2 and 2′.[85] There

is therefore overall symmetry with the exception of the "hybrid" C-alkaloid G (**33**; R = H; R′ = OH). The structure of C-curarine is **33**; R = R′ = H.



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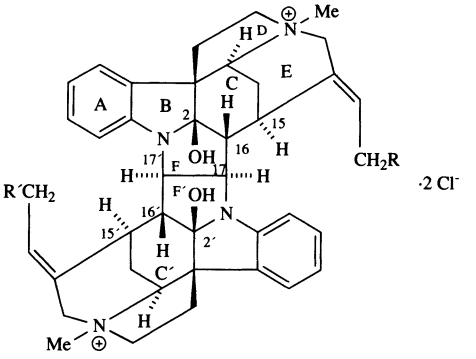
The calebassine group is chemically and stereochemically yet more complex. Ignoring the position-18 substituent, if the basic structure of the dihydrotoxiferine group is inspected, the changes in the calebasinne group are: (i) reduction of the C16, C17 and C16′, C17′ double bonds, creating further asymmetric centers at C16 and C16′; (ii) the presence of an OH group at positions 2 and 2′; and (iii) bondage of positions 17 and 17′, creating further centers of asymmetry (see **34**). By careful consideration of both Dreiding models and NMR spin–spin couplings, it was possible[86] to relate newly created asymmetric centers to known ones, and thereby establish the overall absolute stereochemistry. Thus, Dreiding models indicate that *cis*-fusion alone is allowed for rings C/F, C′/F′, and F/F′ (see **34**). Analysis of coupling data from NMR reveals a *trans*-relationship between protons at C16 and C15, and C16′ and C15′; this *trans*-relationship also applies for protons at C16 and C17, and C16′ and C17′. All aspects have been confirmed by X-ray analysis of calebassine diiodide.[87] Full structures are: C-calebassine (**34**; R = R′ = H); C-alkaloid A (**34**; R = R′ = OH); C-alkaloid F (**34**; R = H; R′ = OH).



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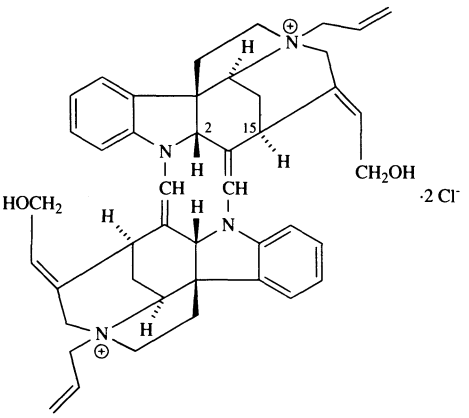
Readers requiring a fuller treatment of the chemistry and stereochemistry of these and related alkaloids may refer to two excellent reviews by Battersby.[88,89]

It is possible to dequaternize toxiferine 1 and related *bis*-quaternaries by pyrolysis. Requaternization of *bis*-nortoxiferine with two equivalents of allyl

chloride yields alcuronium chloride (**35**, "Alloferin"), which is the only compound of this class used in medicine. This agent, *N,N'*-diallyl *bis*-nortoxiferine 1, was synthesized in an effort to reduce the duration of action of potent, long-acting toxiferine 1, by offering the body a potential site for biotransformation in the allyl functionality placed on permanently charged nitrogen.[44] The drug has about 1.5 times the potency of (+)-tubocurarine in man.[90]



35

The calabash curares considered above are generally more potent NMB agents than tubocurarine, and more toxic. Table 10.6 provides the potency and duration of the important calabash agents in the mouse head-drop test.[46,78] Figure 10.3 shows potency and duration data for seven of the agents tested on cat gastrocnemius muscle.[91] The high potency and relatively long duration of action of toxiferine 1 and C-alkaloid E are clear from these data.

TABLE 10.6.
NMB Potency of *Bis*-Quaternary Alkaloids from *Strychnos toxifera*, Together with Duration, in the Mouse Head-Drop Test

Compound (Group classification in parentheses)	Potency (μg/kg)[a]	Duration (min)
Dihydrotoxiferine (i)	30	5.5
C-alkaloid H (i)	16	3.7
Toxiferine 1 (i)	9	12
C-curarine (ii)	30	4
C-alkaloid G (ii)	5	7
C-alkaloid E (ii)	4	18
C-calebassine (iii)[b]	240	3
C-alkaloid F (iii)	75	1.3
C-alkaloid A (iii)	70	2

[a] Dose to produce head drop in the mouse.
[b] Previously called C-toxiferine II.

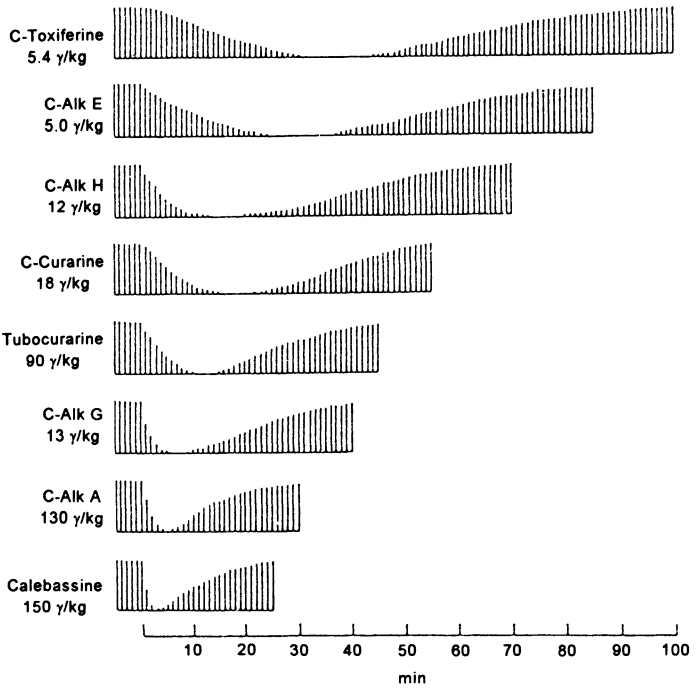
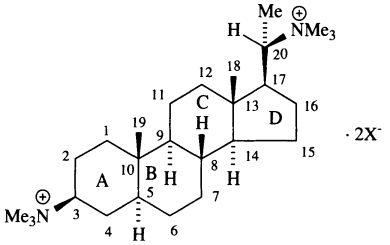


FIGURE 10.3. Potency and duration of action of seven calabash NMB agents, together with (+)-tubocurarine, in the cat gastrocnemius muscle (after Waser).[91]

10.6.4. Steroidal Neuromuscular Blocking Agents

Interest in the steroid skeleton as one on which functionalities encouraging neuromuscular blocking activity might be attached originated from studies on the steroid alkaloid malouetine (**36**), obtained from *Malouetis bequaertiana* E, Woodson.[94,95] This agent is almost as potent as tubocurarine in the rabbit and of shorter duration of action. Full stereochemical detail, including steroid ring fusions, are presented in structure **36**; see Shoppee[96] for a fuller treatment of steroid chemistry and stereochemistry.



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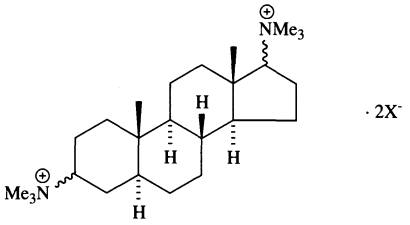
Thus, the proton at position 5 is α (rings A and B are *trans*-fused), the quaternary functionalities at position 3 and 17 are β, methyls 18 and 19 (conventionally presented) on C-13 and C-10 respectively are β, the stereochemistry at C-20 is α, rings B/C are typically *trans*-fused, and rings C/D are also *trans*-fused.

Studies on malouetine isomers (the 3β,20β-, 3α,20α-, and 3α,20β-stereoisomers) reveal that the effect of stereochemistry at these positions is of little consequence.[97,98] This observation concurs with potency data secured for the four stereoisomers possible for the 3,17-disubstituted androstane quaternaries (**37**). Not only do the 3α,17α- and 3β,17β-isomers have similar potencies, but so do the 3α,17β- and 3β,17α-compounds, the latter pair having onium groups projecting from opposite sides of the steroid nucleus.[99,100]



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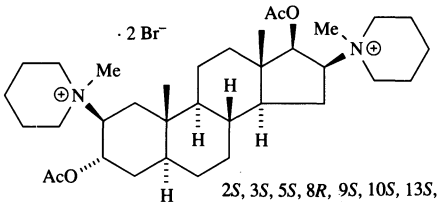
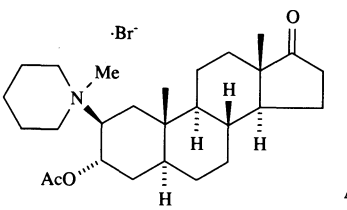
It would seem that among steroidal NMB agents, and within certain limits, interonium distance *per se* is relatively unimportant, and high potency is seen among agents containing two nitrogen atoms (at least one of which is quaternary) appropriately substituted and flanked by compatible additional functional groups. The selection of the drug pancuronium from a large number of related candidates illustrates how precise the requirements are.

The synthesis of pancuronium (**38**) followed from work on 3α-acetoxy-2β-piperidino-5α-androstan-17-one (**39**) which, although possessing a single nitrogen, nevertheless has 1/16th the potency of (+)-tubocurarine.[101] The acetylcholine-like fragment seen in ring A of **39** was considered to assume a preferred conformation resembling a specific molecular conformation of the natural hormone[102] (see acetylcholine adjacent to **39**). However, X-ray analysis of pancuronium bromide water, methylene chloride solvate reveals that ring A adopts a skew-boat conformation to avoid severe N...C10-Me diaxial interactions,[103] and similar conformations are thought to apply for the solute from ^{1}H-NMR evidence.[128]



2*S*, 3*S*, 5*S*, 8*R*, 9*S*, 10*S*, 13*S*, 14*S*, 16*S*, 17*R*

38



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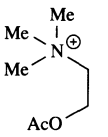
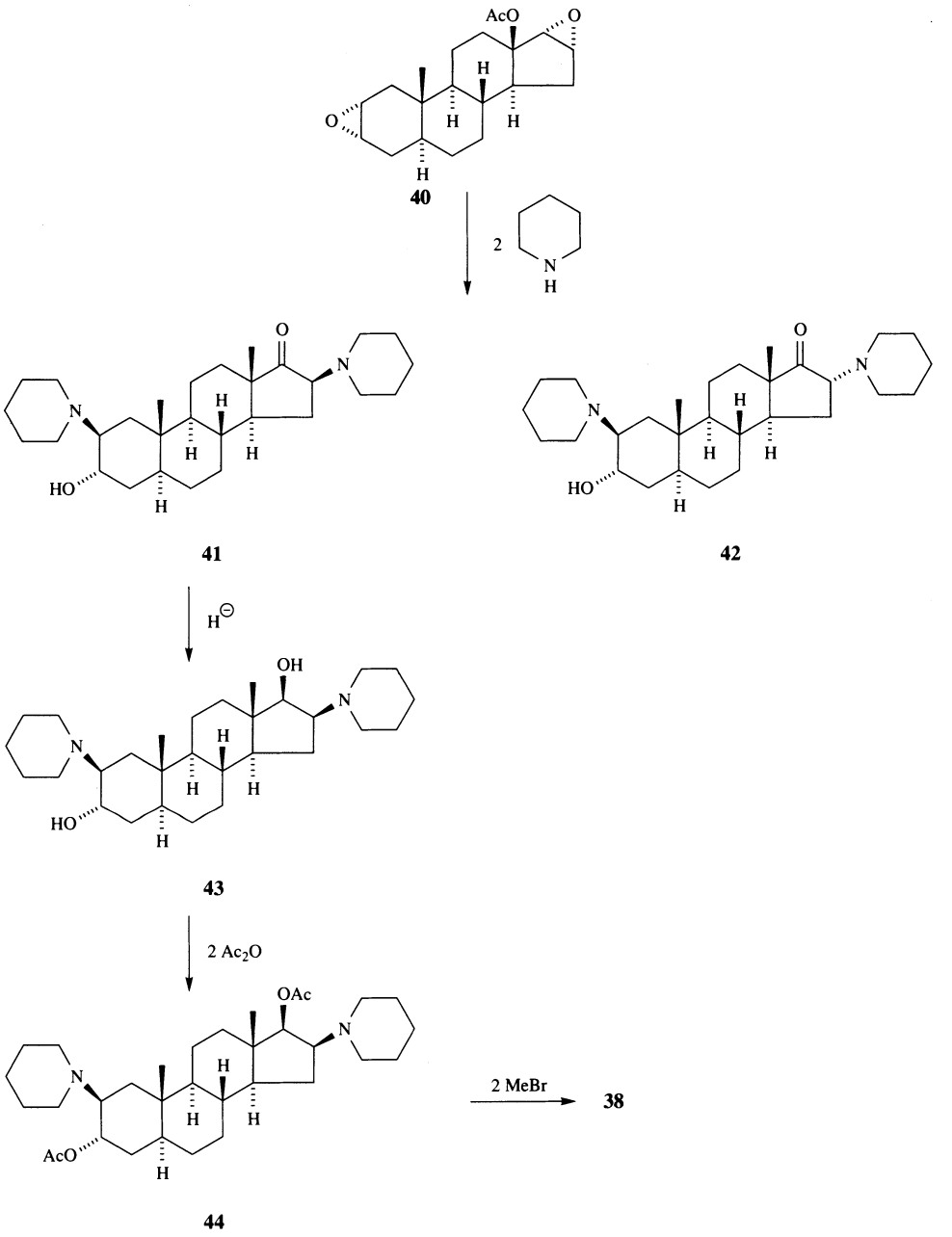


Acetylcholine

Pancuronium was one of a large series of compounds synthesized to extend the acetylcholine-like fragment principle to ring D, thereby giving steroids with two nitrogen atoms and enhanced prospects of high NMB activity. The range included *bis*-quaternary diesters of varying type, corresponding diols, and some *mono*-quaternary agents.[102]

The synthesis of pancuronium illustrates the strategy employed in securing such compounds (see Scheme 10.2). 2α,3α: 16α,17α-Diepoxy-17β-acetoxy-5α-



Scheme 10.2.

androstane (**40**) on treatment with aqueous piperidine yields principally 2β,16β-dipiperidino-3α-hydroxy-5α-androstan-17-one (**41**), contaminated with some 16α-epimer (**42**).[102] The structure of **41** was established from the X-ray analysis of pancuronium itself.[103] Borohydride reduction of C-17 carbonyl in **41** gave the 17β-alcohol (**43**) in 96% yield. Acylation with a range of agents furnishes a variety

of diesters, including the pancuronium precursor *bis*-amine **44**. Full quaternization yields pancuronium (**38**), the full name of which is 3α,17β-diacetoxy-2β,16β-dipiperidino-5α-androstane dimethobromide. However, the ring A nitrogen is more sterically hindered than the ring D one, and it is possible to selectively *mono*-quaternize the C-16 piperidino nitrogen of **44** to give **45**. This latter compound is called vecuronium, a NMB agent introduced into clinical practice in 1983 after being reexamined in 1979.[104]

Table 10.7 provides data obtained from anesthetised cat sciatic–gastrocnemius preparations for a representative group of agents prepared.[102] The table emphasizes the fundamental importance of the presence of two nitrogen atoms and

TABLE 10.7.
Neuromuscular Blocking Activity of Some Quaternary 5α-Androstanes[1]

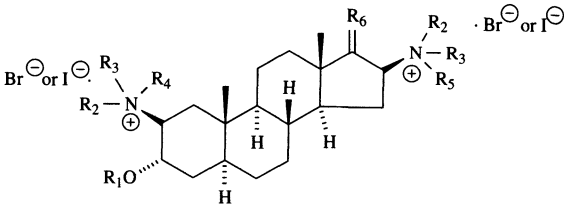
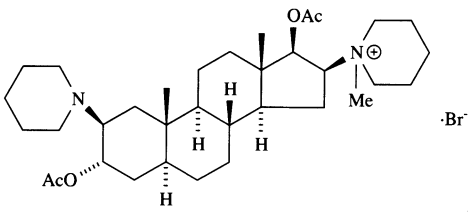


Table entry	R_1	R_2R_3N	R_4	R_5	R_6	Potency (Tubocurarine = 1.0)
a[2]	Ac	Pi	CH_3	CH_3	β-AcO,H	9.41
b[3]	Ac	Pi	—[4]	CH_3	β-AcO,H	6.00
c	Ac	Pi	CH_3	CH_3	α-AcO,H	6.15
d	Ac	Mo	CH_3	CH_3	β-AcO,H	3.44
e	Ac	Mo[5]	CH_3	CH_3	β-AcO,H	0.95
f	H	Mo	CH_3	CH_3	β-OH,H	0.01
g[6]	Ac	Pi	CH_3	CH_3	β-OH,H	1.72
h	H	Pi	CH_3	CH_3	β-OH,H	0.55
i	H	Pi	CH_3	CH_3	β-AcO,H	5.15
j	Ac	Pi	$CH_2C\equiv CH$	$CH_2C\equiv CH$	β-AcO,H	6.31
k	Ac	Pi	CH_3	$CH_2CH=CH_2$	β-AcO,H	7.23
l	Ac	Pi	—[4]	$CH_2CH=CH_2$	β-AcO,H	3.44
m	H	Pi	$CH_2C\equiv CH$	$CH_2C\equiv CH$	β-OH,H	0.77
n	EtCO	Pi	CH_3	CH_3	β-EtCOO,H	7.10
o	Me_3CCO	Pi	CH_3	CH_3	β-Me_3CCOO,H	3.80
p	Me_3CCO	Pi	CH_3	CH_3	β-OH,H	1.70
q	PhCO	Pi	CH_3	CH_3	β-PhCOO,H	1.00
r	PhCO	Pi	CH_3	CH_3	β-OH,H	0.84
s	Ac	—[7]	CH_3	CH_3	β-AcO,H	0.40

[1] Data from Buckett, Hewett, and Savage.[102] Potency is reduced relative to tubocurarine to less than 1.0 in all cases cited where the 16β- and 17β-groups are interposed. The two molecules bearing the ring A functionalities alone or ring D functionalities alone are essentially inactive, as is the diHCl of pancuronium precursor *bis*-amine (**44**).
[2] Pancuronium bromide (**38**).
[3] Vecuronium bromide (**45**).
[4] Not quaternized.
[5] 2α instead of 2β.
[6] Data for dibromide salt quoted.
[7] 2β-piperidino-16β-dimethylamino. Pi is piperidino, Mo is morpholino. 5β-epimers are generally less potent than corresponding 5α-compounds (see, for example, Bamford *et al.*[100]), reflecting receptor preference for the relatively flat steroid nucleus in the latter over the more folded nucleus of the former.

the fact that at least one of these must be quaternary (see footnotes to Table 10.7; also entries a and b for pancuronium **38** and vecuronium **45**, respectively).



45

Diesters are clearly superior to corresponding mono-esters, mono-alcohols, or di-alcohols, with intact position-17 ester being the more important (compare entries a, h, and i, for example). The piperidino group is superior to morpholino (compare entries a and d), and smaller, noncyclic entities are even more detrimental (compare a and s). Potency drops on inverting stereochemistry at C-2 (compare d and e), and does so even more significantly when the 16β- and 17β-groups are interposed (see footnotes). Somewhat in contrast to the tubocurarines,[61] quaternization by higher alkyl halide retains activity (see j, k, l, and m).

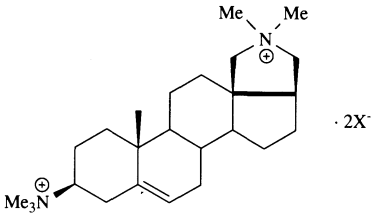
A fuller structure-action analysis on compounds of this type may be found in a review by Riesz *et al.*[105] Related compounds in which the nitrogen-containing ring is enlarged to, for example, hexahydro-1*H*-azepin-1-yl are described.[106]

Pancuronium and vecuronium are both widely used clinically. Pancuronium is some five to six times more potent than tubocurarine, with a somewhat slower onset of action and similar duration of action.[107] Side-effects of the agent include tachycardia, hypertension, and antipseudocholinesterase activity.[108,109] This drug has been in use since 1968 (marketed as "Pavulon") and efforts by the marketing company (Organon) since then have been directed toward developing related substances with a quicker onset and shorter time-course of action. Vecuronium is about equipotent with pancuronium in man, with a comparable onset of action but a duration approximately half that of pancuronium.[110] It is a drug remarkably free of side-effects such as histamine release, ganglion blockade, and vagolytic and cardiovascular effects.[111,112] The background to the emergence of vecuronium from the pancuronium series is reviewed.[113,114] It is considered that the freedom from side-effects in this drug is associated with the absence of the acetylcholine-like feature in ring A. The potency-inducing ring D (see Table 10.7) is known to involve a hydrogen bonding system within the acetylcholine-like fragment showing two quasi-rings, and this is much more complex than the system applying in ring A of pancuronium.[102,103]

The search for a steroidal agent with a more rapid onset of action and shorter time-course to vecuronium continues. However, a paper by Bowman *et al.*[115] suggests that brief duration of action coupled with a fast onset may, in general, only be produced with compounds of relatively low potency. In a subsequent paper[129] two short acting 17-butyryl analogues of vecuronium were studied: Org 9616 (3α-acetoxy-17α-butyryloxy-2β,16β-dipiperidino-5α-androstane 16-*N*-methobromide), which is epimeric at position 17, and Org. 7617 (3α-acetoxy-17β-butyryloxy-2β,16β-dipiperindino-5α-androstane 16-*N*-allobromide). In the cat

tibialis muscle, the compounds were, respectively, one-fifth and one-tenth as potent as vecuronium as NMB agents.

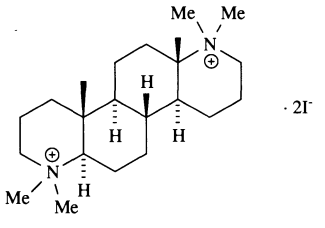
In general, pregnane derivatives, of which malouetine (**36**) is an example, are less potent NMB agents than the androstanes.[105] Potency data for the *N,N*-dimethyl quaternary (**46**) of conessine and its analogues are available.[105,130]



46

Another important group of steroidal NMB agents are the azasteroids, some of which display potencies close to that of pancuronium, but of shorter duration. The first examples have the 4,17a-diaza-D-homo system, and the first agent prepared was 4,17a-4,17-diaza-D-homo-5α-androstane dimethiodide (**47**).[116] This agent is essentially equipotent with tubocurarine, and its action is rapid in onset and of shorter duration.[117] However, an internitrogen distance of 8.17 Å[118] is likely to be responsible for severe ganglion-blocking effects.

This problem with ganglion blockade can be overcome if either the position-4 or position-17a nitrogen is removed from the ring and, as quaternary, becomes attached to it. For example, 4-methyl-17β-dimethylamino-4-aza-5α-androstane dimethiodide (**48**)[119] which has an interonium distance of 9.02 Å,[118] is also of short duration, rapid in onset, and equipotent with tubocurarine. It is only weakly active as a ganglion blocking drug.[120]



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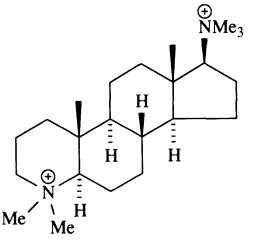

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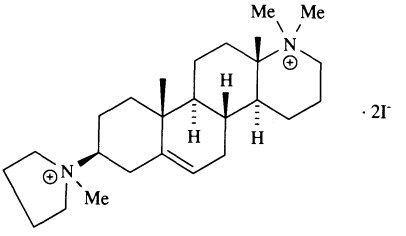
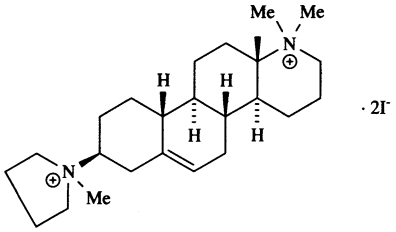
The prototype of the group is chandonium iodide (**49**), which is a 3β-pyrrolidino-17a-aza-D-homo-androstene diquaternary.[119] This agent, which has



49

undergone clinical assessment,[121] is almost as potent as pancuronium in anesthetized cats.[122] X-ray analysis reveals an internitrogen distance of 10.3 Å in chandonium[123] which compares with the value of 11.1 Å obtained from similar analysis of pancuronium.[103]

The 5,6-dihydro derivative (β at position 5) of chandonium has half the potency of the parent,[124] while independent work by Hungarian workers[125] reveals that the 3α-epimer of dihydrochandonium is essentially equipotent with this latter agent. Interestingly, 19-norchandonium iodide (**50**) is equipotent with its mirror image form (**51**); both enantiomers are 3–4 times less potent than chondonium itself.[126] Molecular orbital calculations undertaken on chandonium iodide and some of its relatives concur with conformational data obtained from X-ray analysis.[127]



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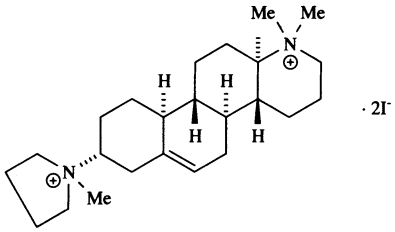
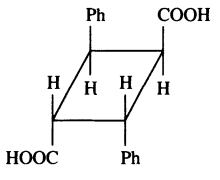


51

10.6.5. Miscellaneous NMB Agents

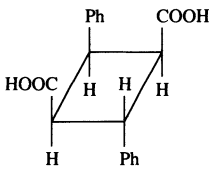
10.6.5.1. Agents Related to Thesine

There is considerable interest, particularly in Russia, in *bis*-tertiary amine salts and quaternaries that are part of esterified truxillic acids. The truxillic acids are isomeric forms of 1,3-diphenylcyclobutane-2,4-dicarboxylic acid, and the α-form has both phenyl groups and both carboxyl groups *trans* (as in **52**).[132] The γ- and ε-isomers are, respectively, **53** and **54**.



α

52



γ

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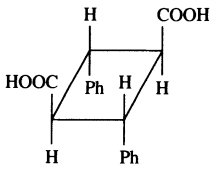
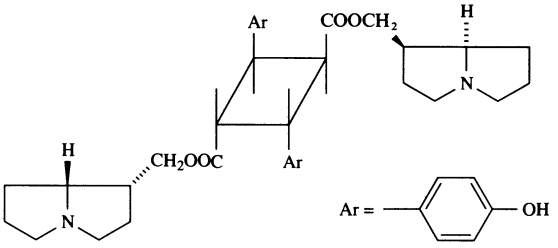


ε

54

The alkaloid thesine, obtained from a central Asian plant called *Thesium minkwitzianum*, is the *bis*-4-hydroxymethylpyrrolizidine ester (**55**) of *p,p'*-dihydroxy-α-truxillic acid, and the NMB activity it displays, as well as its dimethiodide,[131] has encouraged study on simpler amino ester truxillic acids.

Table 10.8 shows the NMB activity of a series of ester and amide *bis*-quaternary derivatives of α-, γ-, and ε-truxillic acids. In all cases, superior activity is observed in derivatives of the α-acid, with ε-derivatives displaying intermediate

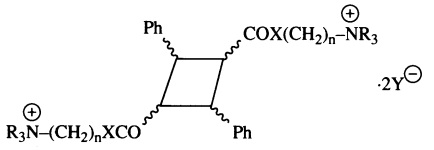


55

potency. It is also notable that whereas the α-amide (**56j**) is more potent than its corresponding ester (**56g**) and related esters (**56a** and **56d**), the γ- and ε-amides are dramatically less potent than their ester relatives (cf. entries k, l with b, c, e, f, h, i).[132,133] These potency orders have inevitably led to attention being focused on ester and amide quaternaries of α-truxillic acid.

Table 10.9 provides the names and NMB activities of the most significant α-truxillic acid ester and amide *bis*-quaternaries studied.[134] The compounds anatruxonium, cyclobutonium, and truxilonium are utilized clinically (mainly in

TABLE 10.8.
Neuromuscular Blocking Activities of Some Stereoisomeric Truxillic Acid Quaternary Esters and Amides



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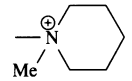
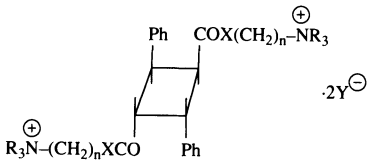
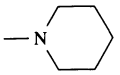
Entry	Isomer	X	*n*	$^{+}NR_3^{-}$	NMB(μg/kg) Rabbit head drop[1]	NMB(μg/kg) Cat[2]
a	α	O	4	$^{+}NEt_2Me$	48	250–350
b	γ	O	4	$^{+}NEt_2Me$	250	800–1,000
c	ε	O	4	$^{+}NEt_2Me$	130	400–500
d	α	O	4		46	150–200
e	γ	O	4		153	400–500
f	ε	O	4		110	200–250
g	α	O	3	$^{+}NEt_2Me$	37.5	150–250
h	γ	O	3	$^{+}NEt_2Me$	260	900–1,100
i	ε	O	3	$^{+}NEt_2Me$	126	400–450
j	α	NH	3	$^{+}NEt_2Me$	21.3	40–50
k	γ	NH	3	$^{+}NEt_2Me$	3600	10,000–12,000
l	ε	NH	3	$^{+}NEt_2Me$	1200	6,000–8,000
Tubocurarine chloride				—	137	180–230

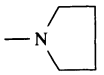
[1] Mean effective doses (ED_{50}). Agents administered by bolus within 3–5 s into auricular vein.
[2] Sciatic nerve–gastrocnemius muscle preparation in cats anesthetized with chloralose/urethane.

TABLE 10.9.
NMB Activities of Some Ester and Amide *Bis*-Quaternaries Derived from α-Truxillic Acid



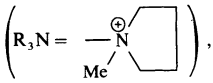
Compound name	*n*	X	$\overset{\oplus}{N}R_3$	NMB activity (μg/kg iv) In the cat[a]	Human myorelaxation[b]
Anatruxonium	3	O	$C_5H_{10}\overset{\oplus}{N}Et$	100–130	100–150
Cyclobutonium	3	O	$\overset{\oplus}{N}Et_2Me$	130–180	120–150
Pyrocyclonium	3	O	$C_4H_8\overset{\oplus}{N}Et$	120–140	—
Truxilonium	4	O	$C_5H_{10}\overset{\oplus}{N}Et$	150–180	120–150
Amidonium	3	NH	$C_5H_{10}\overset{\oplus}{N}Et$	18–22	—
Dipyronium[c]	3	NH	$C_4H_8\overset{\oplus}{N}Me$	18–22	—

$C_5H_{10}N$ is 

C_4H_8N is 

[a] Sciatic nerve–gastrocnemius muscle in cats anesthetized with chloralose-urethane. Tubocurarine chloride has a value of 180–230 μg/kg.

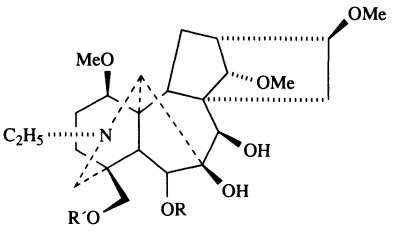
[b] Where appropriate. Tubocurarine chloride has a value of 400–500 μg/kg.

[c] The corresponding *bis*-tertiary amine, called pyrocurinum ($R_3N =$ ), of this agent has a potency in man 2–3 times that of tubocurarine, and sees clinical application in Russia.

Russia), and they are approximately three times as potent as (+)-tubocurarine in man.

10.6.5.2. Delphinium Alkaloids

The delphinium alkaloids are briefly mentioned here as they are an interesting class of NMB agents that lack a quaternary nitrogen function. These alkaloids are obtained from various species of *Delphinium* (for example, delphinine itself is obtained from *Delphinium staphisagria* L), and they are all tertiary bases. The compounds are fairly complex polycyclic diterpenes, and the basis of their structure is the lycoctonine ring system (**57**; lycoctonine itself has R = Me; R′ = H). Fundamental aspects of chemistry and stereochemistry may be obtained from reviews by Stern.[135,136]



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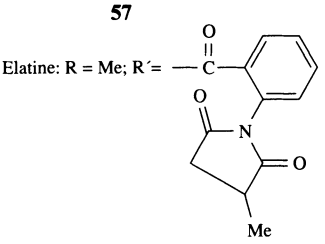
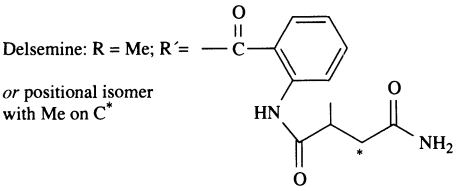


(the two *cis* geminal hydroxyl hydrogens are also replaced by —CH_2— in this molecule)

Methyllyaconitine: R = Me; R´as above for elatine

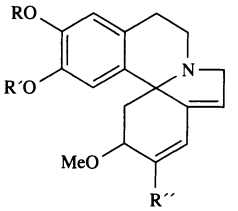


The structures of three pharmacologically active agents are given above,[137,138] and details of other compounds studied may be obtained from a review.[139]

Thus, active compounds require *N*-substituted *O*-anthranoyl derivatization of the parent lycoctonine. All three agents are nondepolarizing and of high toxicity. Elatine has approximately half the potency of tubocurarine in cats, while methyllycaconitine is about five times less potent again. Delsemine is about seven times less potent than elatine. These alkaloids have seen clinical application in Russia for certain extrapyramidal conditions, but have now been displaced.

10.6.5.3. The Erythrina Alkaloids

The erythrina alkaloids, like the delphinium agents, are tertiary bases rather than quaternaries. The compounds come from the seeds of a number of species of

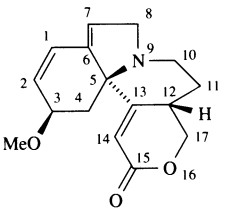


R = R´= Me; R´´= OH is Erythratine

58

the genus *Erythrina*, one example being *E. americana*. The species grow in tropical and subtropical regions of the earth.

Chemically, the alkaloids are of the tetrahydroisoquinoline type (**58**),[140,141] or the lactone bases α-erythroidine (**59**) and β-erythroidine (**60**).[142] The lactones are considerably more potent, and of greater interest, than the tetrahydroisoquinolines, and the former will therefore be considered.



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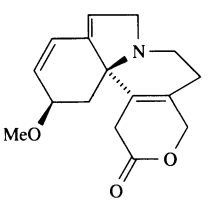

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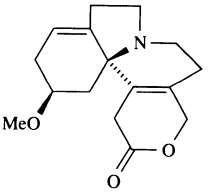
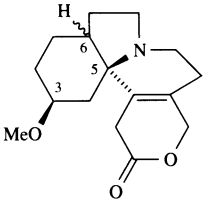
The most active compound is a partial reduction product of β-erythroidine, dihydro-β-erythroidine (**61**), the absolute stereochemistry of which has been established by X-ray analysis[147] and by traditional chemical means[151] as 3*R*,5*S*. The stereochemistry in β-erythroidine itself follows. In addition, the chemical conversion of α-erythroidine into β-erythroidine[152] establishes identical stereochemistry at positions 3 and 5 in the former molecule. The further asymmetric center in α-erthroidine (at position 12) is known to be *S* from work by Hill and Shearer[153] in which (+)-*O*-ethylphenyl-3-tetrahydrofuryl ketone, a degradation product of the parent alkaloid, is related to (+)-methylsuccinic acid, of known stereochemistry.



61

Further reduction of the 1,6-double bond in dihydro-β-erythroidine yields α- and β-tetrahydro forms about position 6 (see **62**). A 1986 paper by Hider *et al.*[154] refers to the two epimers as *cis*- and *trans*-tetrahydro-β-erythroidine (*cis*/*trans* C3-H/C6-H) but the author knows of no correlation of those with the α- and β-forms quoted by Unna *et al.*[143]



62

Table 10.10 provides potency data from frogs for proton salts of β-erythroidine, dihydro-β-erythroidine, α- and β-tetrahydro-β-erythroidine, and the methiodide of β-erythroidine.[143]

TABLE 10.10.
NMB Activity of Derivatives of β-Erythroidine in Frogs[a]

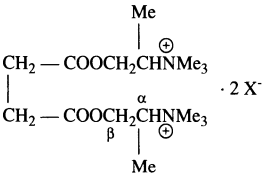
Compound	Paralyzing dose (mg/kg)
β-Erythroidine · HCl[b]	3–8
β-Erythroidine methiodide	200
Dihydro-β-erythroidine · HCl	0.6
α-Tetrahydro-β-erythroidine · HBr	200
β-Tetrahydro-β-erythroidine · HBr	0.5

[a] Administered by lymph sac injection.
[b] α-Erythroidine is a very low potency agent. The potency of (+)-tubocurarine in this test is approximately 2.4 mg/kg.

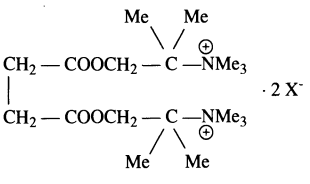
It is interesting that quaternization of β-erythroidine greatly reduces potency, and activity is considerably increased on reduction to dihydro-β-erythroidine. The enhanced potency in dihydro-β-erythroidine is likely to be due to a base-strengthening effect that arises because replacement of the conjugated saturation in the parent by a single double bond in position 1,6 after reduction removes most of the influence of the double bond on nitrogen.[43] In the tetrahydro compounds there is a considerable difference in molecular shape between the two epimers, which may account for pharmacological potency variation. Inspection of models reveals that, in the molecule with *cis*-C3-H/C6-H geometry (6*S*), the spiro-ring is largely above the other three, essentially coplanar, rings. The epimer with 6*R*-geometry, however, has only two coplanar rings, while the remaining two are orientated one above and one below this plane. Dimensionally, the former epimer approximates that of β-erythroidine and dihydro-β-erythroidine which may suggest that it is β-tetrahydro-β-erythroidine.

10.7. Stereochemistry in Depolarizing Agents

As indicated earlier, the structural requirements for depolarizing NMB activity are so precise that only modest variations of the prototype agent decamethonium (**15**; R = Me) or Suxamethonium (succinylcholine; **14**) is needed to change the mechanism to a nondepolarizing one, usually with attendent loss of potency. The α- and β-methyl derivatives of Suxamethonium provide a good example. The relative potencies (suxamethonium = 100; rabbit head drop) of succinyl*bis*-α-methylcholine (**63**), succinyl*bis*-α,α-dimethylcholine (**64**), and succinyl*bis*-β-methylcholine (**65**) are, respectively, 22.5, 6, and >0.36.[155] Additionally, the β-methyl derivative **65** is reported to be tubocurarine-like.[156] In a study of the various isomers of succinyl*bis*-α- and β-methyl-cholines,[157] the interesting observation has



63



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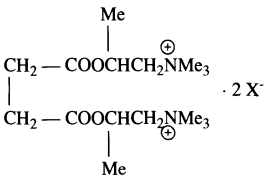

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TABLE 10.11.
Relative Potencies of the Enantiomers of Succinyl*bis*-α- and β-Methylcholines

Isomer	Potency (No. of moles ≡ 1 mole of succinylcholine)
(*S*,*S*)-(−)-α	2.6
(*R*,*R*)-(+)-α	1.8
(*S*,*S*)-(+)-β	890
(*R*,*R*)-(−)-β	1200

been made that, while the (*R*,*R*), (*S*,*S*), and (*R*,*S*) forms of succinyl*bis*-α-methylcholine are decamethonium-like, the (*R*,*R*) and (*S*,*S*) optical isomers of the β-methyl derivative are tubocurarine-like, and the (*R*,*S*) form, decamethonium-like.[158] Isomer potencies of the optical forms of **63** and **65** are given in Table 10.11.[158,159]

10.8. Snake Neurotoxins

Potent neuromuscular blocking activity is observed among various neurotoxic cobra venoms. These neurotoxins, particularly α-bungarotoxin, are highly selective polypeptide probes that bind pseudoirreversibly with AChR.[160] α-Bungarotoxin from *Bungaris multicinctus* has been particularly widely studied because of its ease of radioiodination [^{125}I] and its irreversible binding to receptor. This toxin has been used to establish that brain and muscle receptors are similar but not identical in terms of their physical, structural, and immunological properties.[161]

The most notable neurotoxins possess 61–74 amino acids, as a single chain, and it is significant that they contain substantial numbers of basic lysine and arginine residues, as well as four or five disulfide bridges. α-Bungarotoxin has 74 amino acids and the complete sequence has been established (MW 7983).[162–164] Other widely studied neurotoxins are:

Formosan cobra (*Naja naja atra*) venom: 62 amino acids.[165]

East India cobra (*Naja naja naja*) venom: 72 amino acids.[166]

Siamese cobra (*Naja Siamensis 3*) venom: 71 amino acids.[166]

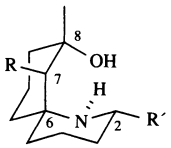
Cape cobra (*naja nivea*) α-, β-, and γ-venoms: 71, 61, and 61 amino acids, respectively.[167,168]

Some of the venoms have identical structures, but all have similar folded structures, the integrity of which is maintained by the four or five disulfide bridges. Masking of the basic amino acids in the venoms through, for example, carbamoylation substantially reduces the lethal curarizing action.[166]

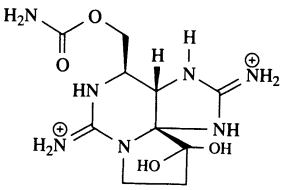
10.9. Other Toxins

Finally, mention should be made of toxins which block Na channel function, i.e., interfere with operation of the channel which forms an integral part of the

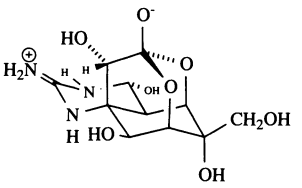
nACh receptor. Witkop and Gossinger[169] state that agents of this kind, in particular histrionicotoxin, antagonize the response to ACh without blocking the binding of the neurotransmitter and hence act in a noncompetitive manner. Such toxins include histrionicotoxin (**66**) and its relatives (from the skins of certain species of South American frogs), saxitoxin (**67**) (a mussel poison), and tetrodotoxin (**68**) (the notorious puffer fish poison).[170]



66 histrionicotoxin
(2*S*, 6*R*, 7*S*, 8*S*)
R = CH=CH–C≡CH (Z-configuration)
R′= CH_2CH=CH–C≡CH (Z-configuration)



67 saxitoxin



68 tetrodotoxin

All are relatively small molecules of complex cyclic nature and with several chiral centers of well-defined geometry. Their absolute configurations have been established by X-ray crystallography: histrionicotoxin,[172] saxitoxin,[171] tetrodotoxin,[170] and there is exact knowledge of the spatial arrangement of their funtional groups. However, little stereochemical SAR study of the group appears to have been carried out—possibly due to the synthetic inaccessibility of stereoisomers. The natural products tetrahydrohistrionicotoxin and allotetrahydrohistrionicotoxin are C-2 epimers.[169]

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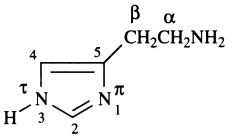
11

Histamine Receptors

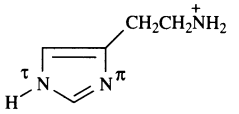
Agonists and Antagonists

11.1. Histamine: Biogenesis and Metabolism

Histamine [**1**; 5(4)-(2-aminoethyl)imidazole] is an achiral molecule which exists under physiological conditions as the monocation **2** in equilibrium with its N^{π}-H tautomer.[1] The amine arises as a result of the decarboxylation of *S*-histidine, and its biogenesis and metabolism (reviews)[2,3] offer several points of chiral interest.

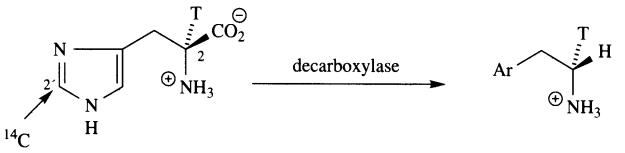


1

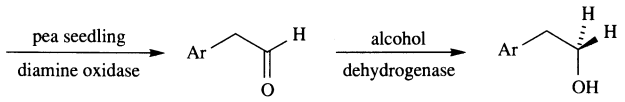


2

Battersby has shown that reactions catalyzed by mammalian histidine decarboxylase (pyridoxal phosphate cofactor) and bacterial enzymes (pyruvate residue cofactor) both proceed with retention of configuration.[4,5] *S*-[2-^{3}H, 2′-^{14}C]Histidine (**2a**) was used as substrate in the sequence **2a** → **2c**; the double radiolabeled substrate was a mixture of α-*S*-[2′-^{14}C]histidine and α-*S*-[α-^{3}H]histidine obtained by the



2a **2b**



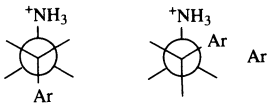
2c

nonstereoselective α-tritiation of *N*-acetylhistidine followed by selective hydrolysis of the α-*S*-amide by hog kidney acylase-I. With knowledge of the steric course of the deamination step (see Chap. 4, page 78), the virtual lack of ^3H in the histaminol **2c** (1.8% retention) establishes the *S*-configuration of the histamine **2b**. *S*-α-Fluoromethylhistidine is reported to be a specific inhibitor of L-histidine decarboxylase.[6]

Methylation of the imidazole ring of histamine at the N-τ-site by histamine-methyltransferase (HMT) forms one of the chief routes of its metabolic inactivation. The availability of certain α-methylhistamines (see p. 371) has enabled chiral aspects of this biotransformation to be investigated[7] by measuring the ability of various antipodes to take up $^{14}CH_3$ from *S*-adenosyl-L[^{14}C-methyl]methionine. At an optimal substrate concentration of 10^{-4}M, % methylation values were: histamine 96, *S*-(+)-α-methylhistamine 98, *R*-(−)-α-methyl antipode 63. The *N*-methylation of NHMe (N^{α}-methyl) substrates was much reduced: N^{α}-methylhistamine 12%; *S*-(−)-N^{α}-dimethyl 3, *R*-(+)-antipode 14. The dimethyl derivatives activated the enzyme in a nonstereoselective manner.

11.2. Receptor Subtypes and Agonist Conformation

There is good evidence that the physiological actions of histamine are mediated by at least three distinct classes of receptor.[8] The type designated H_1 occurs in the smooth muscle of guinea-pig ileum and bronchi and is antagonized by the tertiary amine class of antihistamine. The second receptor type, H_2, mediates the histamine actions of stimulating gastric acid secretion, inhibiting contractions of the rat uterus, and increasing the heart rate in the guinea pig. More recently a third, H_3 subclass has been described[9] which controls the synthesis and release of histamine from histaminergic neurones by a feedback mechanism[10]; such receptors are sited presynaptically and have been detected both peripherally and centrally.[11] To account for the H_1–H_2 dualistic actions of histamine, Kier proposed in 1968[12] that its ligand–receptor interactions involved two separate conformations, namely, *trans*-$^+NH_3$/Ar (**3**) at H_1 receptors and *gauche* (**4**) at H_2 sites (cf. related arguments



3 **4**

2 equivalent forms

in regard to the muscarinic and nicotinic actions of acetylcholine, p. 249). Kier's paper stimulated much interest in the conformation of histamine and its analogues and evidence based molecular orbital (MO) computations,[18] ^{1}H-NMR spectroscopy (solute state in D_2O), and X-ray crystallography (solid state) has been published and is summarized in Table 11.1. Of the computational methods, Ganellin *et al.*[13] believed the EHT procedure to be best suited to the conformational analysis since it led to results similar to those deduced from the NMR evidence. The NMR data for the monocation indicate a mole fraction of *trans*-rotamer close to 0.5, and 0.25 for each of the equivalent *gauche*-forms; preference

TABLE 11.1.
Calculated and Observed Population Percents of the *trans*-Conformer of Histamine by Different Methods (after Ganellin)[21]

Method[a]	Reference	Dication population	Monocation (2) population
EHT	13	64	55
	12		60
CNDO	13	> 99	< 1
INDO	14		< 1
PCILO	15		< 1
^{1}H NMR	13	54	45
		48	
	16	0.55	47
X-ray (acid phosphate dication)	17	100	

[a] EHT (extended Huckel theory); CNDO (complete neglect of differential overlap); INDO (intermediate neglect of differential overlap); PCILO (purtabative configuration interaction using localized orbitals).

for the *trans*-conformer in the dication is somewhat greater than that in the monoprotonated base.

The application of ^{1}H-NMR spectroscopy to investigations of the conformational equilibra of biologically active 1,2-disubstituted ethanes (XCH_2CH_2Y) presents difficulties of two kinds: analysis of the spectra, which are typically of the AA'BB' type, requires second-order treatment, while interpretation of the data in terms of conformation is by no means straightforward[19] (see also Chap. 8, p. 252). Although the two published studies used different ways to calculate individual

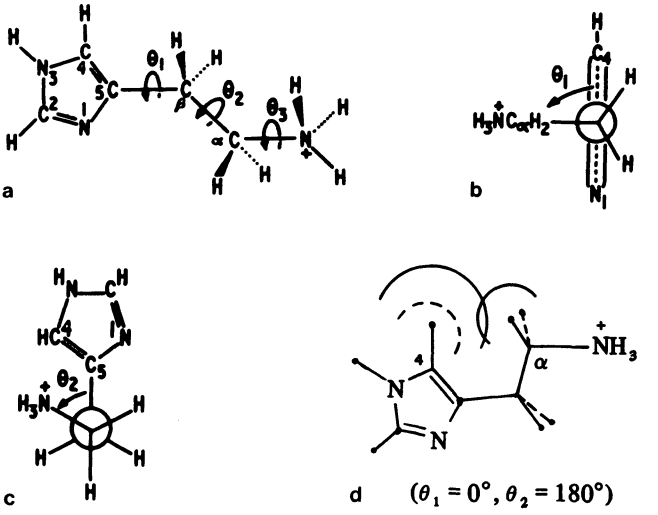
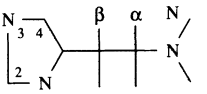


FIGURE 11.1. Histamine monocation showing (a) atom numbering and torsion angles; (b) torsion angle θ_1 viewed along $C_\beta - C_5$ bond from C_β to C_5; (c) torsion angle θ_2 viewed along $C_\alpha - C_\beta$ bond from C_α to C_β; (d) overlap of van der Waals zones: unbroken lines for 4-Me and α-CH_2; dashed arc for 4-H.[13,21]

TABLE 11.2.
Agonist Activities of Methylhistamines[20]



Compound	Position of Me substituent	Activity (95% confidence limits) relative to histamine = 100		Activity ratio
		H_1 (ileum)[a]	H_2 (atrium)[b]	H_1/H_2
1.	2	16.5 (15.1–18.1)	4.4 (4.1–4.8)	4
2.	3	0.42 (0.16–1.0)	0.1[c]	
3.	4	0.23 (0.20–0.27)	43 (40–46)	0.005
4.	N	72 (62–84)	74 (71–78)	1
5.	N,N	44 (38–51)	51 (42–61)	1
6.	β	0.83 (0.42–1.6)	0.89 (0.79–1.1)	1
7.	α	0.36 (0.18–0.73)	0.74 (0.42–1.3)	0.5[d]

[a] In vitro, on guinea-pig ileum in the presence of atropine.
[b] In vitro, on guinea-pig atrium in the presence of propranolol.
[c] Nonparallel dose–response relationship relative to histamine prevents assay.
[d] Not significantly different from 1.

coupling constants in each rotamer (Ham's group applied electronegativity values and Ganellin's group used parameters from model compounds), close agreement was achieved.

There are two dihedral angles of conformational interest, namely ϕ and ϕ_2 (Fig. 11.1). The NMR analyses provide ϕ_2 magnitudes (see above) while the EHT calculations yield both the ϕ_1 and ϕ_2 values of low-energy conformers.

In a series of papers on the conformation of histamine derivatives Ganellin *et al.*[20] presented the biological and conformation properties of a series of methyl histamines. The biological data (Table 11.2) identified the 4-methylderivative as a

TABLE 11.3.
Comparison of *trans–gauche* Conformer Ratio with H_1–H_2 Activity Ratio for Methylhistamines[20]

Molecule[a]	Most stable conformation[b] θ_1/θ_2 (deg)	Conformer ratio *trans/gauche*[c]	Activity ratio[a] H_1/H_2
histamine[d]	120/180 *trans*	1.2	1
2-Me	120/180 *trans*	1.2	4
3-Me	120/180 *trans*	1.2	
4-Me	180/180 *trans*	3	0.005
NMe	120/180 *trans*	1.2	1
N,N Me	120/180 *trans*	3.5	1
β-Me	120/300 *gauche*	0.02	1
α-Me	120/300 *gauche*	0.1	1

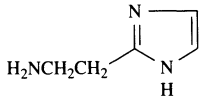
[a] Positions of methyl substituent and activity ratio from Table 11.2.
[b] From EHT calculations.
[c] Derived from population of the *trans*-conformer at 37° C.
[d] First-order analysis of 400 MHz ^{1}H-NMR spectra indicates a lack of conformational preference (Bath data, unpublished).

selective agonist for H_2 (atrium) sites, and the 2-methyl analogue as the agonist of highest H_1 selectivity. Activity ratios H_1/H_2 were next compared with the conformational preferences of the various derivatives (Table 11.3) in order to establish the validity of the corollary to Kier's proposals. This was that "if minimum energy conformations are of importance, then an altered conformational preference should be accompanied by a corresponding change in biological activity". They concluded that the conformer and activity ratios of Table 11.3 appear to be unrelated. Thus the side-chain substituted compounds (α-Me, β-Me), predicted to be conformationally different from histamine, show no selectivity of action (potency relative to histamine is reduced, but to an almost equal degree at both receptor subsites). Conversely, the ring-substituted compounds (2-Me, 4-Me) which do show selectivity of action (pronounced for the 4-Me derivative) have a rotamer composition similar to that of histamine. In a following paper[21] Ganellin argued for the significance of the ϕ_1 dihedral angle in ligand uptake at histamine receptor subtypes, and proposed an H_1-active conformation as one in which the side chain is fully extended (*trans*) and all carbon and nitrogen atoms are coplanar with the imidazole ring (Fig. 11.1a).

Replacement of C_4-H by methyl raises the energy of this conformation by about 10 kcal/mol (EHT prediction) as a result of steric interactions of the side-chain α-methylene protons (Fig. 11.1d). Hence, since the population of the H_1-essential conformation is low in 4-methylhistamine, the failure of this derivative to activate H_1 receptors may be understood. Many H_2 ligands, subsequently developed, include a 4-methylimidazole unit in their structure.

11.3. Chiral and Restrained Analogues of Histamine and Impromidine

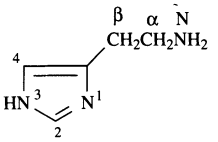
Several chiral analogues of histamine obtained by α- or β-methyl substitution in the aminoethyl side chain have been examined, but their generally low potencies preclude significant identification of receptor stereoselectivities, at least as far as H_1 sites are concerned. Thus the β-methyl racemic mixture retained less than 1% of the potency of histamine at H_1 (GPI) and H_2 (rat atrium) sites while the α-analogue was even less active (Table 11.4). Antipodal forms of the α-methyl derivative (obtained from *R*- and *S*-histidine, Scheme 11.1) showed no difference in activity at GPI sites and only a small difference at GP atrium (H_2) sites (Table 11.4); cf., however, their actions at the putative H_3-receptor described later. Similar results were found for antipodal 4-methyl-α-methylhistamines[26] and α-chloromethylhistamines[25]—*R*-enantiomers of the last kind retained 40–50% of histamine H_2 activity at GP atrium sites and were distinctly more potent than corresponding *S*-isomers. The histamine analogues, isohistamine (**5**) and 1-(2-amino-

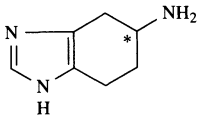


5 isohistamine

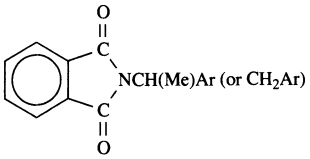
ethyl)thiazole,[30] and 2-(2-aminoethyl)pyridine[27] all suffered large falls in potency after side-chain methylation.

TABLE 11.4.
Histamine Receptor Agonist Activities of Methylated Histamine and Related Derivatives (histamine = 100)



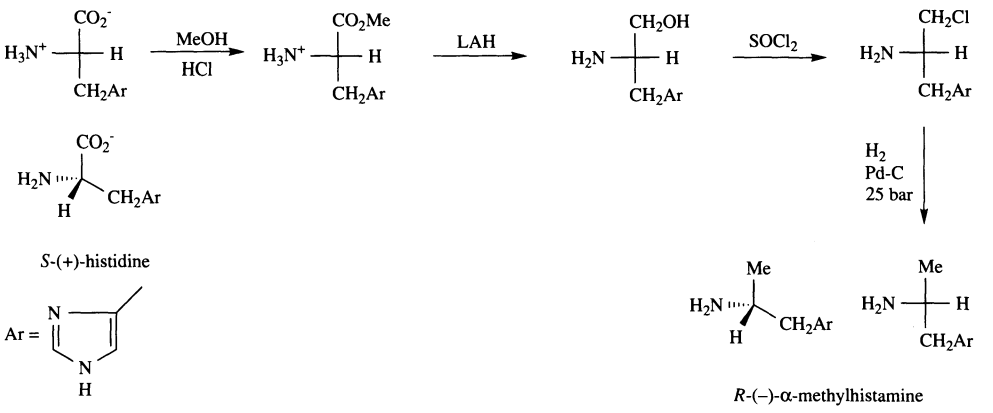
Compound[a]		GPI (H_1)	GP atrium (H_2)	Other	Reference
rac-β-Me		0.83	0.89	0.4[b]	20, 22
rac-α-Me		0.36	0.74	0.8[b]	20, 22
R-(−)		0.49	1.0	—	
S-(+)[c]		0.49	1.7	—	
α, N-Me					
S-(+)		0.69	0.42		24
R-(−)		0.71	0.98		
α-CH_2Cl		0.32			
R-(−)		0.32	42.7		
S-(+)		0.30	17.4		25
α-CH_2Cl, N-Me					
R-(−)		0.30	51.3		25
S-(+)		0.29	7.1		
α-Me, 4-Me					
S-(+)		0.26	8.7		26
R-(−)		0.33	4.78		
2-Pyridyl analog[d]					
α-Me					
rac		0.25			27
(+)		0.38			
(−)		0.12			
	*S*-(−)	0.29	2.09		28
	R-(+)	0.29	0.30		

[a] Configurational assignments based on stereospecific syntheses, e.g., from *R*- and *S*-histidine, and/or the sign of the Cotton effect of related phthalimido derivatives: *S*-antipodes of the general formula shown give + ive, and *R*-antipodes − ive Cotton effects as measured by ORD.[29]

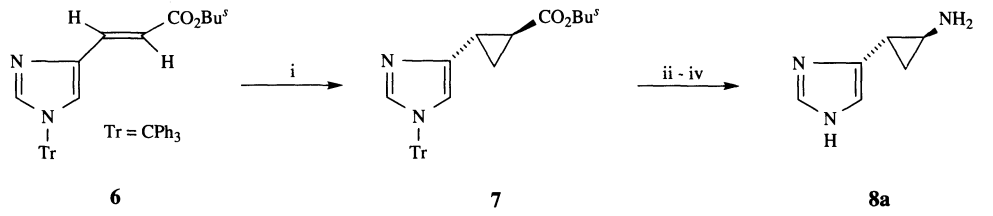


[b] Rat gastric acid *in vivo*.
[c] See page 373 for note on configurational relationships among the α-substituted derivatives.
[d] 2-Pyridyl analog of histamine itself 4.9–5.0.[8]

The conformationally restrained analogue of histamine, 2-(5-imidazoyl)-cyclopropylamine (**8**), in which imidazoly and amino functions are approximately anticlinal (i.e., equivalent to the *trans*-rotamer), proved a feeble agonist at both H_1 and H_2 sites[31]; the cyclopropane ring was constructed by reaction of the *trans*-urocanic acid derivative **6** with dimethylsulfoxonium methylide and conversion of

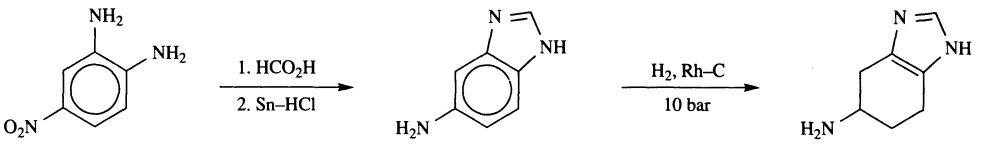


Scheme 11.1. Synthesis of *R*-(–)-α-methylhistamine.[23]



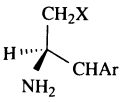
Reagents: i, $Me_2S(O)=CH_2$; ii, KOH; iii, HCl; iv, Et_3N—$ClCO_2Et$—NaN_3.
[a] Activitiy (histamine = 100): GPI, 0.78; rat gastric secretion, 1.6

Scheme 11.2. Synthesis of the *trans*-cyclopropane analogue of histamine.[31]



Scheme 11.3. Synthesis of a tetrahydrobenzimidazole analogue of histamine. [28]

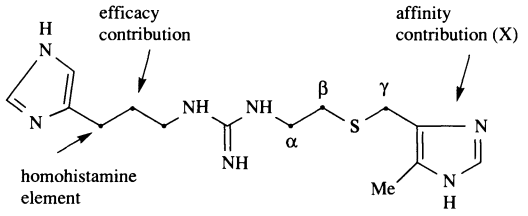
the alkoxycarbonyl group of **7** into an amino group by a Curtius degradation (Scheme 11.2). Conformation restraint was achieved in the tetrahydrobenzimidazole **9** by anchoring the aminoethyl side chain to C-4 of the imidazole ring by a bimethylene chain.[28] The synthetic procedure (Scheme 11.3) gave a racemic mixture which was resolved by use of (−)- and (+)-dibenzoyltartaric acids. Antipodal forms of **9** did not differ in activity at H_1 sites (GPI), but showed a 7-fold difference at H_2 sites with *S*-(−)-**9** the more potent isomer (Table 11.4). Although potency levels in most of the chiral analogues of Table 11.4 are low, it may be of significance that the more potent antipodes all conform in the absolute configuration to the arrangement **10**.



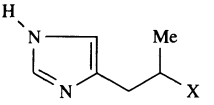
10

X = H (*S*)
X = Cl (*R*)
X, CH_2 linked to C-4 of the imidazole ring

Several chiral analogues of impromidine (**11**, SKF92676), a selective and highly potent H_2-receptor agonist,[32] have been investigated and provide clear-cut evidence of the stereospecific nature of interactions between H_2-receptors and their ligands.[34] Replacement of the homohistamine element of **11** by *R*-(−)-α-methylhistamine gave a product **12** (sopromidine) which was 7.4 times as active as



11



12

histamine on the atrium; the corresponding *S*-antipode showed no intrinsic activity but behaved as an antagonist (24% metiamide). Sopromidine stimulated acid secretion in the mouse to the same extent as histamine, the *S*-isomer again being inactive (or behaving as an antagonist).[38] Neither isomer was active at GPI H_1-sites.[33]

γ-Branching of the side chain of **11** between sulfur and the imidazole ring led to a marked decrease of H_2-agonist activity.[35,36] α- and β-alkylation of the same moiety (see **11**), however, gave H_2 agonists of significant potency (the *R*-α-methyl-isomer was twice as active as the parent) with *R*-antipodes 3 to 7 times more potent than corresponding *S*-forms[36] as shown in Table 11.5.

Histamine inhibits its own synthesis and release from depolarized slices of rat

TABLE 11.5.
Agonist Activity of Some Impromidine Derivatives at H_2 Sites[a]

Impromidine derivatives (see 11)		Guinea-pig atrium assays Relative activity (histamine = 100)	
β-Me	*S*	380	
	R	1660	eutomer 4.4 × *S*
α-Me	*S*	1320	
	R	9550	eutomer 7.2 × *S*
α-Et	*S*	450	
	R	1480	eutomer 3.1 × *S*
γ-Me	*R*	2 (i.a. 0.7)[b]	distomer 0.6 × *S*
	S	3 (i.a. 0.9)	

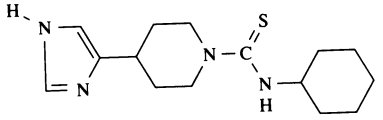
[a] Synthesis of products involved use of intermediates of known chirality.[37]
[b] Intrinsic activity.

cerebral cortex by a negative feedback mechanism. The fact that this action is not blocked by H_1-antagonists while H_2-antagonist inhibit the process, but at potencies different from those found for reference H_2-receptor mediated responses, has lead to the postulation of a third H_3 receptor.[10] The activity of putative H_3-agonists is assessed as follows: cerebral slices are pre-incubated with [^{3}H]histidine (to ensure synthesis of radiolabeled histamine) and then washed to remove excess of precursor and to obtain a constant spontaneous efflux of [^{3}H]histamine. Aliquots of suspensions of these slices are incubated with the depolarizing agent, K^+, and the test agent. The mixture is centrifuged and [^{3}H]histamine present in the pellet and supernatant measured after isolation by ion-exchange chromatography.[38] Data for two chiral analogues of histamine (Table 11.6) reveal the stereoselective nature of the H_3-receptor for compounds of overall low potency. Surprisingly, when antipodal α-methylhistamines were examined in the same test, the *R*-isomer proved 15 times more effective than histamine and 100 times more effective than the *S*-antipode.[39] The preference of *R*-α-methylhistamine for H_3-sites is evident from the following pD_2 values: 4.54 (H_1); 3.96 (H_2); 8.40 (H_3). It is especially noteworthy that H_3 stereoselectivity is the reverse of that displayed by H_2 sites. H_3-actions of *R*-α-methylhistamine were blocked by impromidine and a novel antagonist

TABLE 11.6.
Activities of Chiral Histamines at the H_3-Receptors[38]

Derivative	Activity (histamine = 100)		
α,N^α-dimethyl	*R*-(+)	4.1	
	S-(−)	0.13	*R*/*S* 31.5
N^α-methyl, $2CH_2Cl$	*S*-(+)	1.1	
	R-(−)	0.006	*S*/*R* 183

thioperamide (**13**). In a symposium report[40] substitution in the α- and/or β-position of the histamine side chain is claimed to give full H_3 agonists of varying



13

relative activity. The most potent was the α-*R*, β-*S*-dimethylantipode (pD_2 8.5, 18 × histamine), a compound of high receptor selectivity (H_3 versus H_2/H_1). Details are to be published (*J. Med. Chem.*, in press). Radiolabeled [^{3}H]-*R*-α-methylhistamine has been employed to visualize H_3 receptors in man and monkey brain.[41]

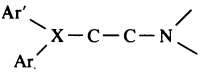
Sopromidine and its *S*-enantiomer acted as H_3-antagonists of similar potencies.[38]

11.4. Antagonists of H_1-Receptors: Testing Methods[90]

While information on stereoisomeric histamine agonists is relatively sparse, there is a rich fund of data relating to antagonist of histamine, specifically to agents which block H_1 receptor sites. Isomers of two kinds have been studied: *optical* derived from molecules with one or more chiral center, and *geometrical* from molecules which include a carbon–carbon double bond.

Typical H_1-agonist actions of histamine relate to the constriction of bronchiolar and gastrointestinal smooth muscle[42] and the compounds discussed here characteristically oppose these actions. Inhibition of contractions of isolated guinea-pig ileum (GPI) induced by histamine is the *in vitro* test most commonly employed for the quantitative assessment of antagonist potency.[43] The antagonist potency of the test substance is expressed either as its pA_2 value, i.e., the negative logarithm of the dose of antagonist that reduces the effects of a double dose of agonist to that of a single dose,[42,44] or affinity (association) constant K_B (or K_A). The two parameters are nominally equivalent (i.e., $pA_2 = \log K_B$) but the K_B value is likely to be more accurate because its measurements involves the use of dose ratios greater than 2. Different antagonists vary in the rates at which they attain equilibrium with the tissues, hence contact times must be taken into account when comparisons are being made. There is evidence that the more potent the antagonist, the longer it takes to reach equilibrium with the receptor that it blocks.[45] Direct study of the binding of antagonist ligands to H_1-receptors has become more frequent over the past decade, and is described later. The most common *in vivo* assays of antihistamines are those that assess the abilities of test compounds to protect guinea pigs against lethal effects of iv histamine or bronchospasm (asthma) produced by inhalation of a histamine aerosol.[42] Protection of rats from the effects of a lethal dose of compound 48/80 (a mixture of oligomers obtained by condensing *p*-methoxy-*N*-methylphenethylamine with formaldehyde) is a more recently devised method.[46] Compound 48/80 causes mast cells to degranulate and release histamine and other mediators of anaphylactic shock—the mechanism of action of compounds that protect animals against its effects is unknown.

Most compounds that are effective at low dose levels (with pA_2 values of 8 or more) in antagonizing H_1-receptor sites may be described by the general structure **14**.[47] In presenting the stereochemical features of specific examples of **14** it is convenient to classify the compounds into distinct groups.

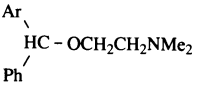


14

Ar: aryl (including phenyl), substituted phenyl and heteroaryl (often 2-pyridyl)
Ar′: arylmethyl ($ArCH_2$) or a second aryl group
X: C,N,C–O, or X–C replaced by C=C
N<: tertiary acyclic (especially NMe_2) or alicyclic (especially pyrrolidino) basic group
Ar and Ar′ may be bridged

11.5. Tertiary Aminoalkyl Ethers

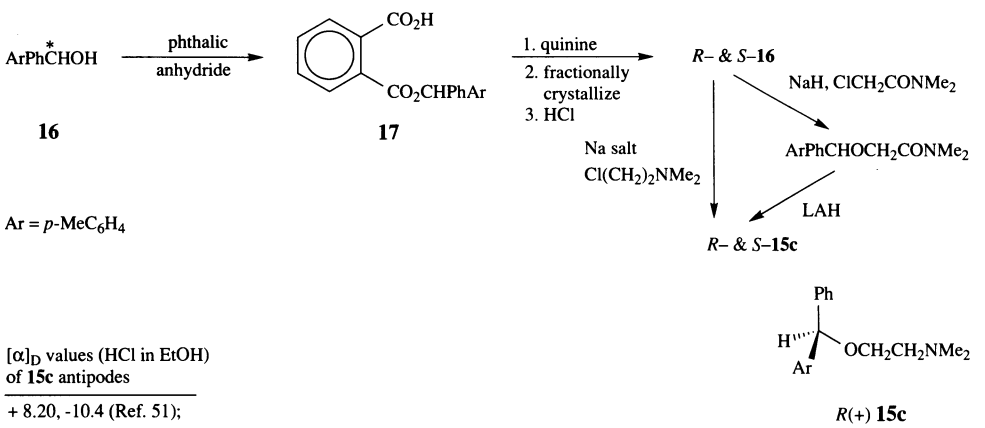
This group is based on diphenhydramine (**15**a, *Benadryl*) introduced as an antihistamine during the late 1940s. The compound also has anticholinergic activity and this property is enhanced at the expense of antihistaminic potency when one of the N-phenyl groups carries an *ortho*-methyl substituent as in orphenadrine



(a) Ar = Ph; (b) Ar = $o-MeC_6H_4$; (c) Ar = $p-MeC_6H_4$

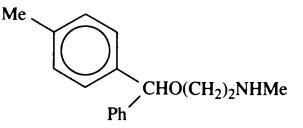
15

(**15**b). *Para*-methyl substitution has the reverse influence on the activity profile, and compound **15**c (*Neobenodine*, *Toladryl*) is a potent antihistaminic (pA_2 7.62 for **15**a, 8.78 for **15**c).[48–50] Antipodal forms of **15**c (obtained from the carbinols **16** resolved



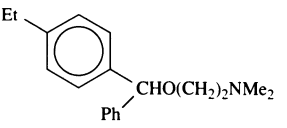
Scheme 11.4. Resolution of neobenodine.[51,52]

as the phthalate ester **17** with quinine; see Scheme 11.4) were first reported by Jarrousse and Régnier in 1951.[51] The French workers identified the dextro isomer as the more potent vs. histamine in guinea pigs (3 × levo, ileum; 4 × levo, bronchospasm; 10 × levo, lethal iv dose of histamine). The low orders of antipodal difference observed probably reflect incomplete resolution of materials used, since Rekker and others,[48] in a later study using antipodes of specific rotations + 13.0 and − 10.3 in ethanol, reported that the dextro form (pA_2 8.7) was 65 times more potent than the levo isomer (pA_2 6.9) in antagonizing histamine at guinea-pig ileum sites. Unexpectedly the (+) and racemic forms were equipotent. The more potent dextro isomer **15c** is reported to have the *R*-configuration in unpublished work,[49] as supported by circular dichroism evidence (see later). Antipodal potency data has also been reported for des *N*-methyl and *p*-ethyl analogues of neobenodine (see **18** and **19**).[49] Antipodal forms of orphenadrine (**15b**) did not differ in their guinea-pig ileum pA_2 values (6.8).[48]



18

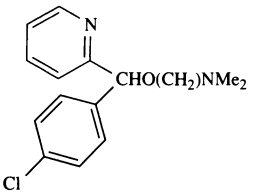
	pA_2
rac	7.7
(+)	8.0
(−)	6.5



19

	pA_2
rac	8.2
(+)	8.7
(−)	7.6

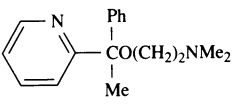
The next example of this class is *carbinoxamine* (**20**, *Clistin*) which carries the nonidentical 2-pyridyl and 4-chlorophenyl aryl groups at its benzylic carbon center. Antipodal forms (obtained by resolution with D- and L-tartaric acids) differ significantly in activity; the data are summarized in Table 11.7.



20 carbinoxamine

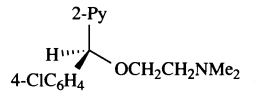
The absolute configuration of the more active levo isomer (dextro tartrate) has been shown to be *S* by a chemical method similar to that applied to antipodal pheniramines and details are presented in the next section (Scheme 11.7) and from its circular dichroism profile (see below).

Doxylamine (**21**, *Decapryn*) is a methylated chiral analogue of car-



21 doxylamine

TABLE 11.7.
Antihistamine Activities of Carbinoxamine Antipodes

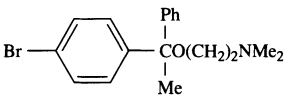


S-(−)-base

Test	Relative potencies etc. of bases[b]
Inhibition of supramaximal histamine-induced contractions of guinea-pig ileum[a]	(−) 29 (+) 1.0 *rac* 15
Protection of guinea pigs against lethal iv histamine (3 mg/kg) given 20 min after ip carbinoxamine[a]	(−) 34 (+) 1.0 *rac* 19
50% blockade of depressor response to iv histamine in anesthetized dog[a]	(−) 42–46 μg/kg iv[c] (+) 11 mg/kg *rac* 13–106 μg/kg
log K_B (GPI, 30° C, 8 min equilibration time)[d]	(−) 9.00 (+) 7.19

[a] Ref. 53.
[b] The levo base is derived from the L-(+)tartrate, and the dextro base from the *D*-(−)tartrate.
[c] ED_{50}.
[d] Ref. 68; optical purities by chiral analysis.

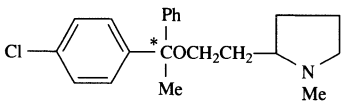
binoxamine[55] but no data on its resolution have been published. The same is true of mebrophenhydramine (**22**, *Mebryl*), recently resolved at Bath via its



22 mebrophenhydramine

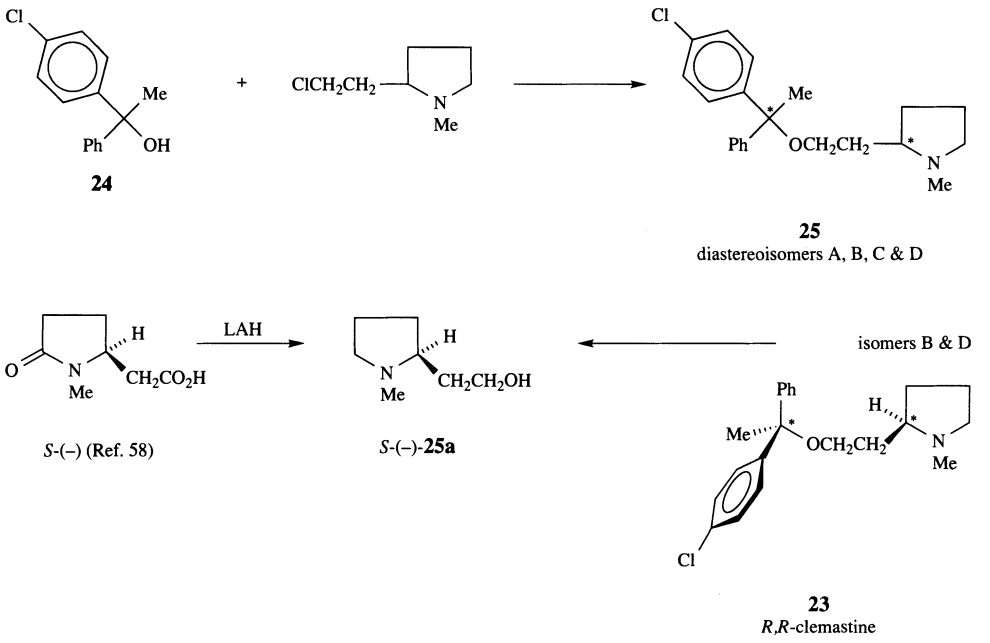
p-toluoyltartrate salts. The levo antipode proved more effective than the dextro isomer at guinea-pig ileum sites [pA_2 (+) 9.95; (−) 8.28; (±)9.92][54,68] and bound the more strongly to H_1 sites of guinea-pig cerebellum and auricle.[56] From CD comparisons with clemastine (see below) the levo isomer may be assigned the *R*-configuration.

Clemastine (**23**, *Tavegyl*), a compound similar to mebrophenhydramine except that its aminoalkyl side chain is terminated by an alicyclic basic group, possess



23

chiral centers at both the benzylic carbon and carbon α to nitrogen. Condensation of the *t*-alcohol **24** with 2-(2-chloroethyl)-1-methylpyrrolidine gave the diastereoisomeric mixture **25** (plus a hexohydroazepin byproduct) (Scheme 11.5).[57] The AB racemate separated as the hydrogen fumarate and was resolved using dibenzoyl-L-



Scheme 11.5. Synthesis of clemastine and its stereoisomers. [57]

tartaric acid. The CD base was recovered from the fumarate mother liquors from which the individual C and D forms could be separated remarkably as maleate salts. The absolute configurations were established by degradation to *R*- and *S*-1-methyl-2-pyrrolidinoethanol **25a** (A and C, *R*; B and D, *S*) of known configuration (see Scheme 11.5), and by X-ray analysis of the methiodide of isomer A (clemastine itself). Data on the antihistaminic activity of clemastine and its isomers,

TABLE 11.8.
Antihistamine Activities of Clemastine and Its Isomers[57]

Isomer[a]	Prevention of histamine toxicity ED_{50} (mg/kg sc)[b]	Prevention of histamine spasm[c]	pA_2[d]
A *RR* (clemastine, **23**)	0.04	*ca* +7	9.45
B *SS*	5.0	*ca* −1.5	7.99
C *SR*	11.0	*ca* −6	8.57
D *RS*	0.28	*ca* +5	9.40

[a] Configuration of benzylic center followed by that of C-2 of the pyrrolidine ring.
[b] Dose which protects 50% of a population of guinea pigs from the lethal effects of a 20 mg/kg sc dose of histamine given 3 h after receipt of the test substance. Animals surviving 12 h later were regarded as protected.
[c] Potency relative to that of the standard drug thenalidine (= 1) in the GPI test. Spasms induced by 5×10^{-8} g/ml histamine HCl applied 5 min after addition of test substance.
[d] GPI data obtained by Nauta and Rekker.[49]

summarized in Table 11.8, demonstrate that chirality close to the aromatic feature of the molecule has a far greater influence on activity than that close to the basic center, since both *RR* and *RS* isomers are potent agents (see also p. 387). Clemastine provides one of the few examples of chiral antihistamines employed clinically in the form of a single isomer (*Martindale* **29**, page 449).

The circular dichroism (CD) features of clemastine (*RR*) and its *SS*-antipode are remarkably similar to those of levo and dextro mebrophenhydramine, respectively, results which provide good evidence for the configuration of the latter antipodal pair (Fig. 11.2). The CD spectra of (+)-neobenodine and (−)-carbinoxamine also correlate in the Cotton effect sign and position with that of clemastine, further proof that all these antipodes have a common absolute chirality (see also Table 11.15).[68]

The achiral *t*-aminoether diphenylpyraline (**26**, *Histryl*), in which the alkylamino chain of diphenhydramine is incorporated in a piperidine ring, is of conformational interest because its ^{1}H-NMR spectrum clearly shows the

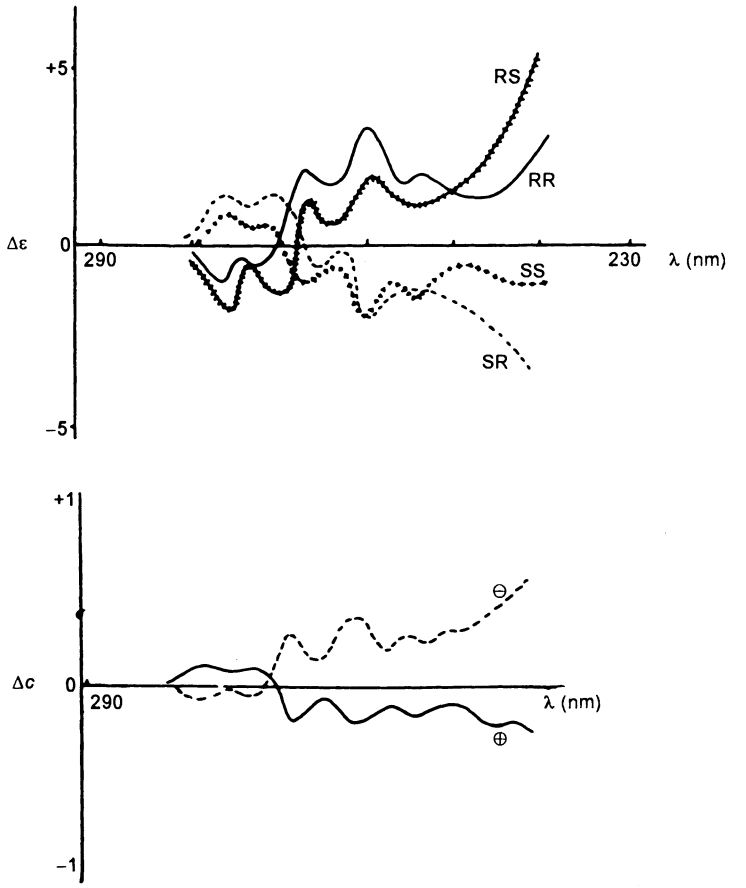
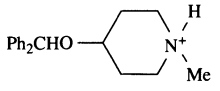


FIGURE 11.2. CD spectra of antihistamine stereoisomers[68]: upper clemastine stereoisomers (fumarates); lower mebrophenhydramine antipodes (maleates). Solvent water. Note close correspondence of spectra of *RR*- and *SS*-clemastine with those of (−)- and (+)-mebrophenhydramine, respectively.



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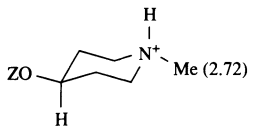

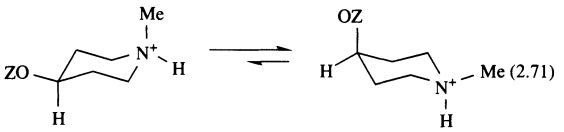

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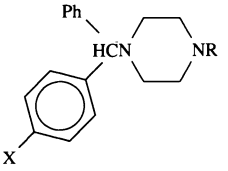
Z = Ph_2CH

400-MHz ^{1}H-NMR spectrum of **26** in D_2O shows series of duplicate signals, e.g. Ph_2CHO singlets 5.58 & 5.69 ppm. 4-H wide multiplet 3.62 (axial 4-H), and 3.78 narrow multiplet (equatorial 4-H, **27**). (Bath, unpublished results.)

Scheme 11.6. Protonated epimers of diphenylpyraline (Histryl).

protonated base to exist as an approximately 1:1 mixture of equatorial 4-Ph_2CHO and axial 4-Ph_2CHO piperidine chairs (details in Scheme 11.6). Analogues anchored either in the eq-Ph_2CHO or ax-Ph_2CHO conformation, e.g., by a 3-methyl substituent, would be interesting target compounds since the epimers differ in the distance between the aromatic groups and basic center (least for **27**) and potency comparisons would identify the separation that is most effective for blockade of H_1 sites. Cyclizine and its analogues may be included in this section (ether oxygen is represented by one of the nitrogen atoms of the piperazino ring); a number of chiral analogues have been reported, such as **28**b, R = CH_2CH=CHPh, cinnarazine (*Stugeron*), and buclizine **28**b, R = $CH_2C_6H_4$-*p*-Bu^n, but no study of antipodal forms has been made.

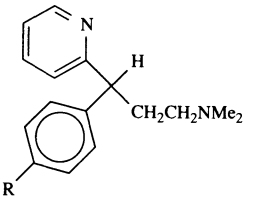


(a) X = H; (b) X = Cl

28

11.6. 3-Amino-1-aryl-1-(2-pyridyl)propanes (Pheniramines)

This group provides three chiral antihistamines in clinical use, namely, pheniramine itself (**29**a, *Trimeton*), chlorpheniramine (**29**b, *Chlortrimeton*, *Piriton*), and brompheniramine (**29**c, *Dimetane*) (*Martindale* **29**, page 448). The resolution of all three compounds by means of fractional crystallization of salts formed with (+)- and (−)-phenylsuccinic acid has been reported in a patent[59]; antipodes of the chloro derivative may also be separated by use of (+)- and (−)-di-*p*-toluoyltartaric acids.[60,61]



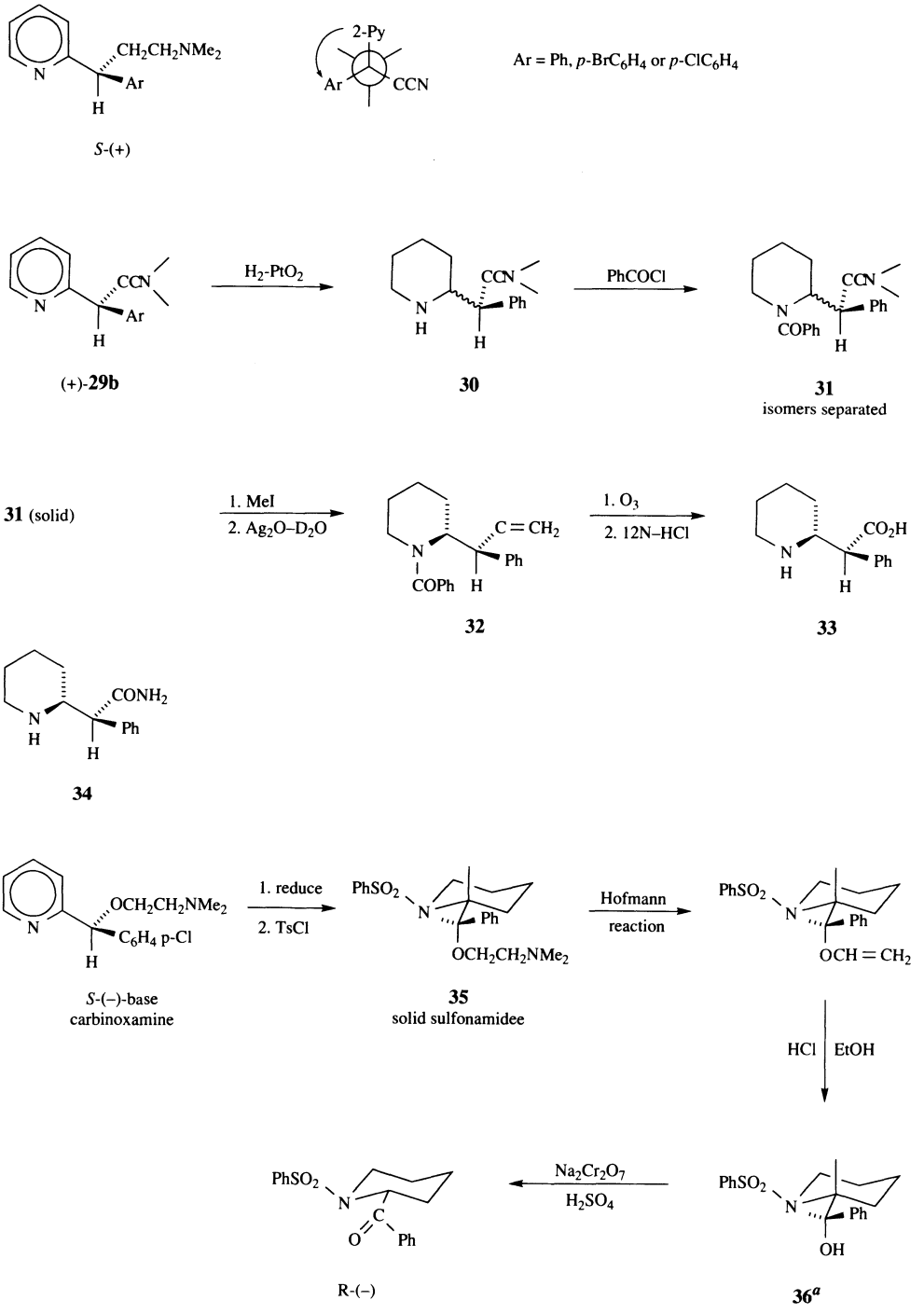
(a) R = H; (b) R = Cl; (c) R = Br
29

The configuration of the dextrorotatory pheniramines has been shown to be *S*, as outlined below by a chemical method (Scheme 11.7)[62] and confirmed by X-ray crystallography.[63] First it was shown that all (+)-pheniramines are related since reduction of (+)-bromo- and (+)-chloro-pheniramine catalyzed by palladium gives (+)-pheniramine. Hydrogenation of (+)-chloropheniramine (**29**b) in the presence of platinum oxide effects reduction of the 2-pyridyl substituent as well as removal of chlorine, to give a diastereoisomeric mixture of 2-piperidine derivatives (**30**) epimeric about the endocyclic asymmetric center. One isomer was isolated as a solid N-benzoyl derivative (**31**) and subjected to the Hofmann degradation to give the alkene (**32**). Ozonolysis of **32** gave the carboxylic acid **33**, which was identical to that produced by hydrolysis of the amide **34** of established stereochemistry.[64,65] Evidence that steps **29**b→**33** did not epimerize the exocylic asymmetric center was provided by conducting the degradation in deuterium oxide and showing that **33** did not contain more than the normal isotopic abundance of deuterium (if epimerization had taken place, a significant replacement of the methine hydrogen by deuterium would result).

Scheme 11.7 also shows how a similar reductive method was applied to solving the stereochemistry of levo carbinoxamine (page 378). In this case the absolute configuration of the piperidine chiral center of the diastereoisomer **35** was first established, and then the benzylic center was deduced from knowledge of the relative configurations of the two centers of **36** based on ^{1}H-NMR and IR evidence.[66]

Many studies of antipodal activity differences have been made in the pheniramine series—especially in regard to chlorpheniramine. Thus Roth and Govier[67] found an *in vitro* potency order: (+) 2 × *RS*, 200 × (−) [guinea-pig ileum test, dose of maleate required to produce 50% inhibition of muscle spasm induced by histamine at 0.2 μg/ml in bath solution: (+) 0.8 μg/l; *RS* 1.7 μg/l; (−) 190 μg/l], and an *in vivo* order: (+) 2–3 × *RS*, 100 × (−) (protection against iv aerosolized histamine lethality). Brittain *et al.*[60] found a smaller potency ratio between chlorpheniramine antipodes at guinea-pig ileum sites (4.6) and reported the pA_2 values (+) 8.47; *RS* 8.10, and (−) 7.81 measured after a 2-min drug-tissue contact time. In a local anaesthetic test on guinea-pig skin, the levo isomer was about 1.5 and the dextro 0.5 times as effective as the racemic mixture. More recent pA_2 values of antipodal chlorpheniramines are listed in Table 11.9. The values indicate that the previous study did not allow sufficient time for equilibration. Table 11.9 also includes pA_2 results for pheniramine and brompheniramine.

In oral tests in guinea pigs the therapeutic indexes (LD_{50}/protective dose 50) of (+)-**29**b and (+)-**29**c were about twice that of the corresponding racemate and many times that of the levo isomer, e.g., **29**b *RS* 1430, (+) 3380, (−) 25.[67]



[a] Configuration of **36** deduced from IR (ν_{OH}) and ^{1}H-NMR (coupling between benzylic CH and α-piperidyl CH) differences between **36** and the corresponding diastereomer epimeric about the benzylic center.

Scheme 11.7. Absolute configuration of the pheniramine [62] and carbinoxamine antipodes. [66]

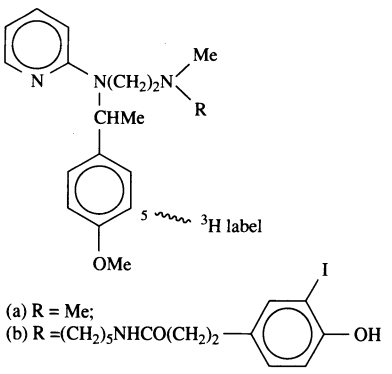
TABLE 11.9.
***In Vitro* Antihistaminic Potency of Pheniramines in the Guinea-Pig Ileum Gut-Bath Procedure**

		pA_2[a]	pA_2[b]	pA_2[c]
Chlorpheniramine	*S*-(+)	9.3	9.3	9.02 (8.89–9.15)[d]
	R-(−)	7.8	7.5	7.11 (6.95–7.26)
		ED_{50} (μg/l)	Relative potency	pA_2[e]
Pheniramine	*rac*	9.0	1.0	7.8
	S-(+)	5.5	1.6	7.5 (7.96)
	R-(−)	170.0	0.05	6.0 (6.74)
Brompheniramine	*rac*	1.4	1.0	
	S-(+)	0.56	2.5	
	R-(−)	88.0	0.016	

[a] Neuten quoted by Nauta and Rekker.[49]
[b] Harms and Nys quoted by Nauta and Rekker.[49]
[c] Casy, Drake, Ganellin, Mercer, and Upton[68]; evidence of optical purity by chiral analysis.
[d] After 8 min equilibration time, 95% confidence limits in parentheses, 3 experiments, (+):(−) potency ratio 80.
[e] pA_2 values quoted by Nauta and Rekker[49] due to Beld and (in parentheses) Harms and Nys.

Dextrochlorpheniramine (*Polaramine*) and dextrobrompheniramine (*Disomer*) have been marketed for clinical use in the USA; USP XXI monographs on these agents relate to both dextro isomers and racemic mixtures. Dextro **29**b blocked phenylephrine (α-adrenoceptor stimulant) and carbachol (muscarinic agent) about 10^3-fold less effectively than it did histamine at sites in the rabbit aorta,[69] results which demonstrate its specificity of action.

Chlorpheniramine antipodes are one of the few pairs of antihistamine isomers whose affinity constants for histamine receptors have been determined from *binding experiments*. The procedure involves measuring displacement of radiolabeled [^{3}H]mepyramine (**37**a) from the membrane fraction of guinea-pig brain deemed to



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contain a high population of H_1-histamine receptors (this is especially true of the cerebellum). Irrelevant contributions due to nonspecific uptake of the radioligand are established by carrying out the binding experiment in the presence of a large excess of a "cold" ligand, e.g., 2×10^{-6} M promethazine. Hill and others[70] found that the affinity constant of dextrochlorpheniramine (1.2×10^9 M^{-1}) was over

TABLE 11.10.
K_i (nM) Values for Binding of Antipodal Chlorpheniramines to Mammalian Brain[a] (Ref. 71)

	Human[b]	Guinea pig	Rat	Rabbit	Monkey
(+)-Chlorpheniramine	4.2 ± 0.7	1.4 ± 0.8	8.0 ± 2.7	21	9.1
(−)-Chlorpheniramine	350 ± 50	130 ± 50	700 ± 140	2100	730

[a] K_i (nM) = IC_{50} divided by $1 + L/K_D$ (see Chap. 2, p. 44); nonspecific binding determined in the presence of 2 μM tripolidine, and K_D dissociation constants obtained from Scatchard plots. IC_{50} is the antipodal concentration that displaces 50% of the specifically bound radioligand, and L the concentration of radioligand (1 nM for GP, 2 nM for the rest). IC_{50} values (nM) for chlorpheniramine antipodes *vs* 4 nM [^{3}H]mepyramine (dextro 4.0, levo 420) were close to those measured *vs* 0.5 nM [^{3}H]doxepin (dextro 4.8, levo 440).[72]
[b] Frontal lobe.

200 times greater than that of the levo isomer (5×10^6 M^{-1}) and that the values (relevant to brain tissue) were close to those measured at guinea-pig ileum sites by the usual gut-constriction technique. An American group[71] also compared the binding affinities of (+)- and (−)-chlorpheniramine and large differences between antipodes were found for a variety of mammalian brains (Table 11.10). Displacement of the recently introduced ligand[^{125}I] iodobolpyramine (**37b**) from cerebellar membranes of the guinea pig by antipodal chlorpheniramines gave K_i (nM) values of 0.21 ± 0.06 for the dextro and 35 ± 9 for the levo isomer.[56]

The solid state conformation of *RS*-brompheniramine maleate is known from an X-ray diffraction study.[73] The propylamine chain is fully extended and adopts an asymmetric disposition toward the two aryl substituents such that the 2-pyridyl ring lies roughtly in the CCN plane (with pyridyl N *cis* to dimethylamino N) while the *p*-bromophenyl group projects away from the rest of the molecule as shown in Fig. 11.3. This conformation is similar to that proposed for 3-aminopropenes of the triprolidine type in the solute conditions.[96] The solid state conformation of (+)-chlorpheniramine is similar to that shown in Fig. 11.3, except that the *p*-halophenyl rather than the 2-pyridyl substituent lies in the CCN plane.[63]

Measurement of formaldehyde formation in parallel incubations containing either *S*-(+)- or *R*-(−)-chlorpheniramine and rat liver microsomes showed that the active *S*-(+)-antihistamine is *N*-demethylated about 35% faster than its distomer.[74] *N*-Demethylation of the same antipodal pair by mice microsomes proved nonstereoselective. GC/MS techniques used to measure *N*-desmethyl (NHMe) and

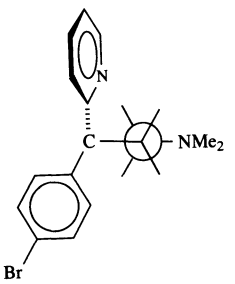
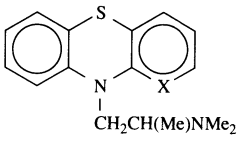


FIGURE 11.3. View along the C−CH_2NMe_2 bond of brompheniramine maleate molecule (adapted from Ref. 73).

N,*N*-desmethyl (NH_2) metabolites produced from a pseudoracemate substrate (deuterated *S*- and unlabeled *R*-chlorpheniramine) gave antipodal ratios similar to those determined by measuring formaldehyde formed in separate incubations. Stereoselectivity with rabbit liver microsomes was higher under pseudoracemate conditions than following the use of separate isomers, suggesting that the *S*-antipode inhibits *N*-demethylation of the *R*-form, or that the demethylated products suffer further biotransformation by routes which favor *R*-substrates.

11.7. Tricyclic Antihistamines

The popular clinical agent promethazine (**38**a, *Phenergan*) and its 1-aza analogue isothipendyl **38**b both contain a chiral side chain and have been resolved. Enantiomers of promethazine had similar antihistaminic and other pharmacological properties.[75] Results with the antipode of isothipendyl[76] produced unexpected



(a) X = CH; (b) X = N

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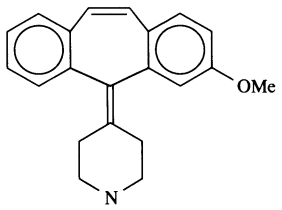
results in that both (+)- and (−)-forms were *less* potent than the racemate in an *in vivo* test (protection against iv histamine in guinea pigs). The levo isomer was half as potent as the dextro form in this test, but the antipodes were equipotent *in vitro* at guinea-pig ileum sites. These results, together with activity comparisons of the clemastine series (page 381) show that an asymmetric center close to the side-chain nitrogen has little if any influence on receptor sensitivity to ligand stereochemistry. When chirality is present through the nonsymmetrical aromatic substitution of a tricyclic antihistamine, as in 3-methoxycyproheptadine **39**, marked activity differences between the antipodes are again observed.[77] It is of interest that the levo isomer is the more potent vs. 5-HT and histamine, and the dextro vs. ACh (see data alongside **39**).



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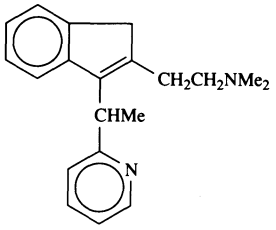
	ED_{50} (mg/kg) *vs* 5-HT oral	*vs* histamine ip	*vs* ACh oral
rac	0.43	1.16	41.2
(+)	23.9	4.45	11.45
(−)	0.38	0.51	80

11.8. Miscellaneous Antihistamines

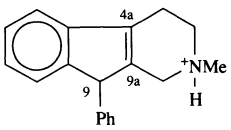
Two indene derivatives of stereochemical interest have long been known in the literature of antihistamines. One of these, dimethindene (**40**, dimethpyrindene,



40

Fenostil), was resolved as the tartrate salts and its pharmacological activity found to reside mainly in the levo isomer.[78,79] More recent antihistaminic data obtained on isolated organs of a guinea pig suspended in an organ bath of 31 °C are presented in Table 11.11.[80] Among these tissues, ileum sites appear to be the least stereoselective (the equilibration time was not given). In experiments at Bath on the guinea-pig ileum, a potency ratio of 50 was observed (pA_2 9.54 for the levo and 7.86 for the dextro isomer).[68] The levo isomer was 70 times (ED_{50}) and 1500 times (ED_{90}) more effective than its antipode in protecting anaesthetized guinea pigs against histamine-induced bronchoconstriction (unpublished results). In binding studies, (−)-**40** proved far more effective than the dextro form in displacing radiolabeled ligands from H_1-sites; K_i (nM) (−) 0.028, (+) 14.4 (cerebellum); (−) 0.05, (+) 7.9 (auricle) versus ^{125}[I]iodobolpyramine.[54,56]

Although the second chiral indene derivative phenindamine (**41**) is used clinically as the tartrate salt (*Thephorin*), the material is a diastereoisomeric mixture



41

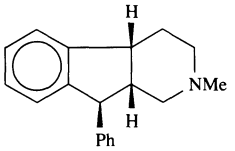
(60:40 approx; Kern, private communication) and no data on optically pure materials are available. The freshly synthesized base is a mixture of 4a–9a and 9a–9 ene positional isomers from which the more active 4a–9a ene may be separated as

TABLE 11.11.
pA_2 Values of Dimethindene Antipodes in Various Tissues[80]

	pA_2 values		
Organ[a] of GP	(−)	(+)	(−):(+) potency ratio
Left atria[b]	9.4	6.3	1600
Trachea	9.3	7.0	200
Aorta	9.9	7.3	400
Ileum	9.1	7.8	20

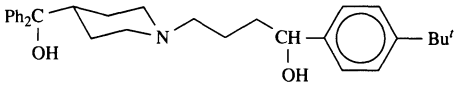
[a] Preparations stimulated by histamine or (trachea only) 2-pyridylethylamine.
[b] Electrically driven at 1 Hz.

a tartrate salt (most other salts are 9–9a enes). These structural relationships have been established by high-field NMR (^1H, ^{13}C) investigation.[81] Dihydroanalogues of phenindamine are less active than the parent and the more potent of the two reported probably has the stereochemistry **42**.[82]



42

The next two examples are piperidine derivatives. Terfenadine (**43**, *Triludan*, *Teldane*), introduced during the early 1980s as a nonsedating antihistamine,[83] has a chiral center well removed from the diaryl (Ph_2COH) feature of the molecule and is thus not expected to display a significant antipodal potency difference (no data; resolution reported in 1992).[120] The second piperidine is cabastine (**44**, R 48756), a compound which bears some relationship to phencyclidine **45** except that the 4-aryl substituent is moved from the 1- to the 4-position of the cyclohexane ring.[84]



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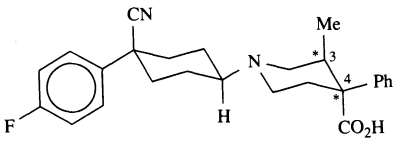

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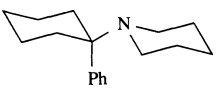


45

Racemic cabastine **44** together with its three diastereoisomers all protected guinea pigs for histamine and compound-48/80-induced lethality in rats. Activity data shown in Table 11.12 demonstrate the remarkable high potencies of cabastine in

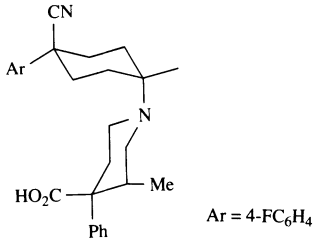
TABLE 11.12.
Protection from iv Histamine-Induced Lethality in Guinea Pigs, and Compound 48/80-Induced Lethality in Rats Afforded by Cabastine and Its Stereoisomers

	ED_{50} (mg/kg)	
Compound (*rac*)[a]	*vs* Histamine	*vs* Compound 48/80
Cabastine (**44**)	0.0025	0.0027
R49389 (**46**)	0.24	2.5
R49429 (**47**)	0.021	0.012
R49549 (**48**)	0.15	2.5

[a] Compounds were administered orally 3 h (iv histamine) or 2 h (Compound 48/80) before challenge.

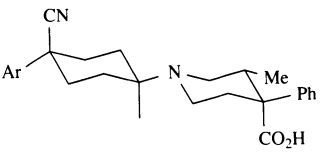
these tests. The 3*S*,4*R* levo antipode of cabastine was twice and ten times as potent, respectively, as the corresponding racemate and dextro isomer vs. histamine in guinea pigs after a 3-h time interval. Initial clinical tests of levocabastine in patients suffering from allergic conjunctivitis show promise and application for a UK license has been made (PJ July 21, 1990).

Diastereoisomers **44** and **46** were obtained by reductive amination of 4-cyano-4-*p*-fluorophenylcyclohexanone with the *trans*-3-Me/4-CO_2R piperidine **49**, while use of the *cis*-analogue of **49** led to **47** and **48**. The isomers were differentiated by



Ar = 4-FC_6H_4

46



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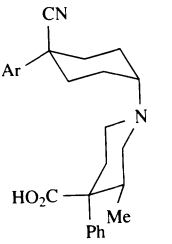

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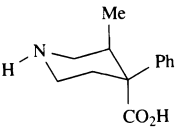
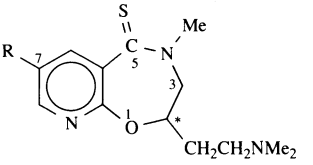


49

the ^{1}H-NMR features of their cyclohexyl 1-H resonances: two large and two small ^3J couplings for the 1-H signal of **44** and **47**, typical of an axial proton; two small couplings for 1-H of **46** and **48**, typical of an equatorial proton.

The compound rocastine (**49a**, R = H) departs from the general requirements



49a

among H-1 antagonists of a pair of aromatic substituents.[118] It possesses a single aromatic feature (pyridyl) fused to an oxazepine ring which carries the ubiquitous 2-dimethylaminoethyl side chain. Rocastine and its analogues are, nevertheless, potent agents (Table 11.13) which display remarkably high eudismic ratios (above 300, *R*-eutomer) in both functional and binding tests. In a series of superimposition exercises using conformations deduced as preferred on the basis of molecular mechanics and ^{1}H-NMR coupling constant data, eutomeric forms of 7-chloro-rocastine (**49a**, R = Cl) and chlorpheniramine (**29b**) gave the best fit when the chloropyridyl ring of the former was juxtaposed with the 4-chlorophenyl feature of the latter (not, as may be supposed, the pyridyl–pyridyl alternative). This interpretation suggest that insertion of 3-chloro into the pyridyl ring of chlorphenir-

TABLE 11.13.
Ligand Activities of Rocastine Antipodes and Related Antihistamines[118]

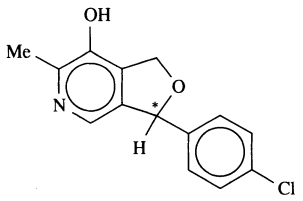
R in **49a**	Configuration	Protection against histamine-induced lethality in guinea pig ED_{50} (mg/kg po), 1 h pretreatment	Inhibition of [^{3}H]mepyramine binding GP cortex IC_{50} (nM)
H	*S*	23 (328)[a]	21000 (700)
H	*R*	0.07	30
Cl	*S*	66.8 (5138)	11900 (6263)
Cl	*R*	0.013	1.9
Cl[b]	*S*	8.28 (1533)	870 (3346)
Cl	*R*	0.0054	0.26
rac-Chlorpheniramine		0.18	8.8
rac-4-ClC_6H_4/3-ClPy analog		> 10	430
rac-2-Py/3-ClPy analog		0.009	24

[a] Eudismic ratio.
[b] $N(CH_2)_3$ analogue.

amine should depress activity, while the analogue with 4-chlorophenyl replaced by 3-chloropyridyl should be of high potency; both predictions were confirmed (Table 11.13).

Antipodal forms of **49a** were obtained by stereospecific syntheses starting from *S*- or *R*-1-methyl-3-pyrrolidinol (*rac*-form readily resolved; *S* may be prepared from *S*-malic acid).

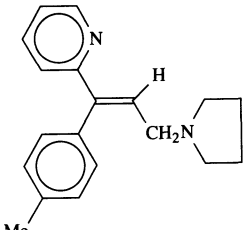
Racemic cicletanide (**50**), the last example of this section, is modestly effective vs. histamine in the guinea-pig ileum (pA_2 6.8) while its levo antipode (pA_2 7.2) was at least 100 times more potent than the dextro isomer.[85] The compound lacks the normal aliphatic basic center of conventional antihistamines.



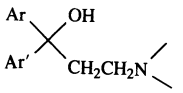
50

11.9. Geometrical Isomers

Triprolidine (**51**, *Actidil*) and its corresponding *Z*-isomer are the best known of several isomeric pairs of 1,1-diaryl-3-aminopropenes that have been investigated as antihistaminic agents. Tripolidine itself has been in clinical use for over 20 years and is a relatively potent antihistaminic agent with a usual dosage of 5 to 7.5 mg daily acting for about 12 h (*Martindale* **29**, page 463). Derivatives of this kind are made by dehydrating precursor tertiary alcohols **52** with acid when isomeric mixtures of alkenes result—the isomeric ratio depends on the reaction time since the dehydration conditions permit isomerization and hence equilibration.[86]



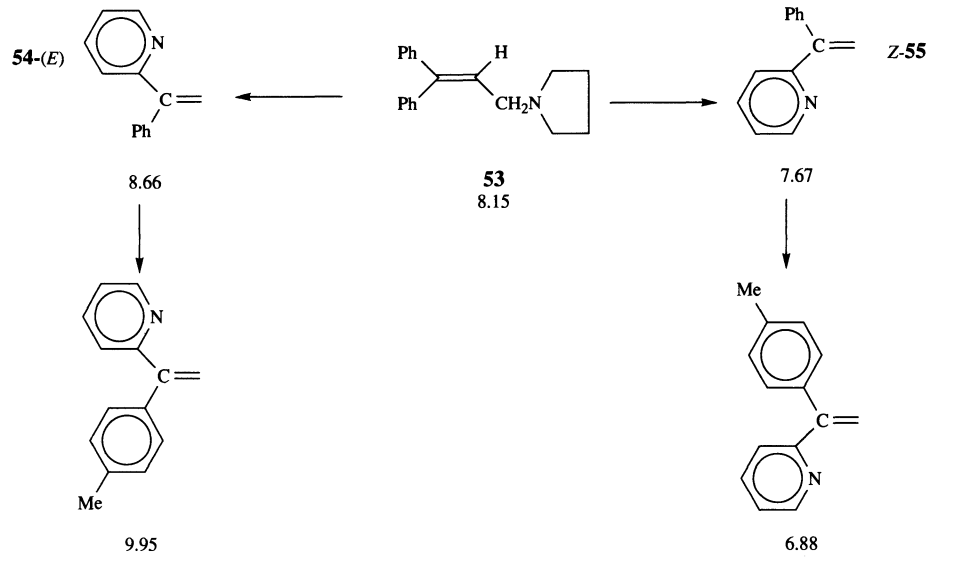
E-**51**



52

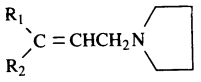
The compounds may be regarded as dehydro analogues of the pheniramine antihistamines, and receptor sensitivity to the disposition of the two aryl groups about a benzylic carbon is found for both groups. Affinity measurements for several *E,Z*-aminopropene pairs, determined in gut-bath tests, are shown in Table 11.14. The variations in affinity ratios must be viewed with reservations in the absence of evidence of isomeric purity, but it is of significance that the 10^3-fold potency difference between triprolidine and its *Z*-isomer has recently been approached in our laboratory using materials shown to be isomerically pure by HPLC. The same materials, in binding experiments performed on guinea-pig cerebellum or cortex, gave association constants (K_a, M^{-1}) of $1.9–2.3 \times 10^9$ for the *E*-isomer and $1.7–2.0 \times 10^7$ for the *Z*-isomer.[121] The fact that the *E*:*Z* affinity ratio of 115 (95–135) was lower than that found in the gut-bath experiments indicates that central and peripheral H_1-receptors are not identical.

Taking 1,1-diphenyl-3-pyrrolidinoprop-1-ene **53** as the standard (Scheme 11.8), it is seen that replacement of phenyl by 2-pyridyl raises the affinity constant when made *trans* to pyrrolidinomethyl but *lowers* it when made *cis* to the same substituent. In sharp contrast, replacement of phenyl by *p*-tolyl in the *E*-isomer **54** further raises potency but reduces activity in the *Z*-form **55**. With phenyl and *p*-chlorophenyl the effects are much less. One of the causes of the large affinity ratio observed for triprolidine and its *Z*-isomer must therefore be due to the com-



Scheme 11.8. Changes in log K_B values among 3-aminopropene antihistamines.[87]

TABLE 11.14.
Affinities of 3-Aminoprop-1-ene Geometrical Isomers for H_1 Sites of Guinea-Pig Ileum (at 37° C and 15–30 min contact time unless otherwise stated)



R_1	R_2	Salt	Configuration	log K_B (pA_2)	Ratio of affinities (E:Z)	Ref.
2-Pyridyl	*p*-Tolyl	Oxalate	*E*	9.945 ± 0.047 (6)[a]	1167	87
2-Pyridyl	*p*-Tolyl	HCl	*Z*	6.878 ± 0.059 (20)		
2-Pyridyl	*p*-Tolyl	Oxalate	*E*	9.0[b]		86
2-Pyridyl	*p*-Tolyl	HCl	*E*[c]	10.034 ± 0.011 (26)[d]	600	121
2-Pyridyl	*p*-Tolyl	HCl	*E*[c]	7.256 ± 0.029 (21)		
2-Pyridyl	*p*-Cl.C_6H_4	Oxalate	*E*	8.611 ± 0.003 (9)	6.8[h]	87
2-Pyridyl	*p*-Cl.C_6H_4	Oxalate	*Z*	7.777 ± 0.065 (9)		
2-Pyridyl	Phenyl	Oxalate	*E*	8.658 ± 0.047 (5)	9.3	87
2-Pyridyl	Phenyl	Oxalate	*Z*	7.688 ± 0.035 (8)		
2-Pyridyl	Phenyl	Oxalate[f]	*E*	7.548 ± 0.021 (6)	17.7	87
2-Pyridyl	Phenyl	Oxalate	*Z*	6.3[e]		
Phenyl	*p*-Cl.C_6H_4	HCl[f]	*E*	8.451 ± 0.052 (6)	14	87
Phenyl	*p*-Cl.C_6H_4	HCl	*Z*	7.3[g]		

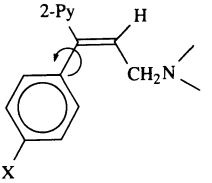
[a] Number of experiments.
[b] 2 min contact time.
[c] Evidence of isomeric purity > 99% by HPLC.
[d] Equilibration time 1–2 h.
[e] Calculated from mean log K_B 6.3736 ± 0.052 (6) for 9:1 mixture.
[f] NMe_2 derivative.
[g] Calculated from mean log K_B 7.956 ± 0.042 (6) for 7.3:2.7 mixture.
[h] Earlier report: 80.[88]

bination of both 2-pyridyl and *p*-tolyl groups being in favorable positions relative to the aminomethyl group in the active isomer (*E*), but being in unfavorable positions in the less active compounds (*Z*).

11.9.1. Configurational Studies

Isomeric 1,1-diaryl-3-aminopropenes are generally separated by fractional crystallization of oxalate and other salts. Isomeric purity may be judged by comparing the UV and ^{1}H-NMR spectra of related *E*- and *Z*-isomers (see below)—these methods are relatively insensitive, however, and inferior to chromatographic techniques. We developed an HPLC procedure suitable for analyzing mixtures of triprolidine and its *Z*-analogue which could detect <1% of the minor component.[121]

Configurational assignments were originally based on differences in the UV spectra of isomers.[89] In *E*-isomers **56**, steric interactions between *cis*-placed phenyl



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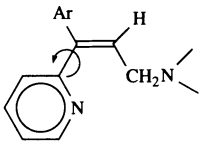
(or substituted phenyl) and the aminomethyl group force the aromatic group out of the plane of the double bond, hence the effective chromophore is of the 2-vinylpyridine type. In *Z*-**57**, however, it is the 2-pyridyl group that is deflected



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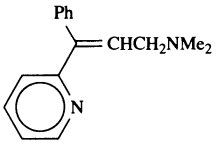
from the double-bond plane, and the chromophore is of the styrene type. Hence, the isomer having a UV spectrum similar to that of 2-vinylpyridine is assigned the *E*-configuration, while that with a spectrum like styrene must be the *Z*-isomer. Typical results (in ethanol) are shown below for the isomers **58**.

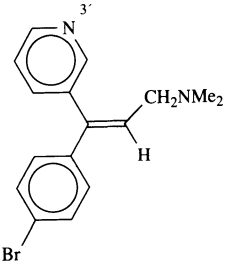


58

	$\lambda_{max}(\varepsilon)$
E	245 nm (7800), 283 nm (5700)
Z	252 nm (10000)
2-Vinylpyridine	235 nm (12600), 278 nm (5010)
Styrene	245 nm (15850)

Differences in the ^{1}H-NMR spectrum of isomers of this kind may also be used for configurational assignments. From analyses of spectra of ethylene, styrene, and 2-vinylpyridine, it is known that a vinylic proton *cis* to 2-pyridyl (as in *E*-**56**) is deshielded about three times as much as that of a proton *cis* to phenyl (as in *Z*-**57**). It follows from this and other considerations that the vinylic signal of *E*-isomers should be at lower field in the spectra of isomeric pairs (from similar arguments, the *Z*-CH_2N signal should take the lower-field position in solvent $CDCl_3$ but not in D_2O).[86] Spectra of triprolidine and its *Z*-isomer (Fig. 11.4) illustrate this method.

The antidepressant agent zimeldine (**59**, *Zelmid*) (now withdrawn in the U.K.),



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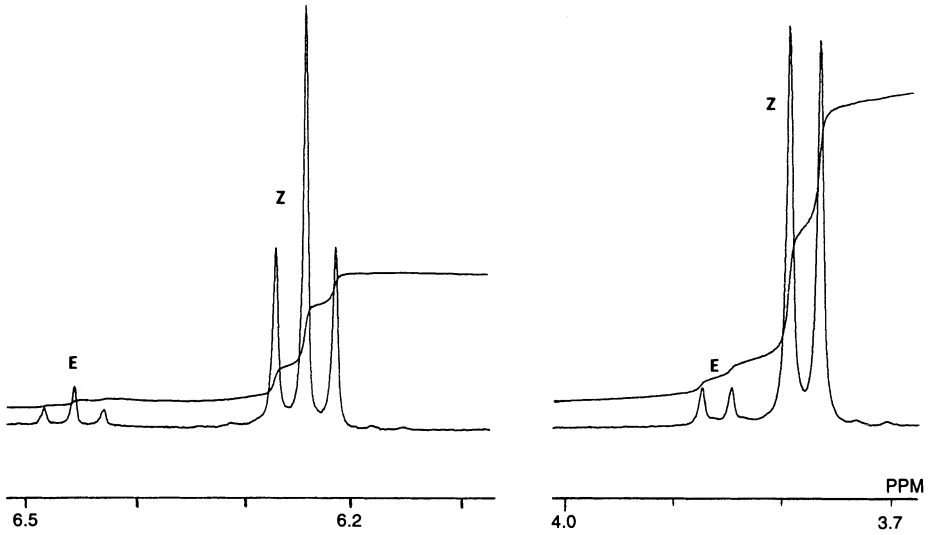


FIGURE 11.4. Part of the 270-MHz ^{1}H-NMR spectrum of the *Z*-isomer of triprolidine HCl in D_2O containing the *E*-isomer as contaminant. The triplet pair between 6.2 and 6.5 ppm is due to the vinylic protons (*E* 6.46, *Z* 6.24 ppm) and the doublet pair near 3.8 ppm to the methylene protons adjacent to nitrogen (*E* 3.86, *Z* 3.78 ppm). Pure isomers displayed single-triplet and single-doublet signals (after Granellin *et al.*[13] and Granellin[21]).

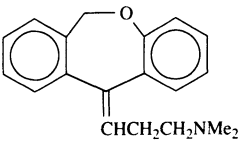
of Z-configuration (see below), and its corresponding E-isomer are 3-pyridyl analogues of E-Z isomers of the triprolidine class. Receptor stereoselectivity is unaltered by this variation although potency levels are much depressed (Table 11.15).[91] Thus the *E*-isomer of **59** was seven times more effective than zimeldine in displacing [^{3}H] mepyramine from rat cerebral cortex, and eleven times more potent at guinea-pig ileum sites. Similar orders of potency differences were found for the corresponding *E*- and *Z*-secondary amines. Brompheniramine was 15–20-fold more potent than *E*-**56** in these tests.

Zimeldine and its nor-metabolite are selective neuronal 5-HT uptake inhibitors, while *E*-analogues are more active in blocking NA uptake.[92] The *Z*-configuration of zimeldine follows from an X-ray analysis of the di-HCl salt,[93] while UV and NMR methods are useful in differentiating the two geometrical isomers.[54,121]

TABLE 11.15.
Biological Data for Zimeldine, Norzimeldine, and Standard Agents[91]

	Binding IC_{50} (nM)	GPI IC_{50} (nM) *vs* histamine
Zimeldine (*Z*)	2900	20000
E-Isomer	400	1800
Norzimeldine (NHMe)	8200	42000
E-Isomer	3400	—
Brompheniramine	20	120
Doxepin	10	260

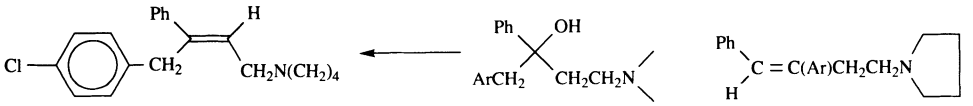
Another antidepressant, doxepin (**60**), is also a potent antihistamine; K_A values: gut-bath 2.6×10^{10} M^{-1}; binding 1.12×10^{10} M^{-1},[94] 3.8×10^9 M^{-1} (from K_D 0.26 nM).[72] The drug consists of a 15:85 ratio of *Z* and *E* isomers. Otsuki *et al.*[95] found the *Z*-isomer to be 3.5 times more potent than the *E*-form vs. histamine in the guinea-pig ileum.



60 doxepin

11.9.2. Pyrrobutamine and Related 1,2-Diaryl-4-aminobutenes

The formerly used clinical agent pyrrobutamine phosphate (**61**, *Pyronil*) is one of the four dehydration products from the tertiary alcohol **62**. The position of its double bond and configuration remained in doubt for some years but was clarified by ^{1}H-NMR and UV spectroscopy in 1970 and established as the *E*-but-2-ene **61**.[96] Differences in the ^{1}H-NMR and UV spectra of isomeric pairs of but-2-enes (and but-1-enes) could be applied to configurational assignments using the same principles as employed in the case of the aminopropenes. A typical set of data and assignments are shown in Scheme 11.9.



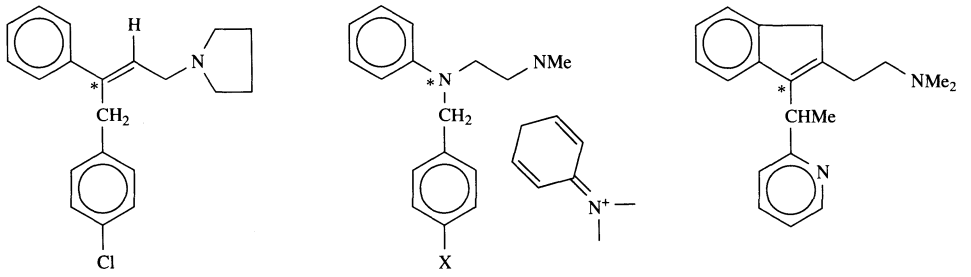
61 **62** **63**

The compound **61** is of high potency (log K_B 10.34 at equilibrium, guinea-pig ileum sites) and significantly more active than its *Z*-but-2-ene and but-1-ene analogues.[96] The but-1-enes **63** are, nevertheless, reasonably potent antihistamines

	Isomer	Vinylic chemical shift (ppm)[a,b]	λ_{max}(nm) (ε)
Ph, H: C = C(Ar)$CH_2CH_2NMe_2$ (but-1-ene)	*E*	6.85 s	263 (16 800)
	Z	6.62 s	255 (11 270)
Ar, $PhCH_2$: C = C(H)CH_2NMe_2 (but-2-ene)	E	6.17 t (7 Hz)	241 (11 500)
	Z	5.588 t (7 Hz)	end absorption only

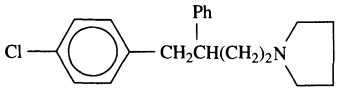
[a] Ar = *p*-$OMeC_6H_4$.[98]
[b] Ar = Ph[99]
(s = singlet, t = triplet)

Scheme 11.9. Spectroscopic evidence of the configuration of some aminobutene antihistamines.[98, 99]



Scheme 11.10. Structural comparisons among some semirigid antihistamines (see also the indole **72**). The asterisk (*) denotes center of rigidity.

with log K_B values 8.12 (*E*) and 8.65 (*Z*). Potency data on a variety of but-2-enes related to **61** and but-1-enes akin to **63** confirm the superiority of *E*-but-2-ene geometry in blocking H_1-sites of the guinea-pig ileum. From this fact, a structural resemblence between pyronil, diamines of the phenbenzamine type, and dimethindene may be discerned (Scheme 11.10). All three compounds possess a degree of rigidity about the starred (*) center which must be important for activity, since when is is removed, as in the dihydroanalogue of pyronil **64**, activity is lost.[97]



64

It is of interest that the pheniramines, dihydroanalogues of the aminopropene class, are potent antihistamines—in this case conformational factors may maintain the rigid orientation of pharmacophoric groups present in the *E*-alkene (see also below).

11.10. Conformational Analysis of Antihistamines

Up to 1986, fourteen H_1-antihistaminic salts had been analyzed by X-ray crystallography and these data have been examined by Borea *et al.*[100] with the aim of discovery a common stereochemical vector of antihistaminic activity. The solid state geometrics are presented in Fig. 11.5. Parameters selected for comparison were the distances d_1, N^+–CG1; d_2, N^1–CG2; d_3, CG1–CG2, where $\geq N^+$ is the protonated nitrogen, while CG1 and CG2 are the centers of gravity of aromatic rings 1 and 2, respectively. Values of d_1 (6.20 ± 0.1 Å) and d_3 (4.90 ± 0.24 Å) fell within narrow ranges while the d_2 values were more variable (5.26–6.83 Å). The d_1 and d_3 values were mostly close to those of low-energy conformers calculated by the force-field energy method. Figure 11.6 shows a suggested pharmacophoric geometry for optimal antihistaminic activity based on these values.

The ^+N ... CG1 (Å) distance (d_1) was considered the more significant parameter, and this was achieved by different compounds in different ways as

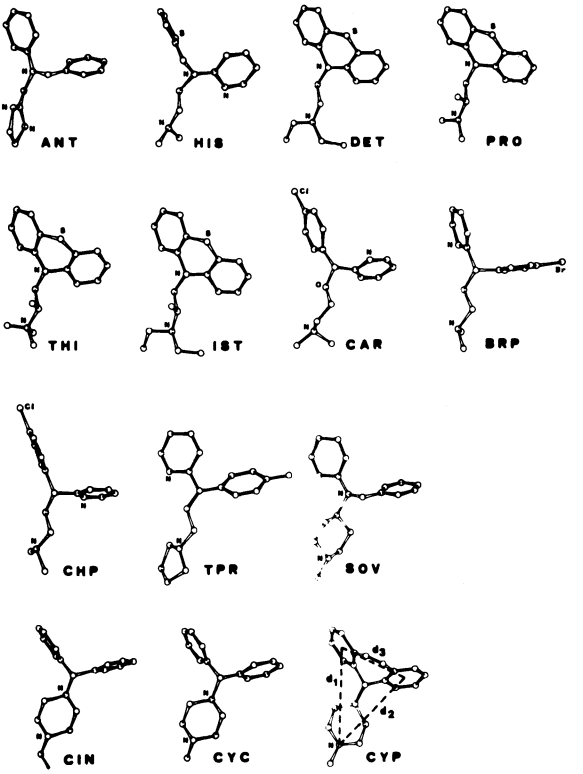


FIGURE 11.5. Geometries of some antihistamines from X-ray data. All molecules are projected on the plane defined by the amino nitrogen and the centers of gravity of the two aryl rings.[100] Abbreviations: ANT antazoline HCl, HIS histadyl HCl (ethylenediamine class); DET diethazine HCl, PRO promethazine HCl, THI thiazinamium methylsulfate, IST isothazine HCl (phenothiazine class); CAR carbinoxamine maleate (aminoethylether class); BRP brompheniramine maleate, CHP chlorpheniramine maleate (propylamine class); TPR triprolidine HCl (propenylamine class); SOV soventol HCl, CYP cyproheptadine HCl (piperidine class); CYC cyclizine HCl, CIN cinnarizine (piperazine class) (after Borea *et al.*).[100]

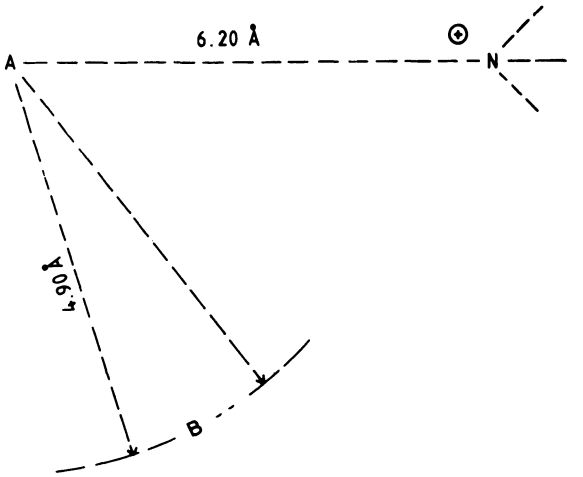


FIGURE 11.6 Suggested pharmacophore geometry for optimum antihistaminic activity. A and B indicate the centers of gravity of Ring A and Ring B, respectively. The spreading of the N–A–B angle is that actually found (after Borea *et al.*).[100]

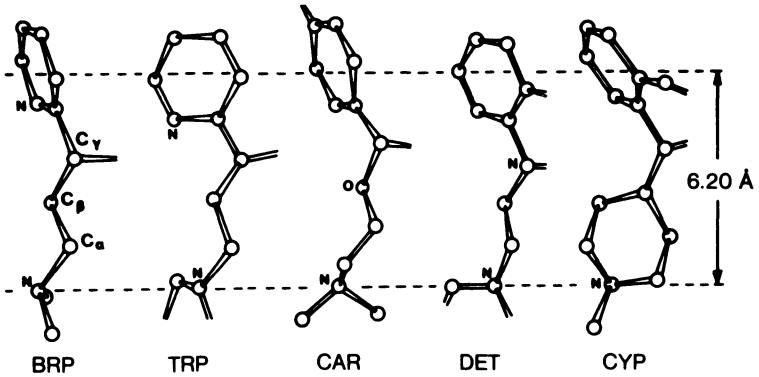


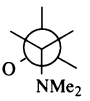
FIGURE 11.7. Conformation of different chains in antihistamines capable of reproducing the same d_1 of distance 6 to 6.40 Å (after Borea *et al.*).[100]

shown for the five examples of Fig. 11.7. In the pheniramines ($\gtrless CCH_2CH_2N^+\lesseqgtr$) and ethylenediamines ($\gtrless NCH_2CH_2N^+\lesseqgtr$) the correct distance is obtained by a zigzag planar conformation of the chain, while in the five-membered aminoethylene ether chain ($\gtrless COCH_2CH_2N^+\lesseqgtr$) of carbinoxamine the same distance requires a helical conformation in which the $OCH_2CH_2N^+\lesseqgtr$ dihedral angle is *gauche*. When the linear chain forms part of a nitrogen heterocycle, the C/NCH_2CH_2 ring moiety mimics the length of the open $CH_2CH_2N^+\lesseqgtr$ chain. The d_2 distance was considered of less importance than that of d_1 to the ligand–receptor interaction, although receptor differentiation between the two aromatic groups is clear from studies on antipodal pairs of chiral antihistamines and *E*/*Z* pairs of the triprolidine type (pages 377 and 393).

When one examines the specific structure of the aromatic ring to which the d_1 distance applies, a lack of consistency is apparent, especially in derivatives of the 2-pyridyl type. Thus while A is 2-pyridyl in *RS*-brompheniramine and triprolidine, it is *p*-chlorophenyl in carbinoxamine and (+)-chlorpheniramine (B being 2-pyridyl). Since all these compounds may be correlated in terms of absolute configuration (p. 401), anomalies of this kind detract from the significance of the solid-state conformational data although these do provide evidence of probable solute state conformations about the linear chain.

Marshall's group[101] carried out a matching exercise by computational methods of seven antihistamines and deduced a three-dimensional pharmacophore with parameter magnitudes similar to those derived from X-ray data, namely, $d_1 = 6.5$ Å, $d_2 = 5.8$ Å, $d_3 = 4.9$ Å. In clemastine, carbinoxamine, and chlorpheniramine the greater $^+$N-aromatic centroid distance d_1 involved *p*-chlorophenyl, while *p*-tolyl (not 2-pyridyl) was involved in triprolidine, results which diverge from some of the solid state data.

The few reported NMR conformational studies of antihistamines have centered on the bimethylene chain linking the aromatic and basic features of the molecule. Ham[102] carried out second-order analyses of 60 and 100 MHz ^1H spectra in D_2O and concluded that *gauche* conformers **65** are favored in the case of diphenhydramine HCl, while little or no conformational preference about C-1, C-2 is exhibited by the ethylene diamines mepyramine, methapyrilene, and tripelennamine as salts (see **66**).



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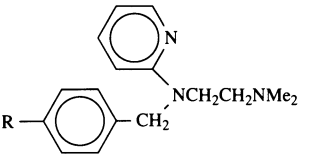

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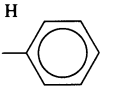
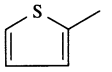
mepyramine R = OMe
tripelenamine R = H

methapyrilene  replaced by 

Testa[103] has advanced evidence for both folded and extended conformations of pheniramine derivatives in D_2O on the basis of CD and 60 MHz NMR data.

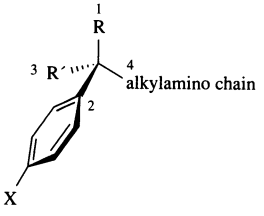
High-field (270 MHz and above) ^{1}H-NMR spectra of antihistamines are now available and exhibit features which must have conformational significance. Thus while the NMe_2 signal of diphenhydramine HCl is a 6-proton singlet, that of carbinoxamine maleate **20** and doxylamine succinate **21** form two well-separated 3-proton singlets, evidence of nonequivalent *N*-methyl environments.[104]

There is NMR evidence that cyproheptadine HCl (**39**, OMe replaced by H) exists in $CDCl_3$ as a 1:4 mixture of conformers with the most populated form corresponding to the solid state geometry.[105]

Conformational considerations of H-2 antagonists of the phenylformamidine series (mifentidine and its analogues) have been presented by Donetti *et al.*[119] as have also those of isocytosine derivatives, which include the dual H-1/H-2 antagonist oxmetidine.[120]

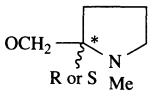
11.11. General Remarks

Data on dissymmetric antihistamines of both chiral and geometrical type presented in this chapter provide ample evidence of the stereoselective nature of H_1-histamine receptors in regard to ligands which block these sites. The work has established the importance of the configuration of a chiral center close to the diaryl unit of the molecule **14** (see pages 377 and 387) as opposed to further removed chiral features. Furthermore, a substantial number of antipodal pairs with chiral centers which may be related meaningfully have now been examined, and results demonstrate that the H_1-receptor is preferentially blocked by a ligand of general absolute geometry **67**. Details of the evidence are summarized in Table 11.16.



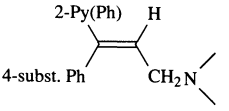
67

TABLE 11.16.
Review of Chiral Geometry for H-1 Antihistamine Activity

Name and configuration of the more potent antipode (eutomer)	Substituent about chiral center (see **67**) 1	2	3	4	References to pharmacology
R-(+)-Neobenodine configuration: Ref. 49 and CD evidence[a]	Ph	4-MeC_6H_4	H	$O(CH_2)_2NMe_2$	49, 51
S-(−)-Carbinoxamine configuration: Ref. 66 and CD evidence[a]	2-Py	4-ClC_6H_4	H	$O(CH_2)_2NMe_2$	53, 54
RR-(+)-clemastine	Ph	4-ClC_6H_4	Me		49, 57
RS-(−)-diastereoisomer configuration: Ref. 57 and CD evidence[a]	Ph	4-ClC_6H_4	Me		
R-(−)-Mebrophenhydramine	Ph	4-BrC_6H_4	Me	$O(CH_2)_2NMe_2$	68
S-(+)-pheniramine and 4-Br and 4-Cl analogs configuration: Ref. 62, 63 and CD evidence[a]	2-Py	4-XC_6H_4 (X = H, Br, Cl)	H	$(CH_2)_2NMe_2$	49, 67, 60, 68

[a] Circular dichroism evidence, Ref. 68 (unpublished).

Receptor sensitivity to the disposition of the two aryl groups about a benzylic carbon is also apparent in antihistamines of the aminopropene type exemplified by triprolidine **51**. Isomers of type **68** (configuration *E* for 2-pyridyl, *Z* for phenyl)



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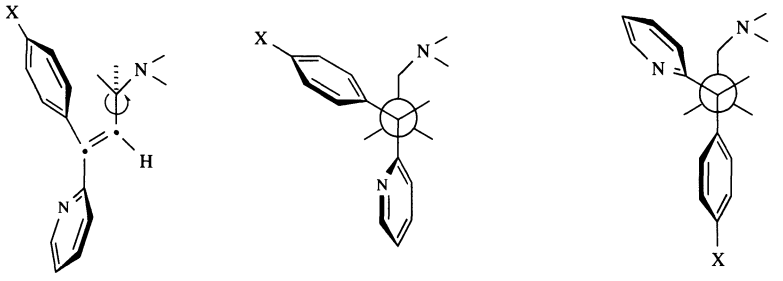
have higher affinities than the corresponding *Z* (2-Py) or *E* (Ph) isomers. Receptor stereoselectivities are maintained in the less potent 3-pyridyl analogues (page 394). It is significant that chiral and geometrical configurational relationships correspond (Scheme 11.11). Thus antipodal pheniramines corresponding to the arrangement of



E-configuration *S*-eutomer *R*-distomer

Scheme 11.11. Triprolidine – pheniramine steric comparisons.

the constrained isomer triprolidine have the configuration B (*S*), while their mirror images C are equivalent to the feebly active *Z*-analogue of the aminopropene. When the double bond of triprolidine is formally reduced, the relative orientations of the two aromatic groups and the aminoalkyl chain alter because of hybridization changes at C-1 and C-2, but overall gross relationships must be maintained in the *S*-antipode because H_1-receptor affinities of *S*-antipodes and triprolidine are of the same order.

From structural features of most agents which block H_1-receptors of histamine, it is evident that ligand–receptor interactions involve the dual aromatic and protonated amino features of the molecules. Of the two receptors which accommodate the aromatic groups, one prefers unsubstituted phenyl (or 2-pyridyl) and the other a *para*-substituted group, as established from the stereochemical investigations detailed above. It also follows from the same studies that the anionic site of the receptor (which associates with ^+NH of the anionic site of the ligand) is closer to the more extended aromatic recognition region. Other conformationally restrained ligands are discussed later.

11.11.1. Isomeric Potency and Affinity Ratios

In the absence of evidence of isomeric purity, one is ill-advised to attach too much significance to numerical differences between potency or affinity ratios obtained from isomeric sets because misleading activity values result even from the presence of small amounts of isomeric impurity (most serious when the less active form is contaminated with the more active isomer; see Ref. 106 and page 307). The optical purity of most chiral antihistamines examined, especially those prior to 1980, are based on measurements of optical rotation, a measured property to which both antipodes contribute. Chromatographic and NMR methods which quantitate each antipode separately are now being applied increasingly to chiral analysis, and a fair degree of confidence may be placed in potency ratio values that relate to isomeric pairs so analyzed (see Chap. 2, page 33).

Isomeric potency data for a series of chiral antihistamines examined at Bath are presented in Table 11.17. While the test procedure is the same for all of them (inhibition of histamine-induced contraction of the guinea-pig ileum), factors such as differences in bath temperature and equilibration time, and, most critically, differences in the optical purity of antipodal pairs, must influence the numerical values recorded. However, from data for isomeric pairs prepared at Bath and shown by HPLC and NMR procedures to be isomerically pure to the extent of 98% or better (i.e., isomeric ratio 99:1 or above), certain trends may be discerned.

1. Ratio magnitudes for brompheniramine and chloropheniramine were notably high (>1500 at 30 °C, >370 at 37 °C) and comparable with those recorded for triprolidine and its *Z*-isomer.
2. Lower orders of potency ratio were recorded for antipodal carbinoxamines (65 at 30 °C, 34 or 37 °C) and mebrophenhydramine (47 at 30 °C, 32 at 37 °C). Molecular flexibility might be one of the factors accounting for the differing range of isomeric potency ratios observed.

Thus the conformational options of the pheniramines (and even more so the triprolidine group) are less than those of the diarylphenhydramines, a group which

TABLE 11.17.
Log Affinity Constants for Antipodal Pairs of Chiral Antihistamines at Guinea-Pig Ileum Sites[68]

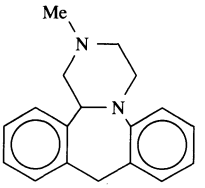
	Temp. (° C)	(+) Mean Log $K \pm$ (S.E.)	(−) Mean Log $K \pm$ (S.E.)	Difference	Ratio of activity[a]
Chlorpheniramine	37	9.339 ± 0.024 (20)	6.555 ± 0.043 (12)	2.784	608
	30	9.975 ± 0.029 (18)	6.749 ± 0.039 (12)	3.226	1683
Brompheniramine	37	9.259 ± 0.027 (25)	6.687 ± 0.034 (10)	2.572	373
	30	10.295 ± 0.023	7.111 ± 0.022 (9)	3.184	1528
		(−)	(+)		
Carbinoxamine	37	8.670 ± 0.024 (26)	7.142 ± 0.016 (15)	1.528	34
	30	9.005 ± 0.019 (24)	7.192 ± 0.026 (15)	1.813	65
Mebrophenhydramine	37	9.715 ± 0.013 (21)	8.206 ± 0.020 (15)	1.509	32
	30	9.949 ± 0.019 (16)	8.280 ± 0.011 (15)	1.669	47

[a] Theoretically, assuming one antipode to be devoid of activity, ratios greater than 100 and 1000 correspond to optical purities above 99.01 and 99.9%, respectively.[106]

incorporates an additional bond in the chain linking the chiral and basic center of the molecule. Another factor to consider is the degree of difference between the dual aromatic features. This aspect appears reasonable when one considers that receptor recognition and differentiation of the two aromatic units must be the prime factor responsible for affinity differences between isomers. In many antihistamines, such as diphenhydramine (**15**a) and the aminopropene (**53**) of Scheme 11.8, the two aryl features are identical. When this is not so, the greater the difference in aromatic nature (homo vs. heteroaryl; homo; vs. homoaryl), the greater is the degree of receptor discrimination between the two aromatic features to be expected. It is therefore significant that the higher potency ratios are found for dissymmetric antihistamines that contain a pyridyl–phenyl (or substituted phenyl) aromatic feature and are of limited flexibility.

11.12. Conformationally Restrained Antihistamines of Cyclic Nature

In addition to antihistaminic agents conformationally restricted by a carbon–carbon double bond, such as triprolidine **51** and pyronil **61**, numerous agents constrained by cyclic systems have been reported. Such antagonists are often of high potency and active at receptors additional to the H_1-site of histamine. Mention has already been made of the indene derivatives dimethindene **40** and phenindamine **41**, and cyproheptadine (**39**, OMe replaced by H) and its relatives (page 387). The latter group are powerful blockers of serotonin (5-HT_2) receptors as is also doxepin (**60**). Mianserin (**69**, Bolvidon), designed as a cyclized analogue of phenbenzamine **70**, is another dual (H_1, 5-HT_2) antagonist[107] and is employed as an antidepressant agent (*Martindale*, **29**, page 372). Other examples include mebhydrolin **71**,[108,109] the indole derivative **72**[110] and the 9,10-dihydro-9,10-ethanoanthracene **73** which duplicates the structural features of diphenhydramine in a rigid molecule (potency ≡ tripolidine).[111] The indole **72**, when drawn in its



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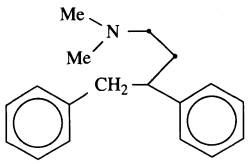

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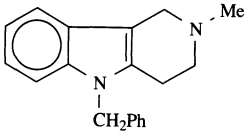

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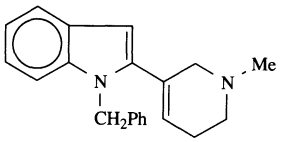

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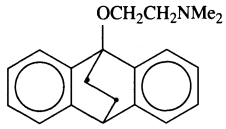
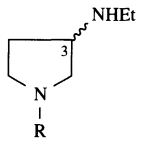


73

s-trans conformation (as shown), overlaps much of the structure of dimethindene and may be included in the set of Scheme 11.9. Its activity in antihistamine and antiserotonin tests fell sharply after reduction. Witiak *et al.*[112] examined some 3-ethylaminopyrrolidines **74** carrying diaryl substituents (bridged and nonbridged) at ring nitrogen. Some potent products resulted (Table 11.18) but receptor

TABLE 11.18.
Antihistamine Potencies of Some Derivatives of 3-Ethylaminopyrrolidine



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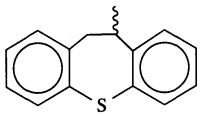
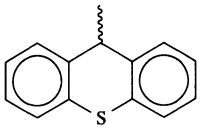
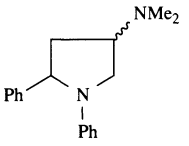
R	Configuration at C-3	ED_{50} (M)[a]
	*R*[b]	5.9×10^{-8}
	S	8.4×10^{-8}
	*R*	6.2×10^{-7}
	S	5.3×10^{-7}
$CHPh_2$	*R*	8.8×10^{-9}
	S	9.2×10^{-9}
Methapyrilene HCl	—	5.7×10^{-9}

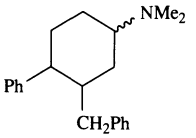
[a] Antagonism of histamine (2.4×10^{-8} M) at GPI sites.
[b] ED_{50} values of corresponding *t*-amines (NEtMe) were 4.4×10^{-8} (*R*) and 4.1×10^{-8} (*S*).

stereoselectivities to *R*- and *S*-antipodes were of low order, as generally found for molecules with the chiral center well removed from the aromatic substituents.

Hanna *et al.*[113] prepared some isomeric 1,2-diphenylpyrrolidines **75**, which also may be regarded as cyclic forms of phenbenzamine **70**. Both *cis*- and *trans*-**75** were potent H_1-antagonists; the *cis*-isomer (5×10^{-8} M) caused a 45–55% inhibition of histamine-induced GPI contractions while *trans*-**75** (1×10^{-8} M) caused an 85% inhibition. The more flexible piperidine analogues **76** were less potent and lacked the receptor selectivity of the pyrrolidines.[114]



75



76

To date, structural and steric correlations among the wide range of conformationally restricted antihistamines now reported have not been carried out. Such a task would seem an ideal problem for investigations by computer graphics techniques.

11.13. Central Effects

It is well known that many antihistamines lead to drowsiness and impaired performance (*Martindale* **29**, page 443). In order to obtain evidence to support the view that these central effects are due to antagonism of H_1 receptors within the CNS, antipodal pairs of chlorpheniramine and dimethindene maleates prepared at Bath were assessed in human volunteers by a double blind study.[115] Performance, subjective assessments, and sleep latencies were measured at 0830, 1000, 1100, and 1230 h, with drug ingestion after the first session at 0930 h. Each subject took, on separate occasions with intervals of at least four days, 10 mg (+)- and (−)-chlorpheniramine, 5 mg (+)- and (−)-dimethindene, 5 mg triprolidine as active control, and two placebos. Changes in the measures of central activity with (−)-chlorpheniramine and (+)-dimethindene (peripheral distomers) were not different from those with placebo. With (+)-chlorpheniramine and (−)-dimethindene (peripheral eutomers), however, marked central effects were observed in all tests applied. Thus the more potent peripheral H_1 antagonist was also the more potent central sedative in the two sets of antipodes, results consistent with the notion that blockade of central H_1 receptors may cause sedation.

Chiral aspects are included in two recent reviews of histamine ligands.[116,117]

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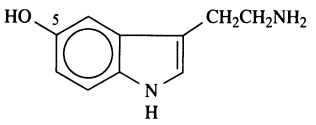
12

5-Hydroxytryptamine Ligands

12.1. Introduction

Although 5-hydroxytryptamine (**1**; 5-HT, serotonin) was detected in the mammalian CNS more than 40 years ago,[1] advances in the understanding of central serotonergic mechanisms lapsed behind those of other neurotransmitters, due chiefly to the relative lack of compounds with selectivity for 5-HT receptors. Hence



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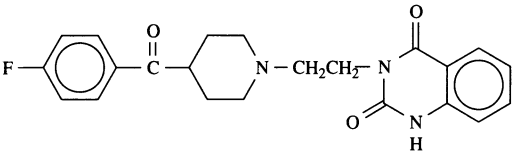
stereochemical study of chiral 5-HT ligands has been limited, with the notable exception of investigations of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) described later. Interest in 5-HT receptors and their ligands has risen dramatically during the past decade and several valuable reviews on the topic are available.[2–7] The detection and subtype classification of 5-HT receptors rests heavily on the results of binding experiments, briefly outlined here. From work with [^{3}H]5-HT, Peroutka and Snyder[8] proposed the existence of two major populations of central 5-HT binding sites, namely, 5-HT_1 sites labeled with high affinity by the natural ligand and 5-HT_2 sites labeled (front cortex especially) by [^{3}H]spiperidone. The ability of the DA-receptor ligand spiperidone to label 5-HT sites was originally reported by Leysen *et al.*[9] The further classification of 5-HT_1 receptors followed observation that a portion of the binding of [^{3}H]5-HT to brain receptor membranes was displaceable by low concentrations of spiperidone—such sites were subsequently termed 5-HT_{1A} while sites insensitive to spiperidone were termed 5-HT_{1B}. Later, 8-OH-DPAT was identified as a ligand selective for 5-HT_{1A} sites (see below) while the quinazoline derivative **2** (R41-468, ketanserin) was shown to have a high affinity for 5-HT_2 sites. Functionally, ketanserin is an antagonist.[10] The 5-HT_{1B} receptor has been labeled with (−)-[^{125}I] iodocyanopindolol (the 2-cyano-3-iodo derivative of pindolol, Chap. 5, page 151) with isoprenaline present



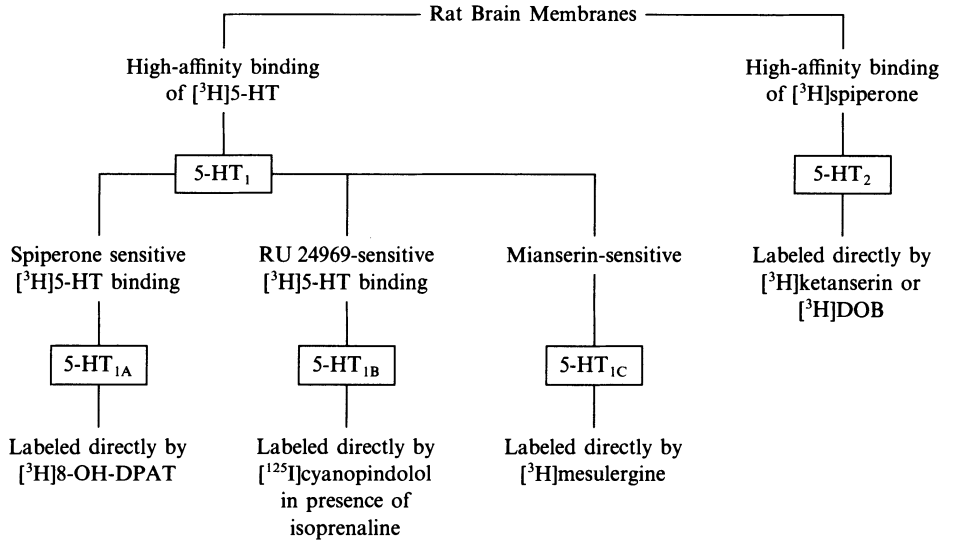
2

to block the binding of this radioligand to β-adrenergic receptors[11]; the affinities of antipodes of this agent differed 100-fold.[12] Pindolol (page 151) itself stereospecifically and selectively (DA and NA receptors were unaffected) inhibited rat brain 5-HT synthesis as judged from assays of 5-HT levels after 4 mg/kg ip dosage.[13] Such action was confined to the levo antipode. The betablockers betaxolol, metaprolol, and ICI 118,551 had no effect in this regard (propranolol has significant affinity for 5-HT_{1A} and 5-HT_{1B} sites as judged from binding experiments; such sites favor the levo antipode).[86]

The current status of 5-HT receptor subtypes is summarized in the *TiPS* receptor nomenclature supplement of January 1991—it includes additional types (5-HT_{1D} and 5-HT_3). Earlier, Fozard provided a useful review in the 100th issue of *TiPS*.[14] Table 12.1, reproduced from a recent monograph on 8-OH-DPAT,[15] is a useful guide to an increasingly complex scenario.

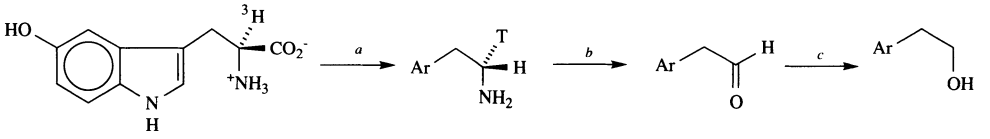
The biosynthesis of 5-HT from the essential amino acid L-tryptophan closely relates to that of dopamine from tyrosine, and involves a tryptophan hydroxylase and L-amino acid decarboxylase—both enzymes require substrates of L-configura-

TABLE 12.1.
Central Serotonergic Receptor Classification[a]



[a] After Dourish *et al.*[15]; the 5-HT_3 subtype, originally named "M" on the basis of the ability of morphine to antagonize the serotonin receptor mediating depolarization of cholinergic nerves in the GPI, is not included in this scheme. Specific 5-HT_3 antagonists are now available (morphine is a nonselective antagonist).

tion.[16] Battersby *et al.*[17] have established that the decarboxylation of tritiated 5-HTP (**3**) takes place with retention of configuration. The fact that the primary alcohol product (**4**) was devoid of radioactivity, after oxidation by pea seedling amine oxidase followed by reductioin, proved that [^{3}H] occupied the pro-*S* position from knowledge of the steric course of the oxidative mechanism (Chap. 4, page 78).

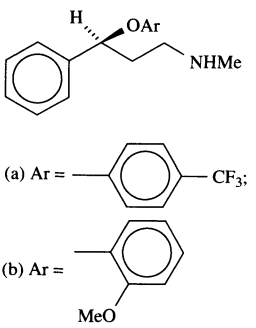


3 *S*[d] **4**

[a] Mammalian L-aminoacid decarboxylase.
[b] Pea seedling amine oxidase (specific for S_i hydrogen).
[c] Liver alcohol dehydrogenase.
[d] *rac*-**3** could be used since only the *S*-antipode is accepted by the enzyme as a substrate.

12.2. Uptake Inhibition

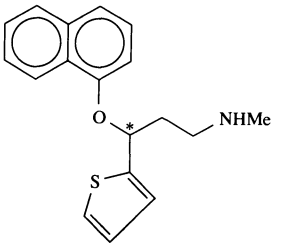
Several nontricyclic derivatives of potential value as antidepressant agents act by preventing the uptake of neurotransmitter amines into presynaptic storage vesicles, including that of 5-HT. Agents specific for 5-HT are of special interest to this chapter. One example is fluoxetine (**5a**),[19,20] closely related to nisoxetine (**5b**) which selectively blocks NA uptake.[18]



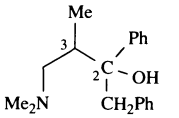
5 *S*-antipode shown

A conformational study of the two agents has been reported using computational methods, and differences in their spatial preferences suggested as a reason for the difference in amine-uptake selectivities.[21] Antipodal forms of fluoxetine differed little in their abilities to block 5-HT uptake (the dextro isomer had the longer duration of action in rats)[20]; the configuration of the somewhat more effective dextro isomer was shown to be *S* by a stereoselective synthesis.[22]

Synthesis of antipodes of the related 2-naphthoxy-2-thienyl derivative **5c** has been described by a method which rests on the asymmetric reduction of a Mannich base intermediate with a complex of LAH and 2*R*,3*S* or 2*S*,3*R* (**5d**).[23] The compound **5c** is a potent inhibitor of 5-HT and NA uptake carriers, and its *S*-antipode



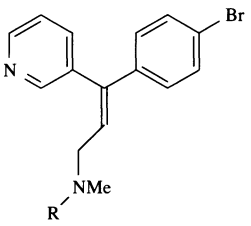
5c

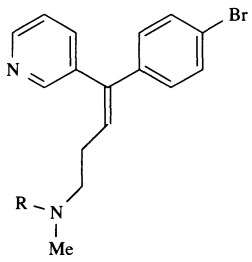


5d
LAH + 2 equiv gives the
Yamaguchi – Mosher – Pohland complex

(related to *S*-fluoxetine) is coded LY 248686—antipodal comparisons were not presented in this paper.

The formerly used antidepressant zimeldine (**6**a) and its *sec*-amine metabolite (both of *Z*-configuration) are also selective neuronal 5-HT uptake inhibitors. In contrast, corresponding *E*-geometrical isomers are more effective in blocking the uptake of NA (Table 12.2). The selectivity profiles of homoallylic **7** and the allylic





(a) R = Me; (b) R = H

Z-**6** **7** Z-homo analogues

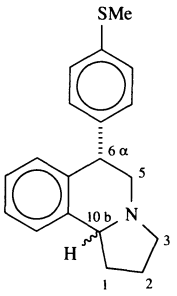
derivative **6** were comparable, with the former series being the less potent. The two series were related stereochemically by UV and ^{1}H-NMR comparisons (cf. Chap. 11, page 394) for zimeldine) and by analysis of europium-induced shifts in

TABLE 12.2.
Inhibition of the Accumulation of (−)-[^{3}H]NA and [^{14}C]-5-HT in Synaptosomes from Rat Brain Cortex[24]

	in vitro IC_{50} (μM)		
	NA	5-HT	Ratio NA:5-HT
Zimeldine **6**a (*Z*)	3.2	0.10	32
E-isomer	0.15	0.22	0.68
Norzimeldine **6**b (*Z*)	0.24	0.038	6.3
E-isomer	0.0046	0.08	0.058
Z-**7**a (homoallyl)	22.5	0.5	45
E-**7**a	1.4	2.6	0.54
Z-**7**b	6.6	0.24	28
E-**7**b	1.0	1.4	0.71

NMR resonances (the *E*-vinylic signal suffered the greater shift, since the relevant proton is *cis* to the pyridine coordination site).

Certain hexahydropyrroloisoquinolines (**8**) also have the property of inhibiting the uptake of biogenic amine neurotransmitters, some with selectivity for 5-HT.[25] The *p*-methylthio derivative (**8**) is particularly effective when of *trans* (6α,10bβ) geometry, with its activity chiefly resident in the dextro antipodal form. Several *c/t* analogues of **8** were examined with the aim of improving selectivity for blockade of 5-HT uptake. A number of potent *trans*-derivatives were obtained (*cis*-forms had feeble action in this respect).5-HT:NA selectivities of 23 and 26.5 were obtained for the 10-bromo and 10-cyano *trans*-derivatives, respectively, but these variants had little activity in an *in vivo* test (potentiation of mouse head twitches).



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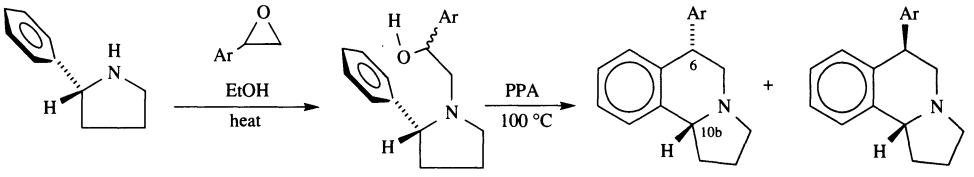
	Uptake inhibition K_i (nM)[a]		
	DA	NA	5-HT
rac-cis	1740	127	16.6
rac-trans	36.8	2.9	0.68[b]
6*S*,10b*R*-(+)-*trans*	23.5	1.8	0.39
6*R*,10b*S*-(−)-*trans*	1450	280	58.4

[a] Method: Ref. 26.
[b] 5-HT:NA selectivity, 4.3.

Stereochemistry. The absolute stereochemistries of (+)-*trans*-**8** and some of its analogues were established by chemical correlation with (+)-2-phenylpyrrolidine (**9**, 75% ee) of known *R*-configuration (Scheme 12.1).[27] Additional evidence was



R-(+)- **9**
75% ee[a]

6*S*, 10*bR*

6*R*, 10*bR*

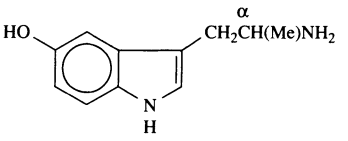
[a] Ref. 28

Scheme 12.1. Synthesis of some hexahydropyrroloisoquinolines. [27]

provided by an X-ray analysis of the 2-chlorophenyl analogue of *trans*-**8**-(+)-HBr-6*S*,10b*R* by anomalous dispersion and CD comparisons. Optical purities of antipodal pairs were investigated by use of (+)-MTPA (Mosher's acid, page 34); antipodal 3-H and 5e-H resonances were well resolved in 360-MHz spectra of these salts.[25]

12.3. Chiral Ligands

The simplest chiral analogue of 5-HT is the α-methyl derivative (**10**). It is listed as a selective agonist for 5-HT_{1C} and 5-HT_2 sites (*TiPS* supplement, Jan. 1991). Nichols *et al.*[32] reported affinities of antipodes of α-methyl 5-HT for 5-HT_{1B} and 5-HT_2 sites of rat frontal cortex homogenates (in this tissue [^{3}H]-5-HT labels primarily the 5-HT_{1B} subtype).[33] Data are shown in Table 12.3. The affinities of 5-HT at both sites were reduced somewhat by α-methylation — less so in the *S*- than the *R*-case and with low orders of stereoselectivity (2.6-fold for α-Me 5-HT antipodes). 4-Hydroxy analogues of **10** had lower affinities than the parent, and



10

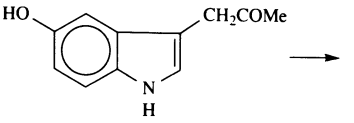
displayed a reverse stereoselectivity to the 5-OH antipodes (pK_i *R* 5.81, *S* 5.39 at 5-HT_{1B} sites). The authors explain these results in terms of interactions between α-methyl and hydroxyl in *trans*-Ar/NH_2 conformers, shown to be the low-energy rotamer of 5-HT by computation[34] and NMR spectroscopy.[30,31] and to adopt this form in the solid state.[35] In the course of examining the receptor subtype selectivities of some methylated 5-HT analogues, a Sandoz group discovered that *S*-(+)-α-methyl 5-HT equaled 5-HT itself as an agonist in the rat uterus (a 5-HT_2 preparation), while its *R*-antipode was about 23-fold less potent.[29,37]

Chemistry. Antipodes of **10** and related compounds were obtained by reductive

TABLE 12.3.
Radioligand Binding Data for α-Methyltryptamine Antipodes using Rat Frontal Cortex Homogenates[32]

	vs [^{3}H]serotonin(5-HT_{1B}) pK_i	[^{3}H]ketanserin(5-HT_2) pK_i
Serotonin	8.46	6.01
5-Methoxytryptamine	8.19	5.99
R-**10** (5-OH)	6.92	5.50
S-**10** (5-OH)	7.34	6.01
R-**10** (5-OMe)	6.42	5.89
S-**10** (5-OMe)	6.38	6.15

amination of the propanones **11** with *R*- or *S*-α-methylbenzylamine, followed by chromatographic separation of the resultant diastereoisomers **12**. Catalytic debenzylation of the latter gave the primary amine antipodes (optical purity by chiral HPLC analysis of the 2-naphthoylamide derivatives using a Pirkle column). Optical rotational comparisons with materials prepared from *R*- and *S*-tryptophan established the absolute geometries (via $CO_2H \rightarrow CH_2OH \rightarrow CH_2OTs \rightarrow Me$).[36]



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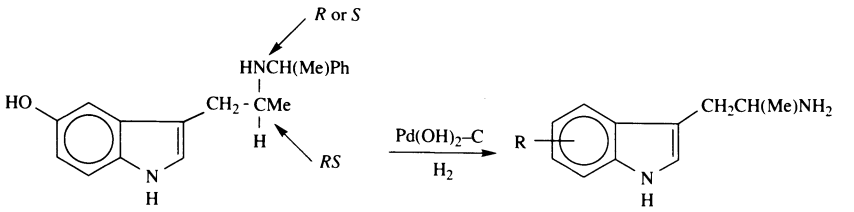
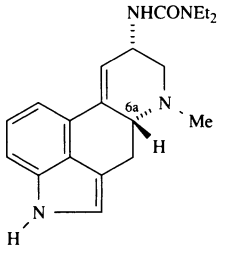


12

Many *ergoline* derivatives show moderately high affinities for both 5-HT_1 and 5-HT_2 sites, e.g., lisuride (**13**) K_i 6.2 nM for 5-HT_1, 11 nM for [^{3}H]spiperidone labeled 5-HT_2 sites[38] while [^{3}H]LSD was one of the first radioligands used to detect 5-HT sites.[39] The high affinities of ergolines for adrenergic and DA sites, however, make them unsuitable as probes of 5-HT receptors. Although all agents used are homochiral and of natural 6a*R*-configuration (see **13**), antipodes are not available to assess the stereoselectivity of their binding to 5-HT receptors. A 5-HT pharmacophore which lacks a phenolic hydroxyl may be discerned within the ergoline molecular framework (see **14** and later). It is remarkable how ergolines



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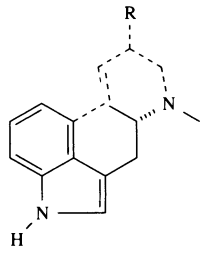


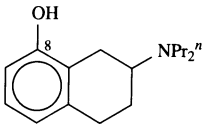
14 showing 5-HT pharmacophore

include both DA (page 185) and 5-HT structural units within their polycyclic system, a fact which helps to account for their duality of action at dopaminergic and serotoninergic sites. The alkaloid yohimbine (Chap. 5, page 133) also includes a tryptamine fragment as part of its polycyclic framework, and its serotonin-related properties are well documented.[40] Yohimbine and its diastereoisomers were all

moderate to strong inhibitors of 5-HT-induced contractions of the rat fundal strip; $-\log K_B$ values of isomers which acted competitively were: yohimbine 6.01, β-isomer 7.24, α-isomer 7.48.[41]

12.4. 8-Hydroxy-DPAT

The ability to study the stereochemistry of ligand binding to 5-HT receptors advanced dramatically following the development of 8-hydroxy-2-(di-*n*-propylamine) tetralin (**15**, 8-OH-DPAT) by Swedish workers,[42] a compound discovered during the course of studies of hydroxylated 2-aminotetralins with



15 8-OH-DPAT

DA agonist activities (Chap. 6, page 174). The value of 8-OH-DPAT as a 5-HT receptor probe received a further impetus when the compound was found to bind selectivity to the 5-HT_{1A} receptor subtype[43] as judged by its high affinity for spiperidone-sensitive 5-HT_{1A} sites. The unique properties of 8-OH-DPAT came to light during biochemical tests for inhibition of DA and 5-HT synthesis and utilization by stimulation of autoreceptors, and in behavioral tests in rats for which both a DA- and 5-HT-syndrome has been characterized (the 5-HT syndrome includes "piano playing" and flat body posture).[15] Biochemical methods followed those already described for DA agonists (page 183). While 8-OH-DPAT had little influence on DOPA levels, it proved effective in reducing those of 5-HTP (5-hydroxytryptophan) when given at a low sc dose level.[44] Data for *rac*-**15** and the 2*R*- and 2*S*-antipodes are given in Table 12.4. Absolute configurations were established by chemical correlation with *R*-2-(di-*n*-propylamino)tetralin[44] and by X-ray crystallography of the (+)-HBr salt.[45]

A point of special interest is the low degree of stereoselectivity displayed by 8-OH-DPAT—less than 2-fold with preference for the *R*-(+)-antipode. This result stands in contrast to methylated analogues described below, and appears to

TABLE 12.4.
Effects of 8-OH-DPAT on the Accumulation of 5-HTP in Rat Brain[44]

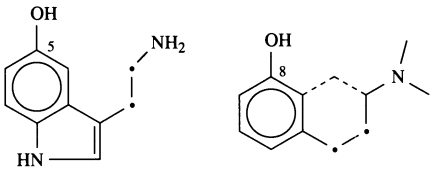
	ED_{50} (mol/kg sc)[a]		
	limbic	striatum	hemispheres (cortex)
rac-**15**	0.052	0.052	0.063
R-(+)-antipode	0.036	0.047	0.050
S-(−)-antipode	0.061	0.065	0.077
(+)-LSD	0.036	0.035	0.036

[a] Dose giving a half-maximal decrease

contravene Pfeiffer's rule (page 5) since potencies involved are relatively high. See, however, a discussion of results based on use of the forskolin-stimulated adenylate cyclase assay, a test of intrinsic 5-HT_{1A} activity (page 420).

In later work, antipodes of 2-NMe_2, 2-NEt_2, and 2-NBu^n_2 analogues of **15** were evaluated in the 5-HTP accumulation test.[46] All were less effective than 8-OH-DPAT, and low eudismic ratios (the greatest value was 10) were recorded with the *R*-form as eutomer except for the NBu^n_2 pair. Binding affinities for rat brain sites labeled by *rac*-[^{3}H]8-OH-DPAT concurred with the biochemical potencies; the IC_{50} nM value for *R*-(+)-**15** was 4.84, and 6.54 for the *S*-(−)-antipode (ratio 1.35).[47]

Modeling studies (page 425) reveal that the structure of 8-OH-DPAT duplicates the pharmacophore of 5-HT itself with the exception of the pyrrole unit (see **16**).

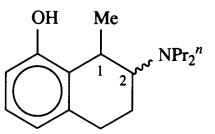


16

Work on 1-methyl and 3-methyl derivatives of 8-OH-DPAT followed, much as that carried out on methylated 5-OH-DPAT analogues with dopaminergic properties (page 178).

12.4.1. 1-Methyl Analogues

Of the *rac*-1-methyl diastereoisomers **17**, only the *cis*-isomer was effective in depressing the accumulation of 5-HTP. Antipodes of *cis*-**17**, unlike those of 8-OH-DPAT itself, differed markedly in their potencies in the same test with ED_{50} values (μmol/kg sc) of 0.09 for the dextro and >5.0 for the levo isomer from analysis of the limbic area.[48] The 1-methyl eutomer had a 1*S*,2*R*-configuration (**18**) and hence relates in chirality to (+)-8-OH-DPAT; potencies of the two eutomers were similar. Molecular geometries were established by X-ray crystallography.[49]



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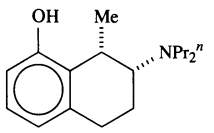
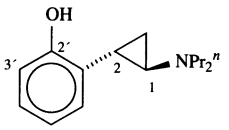


18 1*S*, 2*R*-eutomer

The 5-HT_{1A} binding affinities of antipodes of **17** differed markedly (K_i nM: 1*S*,2*R* 2.9; 1*R*,2*S* 2920), but the intrinsic activity of each isomer was low as judged by the FSC assay (page 420).[82]

It was possible to predict the stereochemistry of the eutomeric form of the 1-methyltetralin **18** from comparisons of eutomers of 8-OH-DPAT and the *trans*-

arylcyclopropylamines **19**.[50] The 1*R*,2*S*-antipode of **19** was a potent agent in the 5-HTP accumulation test (ED_{50} 0.26 μmol/kg sc in rat limbic region) while its antipode was much less effective ($ED_{50} > 50$). Superimposition of the eutomers 1*R*,2*S*-**19** and 2*R*-8-OH-DPAT with the hydroxyl groups, the nitrogen atoms, and the benzene ring coinciding, revealed that the methylene group of the cyclopropyl ring of **19** would correspond to a *cis*-C_1-methyl group in 2*R*-8-OH-DPAT (producing **18**).



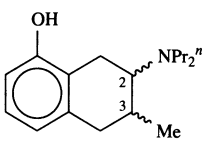
19 1*R*, 2*S*-isomer shown

Curiously, in view of the poor showing of 7-OH-DPAT as a 5-HT agonist, a shift in the hydroxyl position of **19** from the 2′ to 3′ position gave a product with retained 5-HT activity (5-HTP test and 5-HT syndrome) (ED_{50} as above: 0.34 for the levo isomer 1*R*,2*S*, < 50 for dextro isomer). This discrepancy has been explained by assuming that different conformations of the 2-hydroxy and 3-hydroxy isomers activate the 5-HT receptor.[49]

Chemistry. The cyclopropylamines **19** were obtained by subjecting the appropriate *t*-2-arylcyclopropane carboxylic acids to a Curtius rearrangement (cf. page 115). *Trans*-stereochemistry of the amines was established from ^{1}H-NMR coupling data (comparisons with a *trans*-1-dimethylamino-2-phenylcyclopropane standard)[51] and X-ray crystallography which also provided the absolute stereochemistry of the levo HBr salts. Resolution was carried out at the carboxylic acid stage of the synthesis.

12.4.2. 3-Methyl Analogues

All four 3-methyl diastereoisomers **20** showed reduced 5-HT activity in comparison with the 8-OH-DPAT parent,[52,53] as reflected in the binding IC_{50} values



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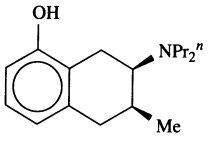
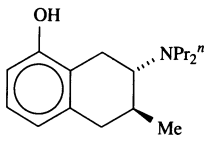


(–)-*cis*-2*R*, 3*S*



trans-2*S*, 3*S*

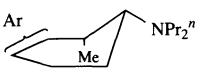
of Table 12.5. *Trans*- and *cis*-eutomers had the 2*S*,3*S* and 2*R*,3*S* configurations, respectively. There was a reasonably large difference beween the receptor affinities of the *trans*-pair (×28) but only a 5-fold one for the more weakly bound *cis*-pair. The activity of the *trans*-2*S*,3*S*-antipode, which has the same C-2 chirality as (+)-8-OH-DPAT, was abolished when C-3 carried both a β- and an α-methyl group (*rac*-product tested).

Reasons for activity differences among stereoisomers of 1-methyl and 3-methyl 8-OH-DPAT have been advanced on the basis of conformational analyses carried

TABLE 12.5.
Affinities of 8-OH-DPAT and its 3-Methyl Analogues for Rat Brain (Cerebral Cortex) [^{3}H]8-OH-DPAT Sites [52]

	IC_{50} (nM)	Eudismic ratio
rac-8-OH-DPAT	3.98	
trans-**20** (3-Me)		
rac	129	
2*S*, 3*S*	60.5	28
2*R*, 3*R*	1680	
cis-**20** (3-Me)		
rac	774	
2*R*, 3*S*	486	5
2*S*, 3*R*	2420	

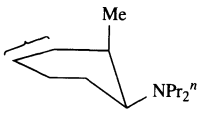
out by computational and NMR spectroscopic methods.[49,52] In low-energy conformations of the 1*S*,2*R*(*cis*) eutomer **18**, the saturated ring adopts a half-chair conformation (**21**) with a pseudoaxial 1-methyl and pseudoequatorial 2-NPr^n



21

substituents. The nitrogen atom is close to the plane of the aromatic ring (~0.6 Å). Tetralin geometry of this kind is also found in (+)-LSD and *R*-(+)-8-OH-DPAT, and hence deemed important for interaction with 5-HT$_1$ sites. In contrast, the N-aromatic plane distance is considerably greater (>1.8 Å) in the inactive *trans*-analogue of **18** which preferentially adopts conformations with pseudoaxial C-1 and C-2 substituents.

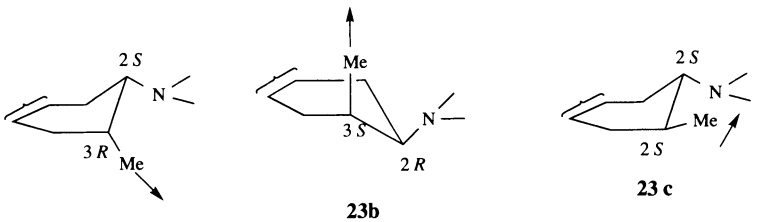
The approximate equipotency of 2*R*-8-OH-DPAT (**15**) and 1*S*,2*R*-**18** demonstrates that the steric bulk of the C-1 methyl group of **18** does not interfere with a proper alignment of the ligand with the 5-HT receptors. Similarly, the methylene group of the cyclopropane ring of **19**, which may be located in a similar spatial position, does not appear to be a steric obstacle. However, the high stereoselectivities of **18** (1-methyl) and **19** (cyclopropane) indicate that, with 2*R*-8-OH-DPAT as common frame of reference, the space below the C-1 position is part of the 5-HT receptor essential volume (see page 426 for definition of this term). In the feebly active 1*R*,2*S*-antipode of **18**, the amino nitrogen atom remains close to the aromatic plane but the 1-methyl group points upward and is outside the receptor essential volume (see **22**).



22

The same arguments explain the differing activities of the *trans*-3-methyl pair **20** in regard to 3-methyl lying withing (2*S*,3*S*) and outside (2*R*,3*R*) the essential

volume in the eutomer and distomer, respectively; in this case the affinity of 8-OH-DPAT is reduced by 3-methyl in either orientation. In regard to the *cis*-3-methyl pair, of substantially lower receptor affinities, the position of the pseudoaxial 3-methyl group may again be invoked to account for the small (× 5) difference in the affinity of antipodes. In the *cis*-case, 2*R*,3*S* (**23b**) is preferred to 2*S*,3*R* (**23a**), since 3-methyl points upward in the former as it does in the 2*S*,3*S*-*trans*-antipode (**23c**).

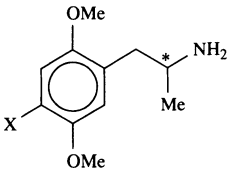


23a **23b** **23c**

Further light has been thrown on the stereochemical demands of the 5-HT_{1A} receptor by use of the forskolin-stimulated adenylate cyclase (FSC) assay—a test of intrinsic 5-HT_{1A} activity since this receptor subtype has been shown to be negatively coupled to adenylate cyclase in rat hippocampal membranes. In tests on a variety of enantiomeric pairs of 8-OH-DPAT analogues, eutomers of a particular pair generally corresponded in regard to binding affinity (vs. [^{3}H]8-OH-DPAT) and activity in the FSC assay.[82] However, antipodes of 8-OH-DPAT itself, which had virtually identical affinities, differed sharply in their intrinsic activities. While the *S*-antipode was only a partial agonist inhibiting FSC by about 50% of the maximum (EC_{50} 135 nM), the *R*-isomer was a full agonist (EC_{50} 57.1 nM). These findings have implications for the use of the commercially available racemic mixtures, widely regarded as a full agonist. In the case of antipodal forms of the 5-fluoro analogue of 8-OH-DPAT, the *R*-isomer (of greater 5-HT_{1A} affinity) was a weak partial agonist while the *S*-form appeared to be a putative 5-HT_{1A} antagonist because it failed to inhibit FSC and produced full reversal of 5-HT-induced inhibition of the same compound.

12.5. Phenalkylamines

Phenalkylamines **24**, which may be regarded as ring-opened analogues of 8-OH-DPAT, generally display a low affinity for 5-HT binding sites. 2,5-Dimethoxy derivatives with halo groups at C-4 have reasonable affinities for 5-HT_2 sites (vs.



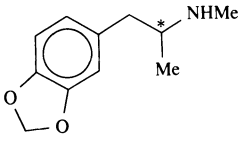
(a) X = Br (DOB); (b) X = I (DOT)

24

[^{3}H]ketanserin) and exhibit low-order stereoselectivities: K_i (nM) for **24**a (DOB) *R*-(−) 4200 (5-HT$_1$), 24 (5-HT$_2$), *S*-(+) 5000 (5-HT$_1$), 145 (5-HT$_2$); **24**b (DOI) *R*-(−) 2290 (5-HT$_1$), 10 (5-HT$_2$), *S*-(+) 920 (5-HT$_1$), 35 (5-HT).[3,54] Eutomers of **24**a, **24**b, and 8-OH-DPAT share *R*-chirality at related centers. Absolute configurations were established by use of intermediates of known stereochemistry in the synthetic methods, such as *R*-(−)-3,4-dimethoxy-α-methylphenethylamine (Glennon, private communication and Ref. 55).

More potent displacers of [^{3}H]ketanserin have been obtained by replacing halogens at C-4 of **24** by *n*-hexyl (K_i 2.5 nM) and *n*-octyl (K_i 3.0); data are only available for racemic mixtures at present.[56] In a paper advocating use of tritiated (−)-DOB as a radioligand for 5-HT$_2$ sites, the following K_i (nM) values were reported: (−)-DOB 0.72, ketanserin 1.33, and spiperidone 1.82.[57]

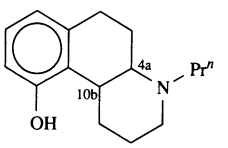
3,4-Methylenedioxymethylamphetamine (**24c**, MDMA), a popular drug of abuse, is closely related to DOB and DOI (**24**). In addition to its behavioral effects, it acts as a selective serotonin neurotoxin in rats and monkeys and brings about long-term destruction of 5-HT neurons.[58] In rats, the *S*-(+)-antipode is the causal



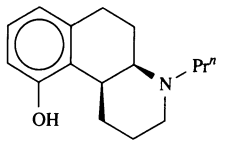
24c

agent—neurotoxicity is not evident with the *R*-(−)-isomer.[59] Remarkably, both antipodes of its demethylated analogue (MDA, a metabolic product)[60] cause long-term serotonin neurotoxicity.[61] 5-HT$_2$ receptor antagonists block MDMA-induced toxic effects of this kind. Schmidt *et al.*[62] employed antipodal forms of the novel 5-HT$_2$ antagonists MDL 11,939 (**32**) to examine the hypothesis that such agents act by interfering with the acute stimulation of striatal DA synthesis caused concurrently by MDMA. Only the *R*-(+)-isomer both reversed the rise in DA levels and prevented the deficit in rat forebrain 5-HT levels measured one week after dosage with MDMA.

Octahydrobenzo[*f*]quinolines (**25**) with a 10-hydroxyl substituent possessed 5-HT receptor activity and lacked action at DA sites (cf. Chap. 6, page 188).[63] In the 5-HTP accumulation test, ED$_{50}$ values (nM/kg sc) were 230 for *trans*-**25**, 3400 for *cis*-**25**, and 48 for *rac*-8-OH-DPAT. When the less potent *cis*-derivative was resolved, the 4a*R*,10b*S* isomer was found to be a full agonist (about 30 times less effective than 1*S*,2*R* 1-methyl-8-OH-DPAT **18**, of related configuration) while its antipode behaved as a partial agonist. X-ray analysis of a NH/OMe precursor provided evidence of configuration.[64] The relatively low potency of 4a*R*,10b*S*-**26** was attributed to the unfavorable direction of its nitrogen lone-pair (or $^+$NH) in comparison with other agonists.

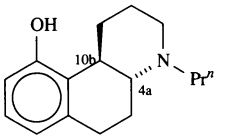


25



26 4a*R*, 10b*S* antipode

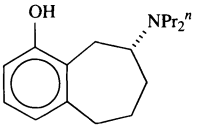
Data on the *trans*-analogue of **25** became available later.[77] In both binding assays (displacement of [^{3}H]-8-OH-DPAT) and the 5-HTP accumulation test, the 4a*S*,10b*S* antipode proved unexpectedly more effective than the 4a*R*,10b*R* isomer **26a**; the eudismic ratios were not large, however (see Ref. 82 for results of FSC assay). X-ray analysis established the configuration of **26a** HCl. Corresponding octahydrobenzo[*g*]quinolines (with a linear arrangement of the three rings) had low affinities for 5-HT$_{1A}$ sites and were mostly inactive in functional tests.



26a
4a*R*, 10b*R* isomer

K_i (nM) vs.[^{3}H]-OH-DPAT	
4a*R*, 10b*R*	32.2
4a*S*, 10b*S*	3.87

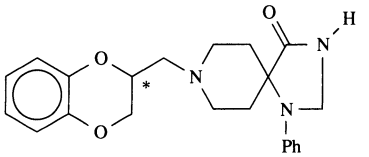
Seven-membered ring analogues of 8-OH-DPAT have been examined.[65] The stereoselectivity of the interaction of **27** with 5-HT$_{1A}$ receptors was more pronounced than that of the parent as judged from displacement of [^{3}H]8-OH-DPAT from rat brain tissue (K_i nM 29.7, 46.3 for *R*-**27**, >300 for *S*-**27**, and 1.0 for *rac*-8-OH-DPAT) and 5-HTP accumulation tests. Preferred conformations of the eutomer **27**, studied by NMR and molecular mechanics, were similar in shape to those of the stereoselective agonist 2*R*,3*S*-**20** (3-methyl).



27 *R*-antipode shown

12.6. Chiral 5-HT Antagonists

There are several reports on chiral 5-HT antagonists. Spiroxatrine (**28**), a close analogue of spiperidone, is 75000 times more potent at 5-HT$_{1A}$ than 5-HT$_{1B}$ sites, and 30 times more potent at 5-HT$_{1A}$ than 5-HT$_2$ sites.[66] Antipodes of **28** were obtained by stereoselective synthesis involving a Sharpless epoxidation of an allylic alcohol **29**, with absolute geometry controlled by (+)- or (−)-diethyl tartrate.[67]



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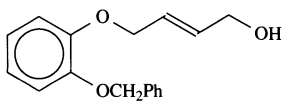
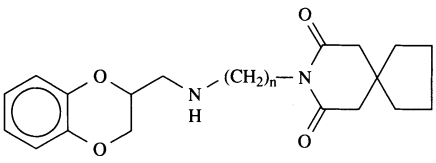


29

In binding tests, the *S*-levo isomer was the eutomeric form (7–11 times more effective than the *R*-dextro isomer) and apparent K_i (nM) values were 1.9 vs. [^{3}H]8-OH-

DPAT (5-HT_{1A}), $>10^{-5}$ M vs. [^{125}I] ICYP (5-HT_{1B}), and 113 vs. [^{3}H]ketanserin (5-HT_2).

The benzodioxanes **30**a (MDL 72832) and **30**b (MDL 73005) share the 5-HT_{1A} selectivity of the closely related compound spiroxatrine (**28**).[68,69] The data of Table 12.6 demonstrate the specificity of MDL 72832 and the eutomeric nature of the



(a) $n = 4$ (MDL 72832); (b) $n = 2$ (MDL 73005)
30

S(levo) isomer which is related in configuration to levo **28**. According to Hibert *et al.*[7] the eudismic ratio of 36 at 5-HT_{1a} sites, which follows from the data of Table 12.6, should be higher since the *R*-(+)-sample used was contaminated with 3% of the *S*-(−)-isomer. Evidence of configuration has not been published. Potencies in functional tests correlated with results of the binding experiments. As anticipated (Chap. 5, page 129), presence of the benzodioxane unit within the molecule conferred significant α_1-adrenoceptor affinity on **30**a.

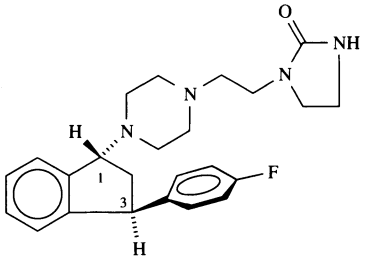
Two further chiral examples are the indanyl derivative (**31**) and the piperidine **32** (MDL 11939), both 5-HT_2 receptor antagonists. The (+)-isomer **31** (irindalone) proved a far more effective inhibitor of 5-HT-induced contractions of isolated rat thoracic aorta (a 5-HT_2 preparation) than the (−)-form (pD_2 values were 7.73 for the dextro and 5.82 for the levo isomer).[70] Antipodes of **31** also blocked NA-induced contractions, again with dextro the eutomer. The absolute geometry of irindalone is as shown—details of methodology are not available (Mikkelsen, private communication).

TABLE 12.6.
Affinities of MDL 72832 and its Enantiomers for Central Neurotransmitter Recognition Sites[68]

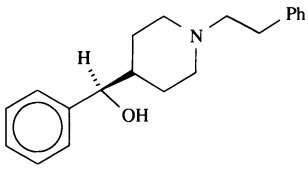
	pIC_{50} values[a]				
	5-HT_{1A}	5-HT_{1B}	5-HT_2	α_1	D_2
rac-**30**a	9.1	6.2	6.2	7.8	6.8
S-(−)-Antipode	9.2	6.1	6.7	8.0	7.1
R-(+)-Antipode	7.7	5.3	6.1	7.2	5.6
8-OH-DPAT	8.5	—	—	—	—
Cyanopindolol	—	7.8	—	—	—
Ketanserin	—	—	8.3	—	—
Prazosin	—	—	—	9.2	—
Spiperidone	—	—	—	—	9.9

[a] Negative log values of concentration giving 50% inhibition of specific binding of tritiated 8-OH-DPAT (5-HT_{1A}), 5-HT (5-HT_{1B}), ketanserin (5-HT_2), prazosin (α_1), and domperidone (D_2). Affinities of **30**a for α_2 (clonidine), D_1 (flupenthixol), and β (dihydroalprenolol) sites were low.

The compound MDL 11939 (**32**)[71] has already been mentioned (page 421). Ligand binding results quoted by Schmidt *et al.*[62] show that the action of this antagonist is due solely to the *R*-(+)-enantiomer. There is no apparent configurational relationship between **31** and **32**.

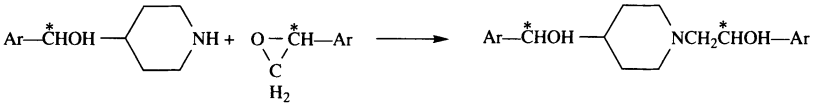


31 (+)-1*R*,3*S*-antipode shown



32 MDL 11939 (*R*-antipode shown)

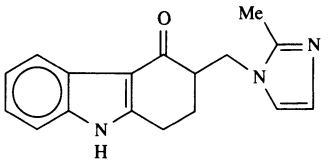
Action of a lipase from *Aspergillus niger* on the *rac*-acetate of **32** gave a mixture of the easily separated *S*-(−)-alcohol and the *R*-(+)-acetate from which *R*-(+)-MDL 11939 was derived (configurational assignments were made by NMR analysis of corresponding *O*-methylmandelate esters).[83,84] All four stereoisomers of **32a**, a bichiral fluoro analogue of **32**, were synthesized in a convergent manner from the chiral precursors **32b** and **32c**; the preparation of each intermediate was dependent on a stereoselective acetate hydrolysis catalyzed by a lipase.[85] All 5-HT_2 antagonist activity of the diastereoisomers **32a** resided in those derived from *R*-(+)-**32b** (Nieduzak, private communication).



32b **32c** **32a** (Ar = 4-FC_6H_4)

12.7. 5-HT_3 Ligands

The development of 5-HT_3 antagonists, agent of more immediate clinical potential than 5-HT ligands of other subtype specificities, has so far led to few examples of stereochemical interest. Members of this class are typified by ondansetron (**33**) and ICS-205-930 (**34**), which are effective clinically for the blockade of chemotherapy-induced emesis.[72,73] Giger *et al.*[37b] reported that the relative affinities of **34** and its pseudotropyl analogue (C-3 epimer) for vagal sites were 1:0.001 (the desheathed rabbit vagus nerve is rich in 5-HT_3 sites).



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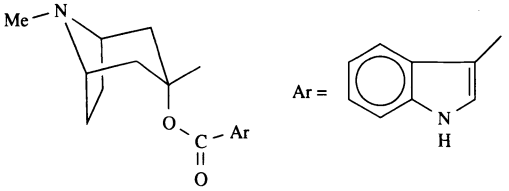
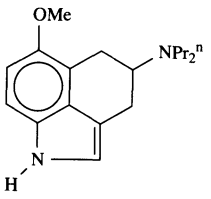


34

12.8. Mapping of Agonist Sites

Hibert *et al.*[74] carried out a computer-aided mapping of 5-HT_{1A} receptors based on the comparison of a series of ligands with the rigid ergot skeleton of lisuride (**13**). Attention was focused on the relative positions of two reference features: the vector representing the basic nitrogen lone-pair of electrons and the aromatic nucleus. The activity of all the 5-HT_{1A} receptor agonists could be accounted for a common structural feature of an aromatic ring with an almost coplanar basic nitrogen at a distance of 5.15 Å (Fig. 12.1).

The authors noted the identical chirality of *R*-(+)-8-OH-DPAT and the ergolines LSD and lisuride. If the aromatic rings of both enantiomers of the tetralin occupy the same position in the receptor site, the nitrogen atom of the somewhat less strongly bound *S*-isomer would be at a distance of 1.4 Å from the corresponding position of *R*-(+)-8-OH-DPAT or lisuride. The hydroxyl groups of 5-HT and 8-OH-DPAT corresponded in the spacial map obtained by superimposition of 5-HT, 8-OH-DPAT, lisuride, and other ligands. The model received support from the disclosure that the compound BAY R1551 (**35**), which includes the pyrrole unit absent from the structure of 8-OH-DPAT, has high affinity for the 5-HT_{1A} recognition site.[75] In fact, compounds related to **35** had been reported in 1988[78]; of these the carboxamide analogue (**35**, OMe replaced by $CONH_2$) proved a potent 5-HT_{1A} agonist when examined as the *rac*-form. The stereospecific synthesis of antipodes of this compound (LY 228729, isomer?) has been reported.[79]



35

Antipodes differed little in their affinities for 5-HT_{1A} sites of rat hippocampus (K_i nM: *R* 0.42, *S* 0.19) or influence on 5-HIAA levels (similar results were obtained for the less potent pair with a 6-CO_2Me function), behavior which mirrored that of 8-OH-DPAT itself except that eutomers had the *S*-configuration (Flaugh, private communication).

A 1991 model developed by Hacksell and his colleagues[77] follows the active analogue approach first employed by Marshall for the steric mapping of the

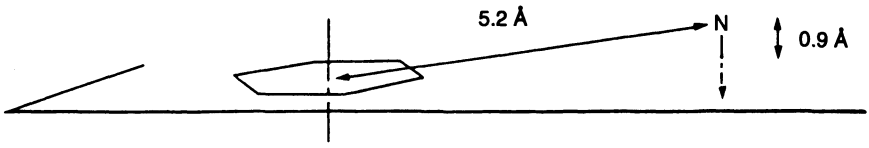


FIGURE 12.1. Pharmacophore of the 5-HT_{1A} agonist recognition site (after Hibert *et al.*).[74]

binding site of ATP:L-methionine *S*-adenosyltransferase.[80] The original method required a set of rigid amino acid analogues each member of which contained pharmacophoric features essential for recognition (in this case an α-CNH_2CO_2H unit). Computer-generated overlap of *active* members provided the *enzyme-excluded* volume map, i.e., the volume adjacent to the active site not occupied by the receptor. Comparison of this map with that generated from inactive members of the set revealed (by difference) volumes assumed to contain the *enzyme-essential* volume, i.e., a region occupied by the enzyme and therefore not available for binding by other molecules. From these parameters, predictions are possible of whether novel analogues (with appropriate pharmacophoric functions) should be active (of volume outside) or inactive (of volume inside) the enzyme-essential volume.

The Uppsala group employed a set of reasonably rigid 5-HT_{1A} agonists together with inactive stereoisomers. Pharmacophoric elements were reduced to two, namely, an aromatic site and a dummy atom–nitrogen vector [it was not necessary to include a phenolic function since nonphenolic 2-(propylamino)tetralin possesses a fairly high affinity for the 5-HT_{1A} receptor].[81] The dummy atom was assumed to mimic a carboxylate ion within the active site of the receptor. The model was constructed by use of potent agonists and is defined (Fig. 12.2) by *y*, the distance (2.1–2.6 Å) from the dummy atom (D) to the plane of the aromatic ring; *x*, the distance (5.2–5.7 Å) from the normal of the aromatic center to the dummy atom; α, the angle ($-28°$ to 28°) between a vector connecting nitrogen (N) and D and a vector bisecting the triangle formed by the corresponding vectors for enantiomers of **26a**; and β, the angle ($-4°$ to 0.4°) between the aromatic plane (also defined by antipodes of **26a**). The model was found to accommodate a large range of compounds of different stereoselectivities and agonist potencies. It defines the limits within which the relative positions of an aromatic nucleus and a nitrogen–dummy atom vector may vary, and also specifies a partial 5-HT_{1A} receptor-excluded volume. Presumably both antipodes of 8-OH-DPAT fall withing this volume since both are active, while the inactive antipodes of agents, such as the 1-methyl derivative **17**, intrude upon the receptor essential volume.

Further discussion of the model of Mellin *et al.*[77] can be found in a paper reporting application of the FSC assay (page 420) to the same group of 5-HT_{1A} ligands.[82]

A conformational-activity relationship study of 5-HT_3 receptor antagonists has also been carried out, based on the structures of the relatively few ligands of this kind so far characterized—these include several derivatives of 3-tropanol.[76]

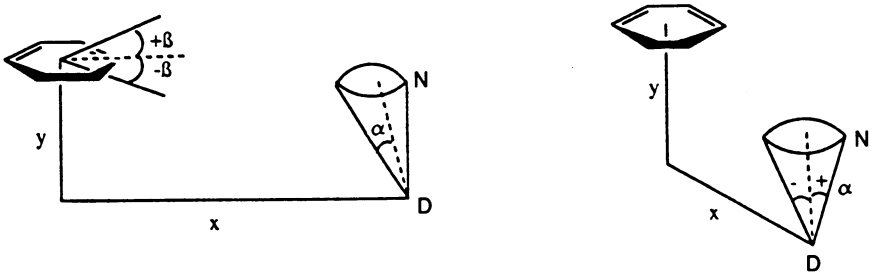


FIGURE 12.2. Pharmacophoric elements and 5-HT_{1A} model of the Uppsala group (after Mellin *et al.*).[77] See text for definitions.

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13

Opioid Ligands

Part 1

13.1. Introduction

The range and diversity of chiral ligands that interact with opioid receptors is so great that their discussion in a single chapter would be burdensome to the reader. For this reasen relevant material has been used to provide the subject matter of two chapters. Although a clear-cut division of material is not possible, the group comprising morphine and its analogues, morphinans, benzomorphans, and 4-arylpiperidines plus some related compounds may be structurally correlated in greater or lesser degree and these ligands are discussed in Part 1.

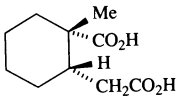
As in many of the pharmacological receptor systems dealt with in previous chapters, most of the work on opioids reported prior to 1970 relates to the single receptor concept, and assays employed to evaluate test compounds largely involve functional (antinociceptive in the opioid case) procedures carried out *in vivo*. In later work, not only do *in vitro* functional tests and binding experiments come to the fore, but recognition is given to the existence of subtypes of opioid receptor. In the *TiPS* supplement of January 1991, three subdivisions are listed, namely, mu (μ), delta (δ), and kappa (κ) together with their selective agonists and antagonists.

An account of receptor subtype investigation is included in a recent review,[1] while progress in the cloning of opioid binding proteins has been presented by Loh and Smith.[2]

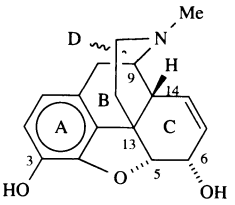
13.2. Morphine and Congeners Derived from Opium Alkaloids

Morphine, the active pain-relieving component of opium, forms the starting point of this chapter not only because of its status as the classical central analgesic, but also due to the fact that its structure has inspired the synthesis of a veritable host of analogues of stereochemical interest. Conclusive evidence of the relative stereochemistry of the five chiral centers of the molecule was provided by the X-ray

crystallographic study of the hydriodide dihydrate salt by Mackay and Hodgkin,[3] following chemical investigations. Proof of absolute configuration was provided by optical rotatory data,[4] degradation of thebaine (linked stereochemically to morphine) to the cyclohexane **1** of known configuration,[5] and finally from an X-ray analysis of codeine hydrobromide dihydrate which employed anomalous scattering by bromine[6]; details of absolute geometry are depicted in structure **2**.

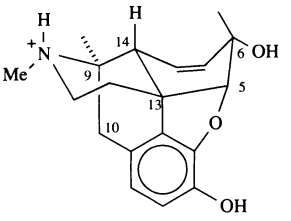


1



2
levo 5(*R*), 6(*S*), 9(*R*), 13(*S*), 14(*R*) morphine

The numerous X-ray crystallographic studies of morphine and its relatives which have subsequently been reported throw light on the solid-state conformation of opiate molecules (summary in a 1986 monograph).[7] All of the compounds have a common "T" shape in which rings A and B form the vertical part and rings C and D the horizontal portion. In morphine and its relatives with a 7–8 double bond, ring C adopts a boat conformation while a chair is favored in analogues without this feature, as in naloxone and azidomorphine (see pages 432 and 440). The conformational represenation of (−)-morphine in the protonated state (**3**) illustrates the T-shape and emphasizes the 4-arylpiperidine feature of the molecule, a fragment on which pethidine (meperidine) and many other synthetic opioids are based.[8]



3

Conclusions as to the solute conformations of morphine and other opiates derived from ^{1}H-NMR analyses generally concur with the solid-state data. Thus Perly, Pappalardo, and Grassi[9] have analyzed 600-MHz spectra of morphine, nalorphine, and oxymorphone hydrochlorides and obtained evidence of conformation from ^3J coupling magnitudes.

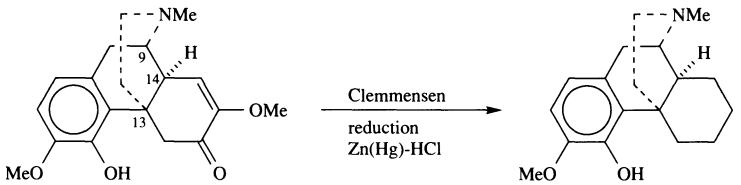
The ^{1}H-NMR spectra of salts of morphine and its congeners are complicated by the existence of protonated epimers, evident from signal duplication and first reported in a 600-MHz study carried out at low pH to slow the interconversion rate. The usual preference of N-methyl for the equatorial conformation in piperidine derivatives is reduced in the fused-ring system of morphine by nonbonded interactions with the β-10-proton, and an eq-*N*Me/ax-*N*Me ratio of about

5 is found for morphine and nalorphine salts based on integration of major and minor 9-H resonances.[10]

Many computational and molecular modeling studies of morphine and other opiates have been reported, as summarized by Lenz *et al.*[7] and Casy and Parfitt.[8]

Isomeric activity comparisons among the morphine group comprise (1) antipodes of natural and unnatural kind, (2) pairs with B/C ring junctures of *cis* (natural) and *trans* (synthetic) geometry, and (3) diastereoisomers resulting from substitution in ring C or with chiral substituents attached to nitrogen.

In principle, access to the dextro antipode of morphine follows from resolution processes carried out as part of the several schemes of total synthesis that have been described (for a review, see Ref. 8). In practice, all pharmacological studies of (+)-morphine have employed material obtained by interconversions of the natural alkaloid (−)-sinomenine (**4**). Remarkably, the configurations of the C-9, -13, and -14 centers of (−)-sinomenine are all opposite to those of the equivalent carbons of (−)-morphine, as established many years ago by the conversion **4**→**5**. The

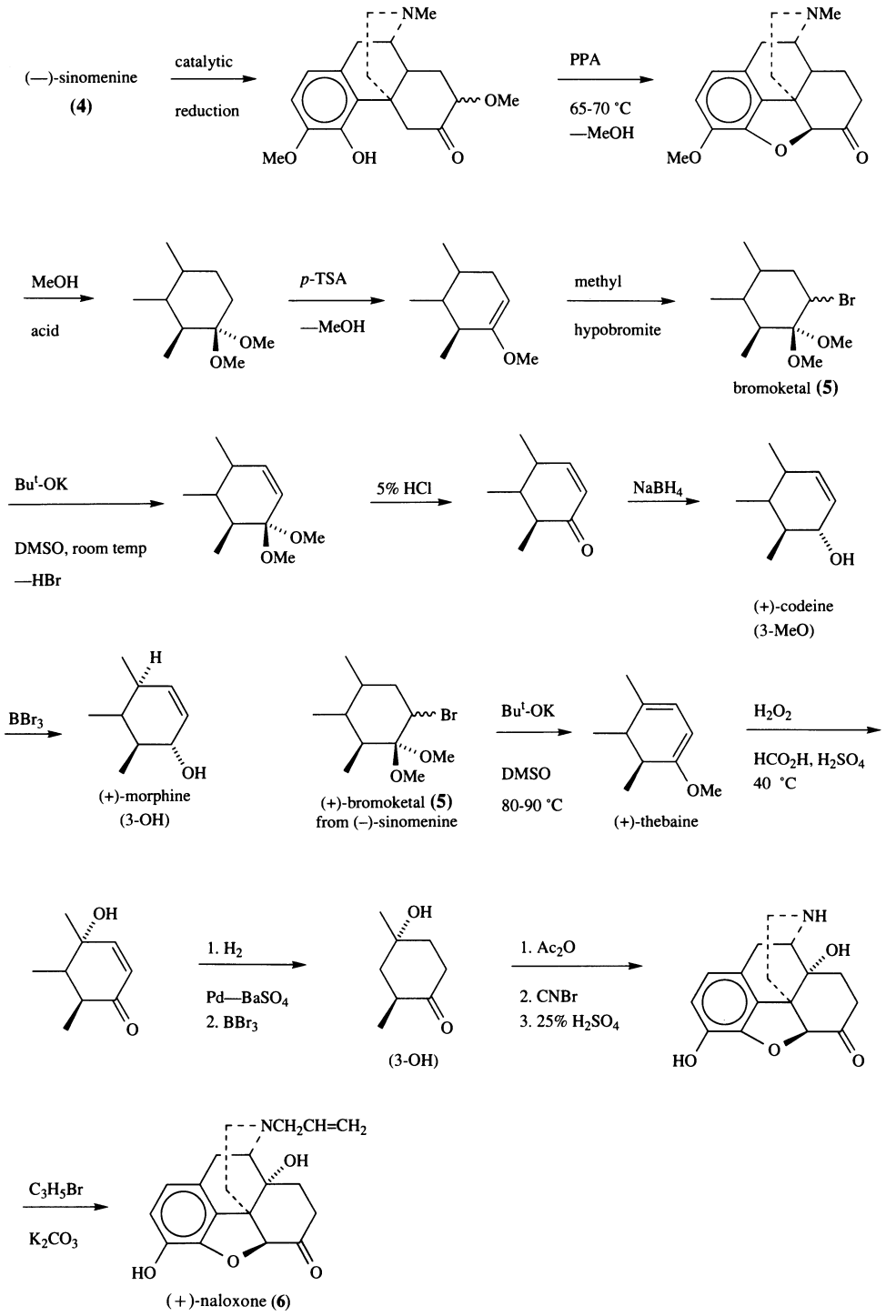


4 **5**

product **5** had a specific rotation equal in magnitude to that derived from morphine but of opposite sign.[11] The transformation of (−)-sinomenine to (+)-morphine was first described by Japanese workers in 1954,[12] and later modified by an NIH group to give a 25–27% yield of product.[13] Details are provided in Scheme 13.1. A key intermediate was the bromoketal (**5**). When treated with potassium *t*-butoxide in dimethylsulfoxide at room temperature, the ketal function was retained with loss of HBr to yield a 7-ene convertible to (+)-morphine. At a higher reaction temperature the bromoketal lost 1 mole each of HBr and methanol to give (+)-thebaine, which was transformed to (+)-naloxone (**6**) by reactions previously established in work involving the natural (−)-alkaloid.[14]

The original reports of Goto and Yamamoto[12] of the failure of (+)-morphine to act as an analgesic were confirmed by the NIH group, dextro antipodes of morphine, codeine, and heroin all being devoid to antinociceptive action in mice by routine screening.[13] While (−)-codeine had sc ED_{50} values (mg/kg) of 4.09 and 20.7 in tail-flick and hot-plate tests in mice, respectively, doses of 100 (TF) and 75 mg (HP) of the (+)-antipode were ineffective; both isomers had antitussive activity in cats with (−)-codeine 6 times more potent than (+)-codeine.[15] *In vitro* test data for (+)-morphine are as follows[16]:

1. It was 10^4-fold weaker than (−)-morphine in its ability to displace [^{3}H] dihydromorphine from binding sites in rat brain homogenates (levo codeine had an IC_{50} value of 16 μM while 10 mM (+)-codeine had no effect).[5]
2. In electrically stimulated GPI, (+)-morphine did not inhibit contractions at a dose 100 times greater than effective doses of (−)-morphine or of (−)-normorphine [the latter pair were not antagonized by (+)-morphine].

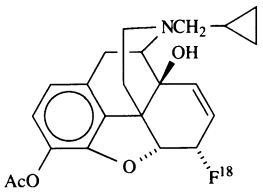


Scheme 13.1. Conversion of (–)-sinomenine to (+)-morphine and other opiates of unnautral antipodal form, including (+)-thebaine and (+)-naloxone.[13,14]

3. In the assay of adenylate cyclase activity in neuroblastoma X glioma hybrid cell homogenates, (+)-morphine had less than 10^{-3} of the inhibitory potency of (−)-morphine and did not antagonize the active antipode.

In some tests on (+)-naloxone (**6**), i.e., the antipode of the potent levo agent classified as a "pure" antagonist,[8] the (+)-isomer displaced [^{3}H] (−)-naloxone only at concentrations 10^4 times higher than those of (−)-naloxone necessary to carry out the equivalent displacement; (+)-naloxone had no effect either alone or as an antagonist of (−)-normorphine at concentrations below 5×10^{-5} M in the GPI assay, and failed to influence the morphine-sensitive adenylate cyclase activity of neuroblastoma X glioma hybrid NG 108-15 whether in the presence or absence of added morphine.

Since (+)-naloxone has no more than 10^{-3} to 10^{-4} of the action of (−)-naloxone, its availability for use in parallel experiments allows the stereospecificity of action of naloxone to be determined. A good example is provided by the pattern of opiate receptor distribution in baboon brain as revealed by PET after iv 3-acetyl [^{18}F]cyclofoxy (**7**)—this pattern failed to appear after (−)-naloxone (1 mg/kg) but was unimpaired by the same dosage of (+)-naloxone.[17]



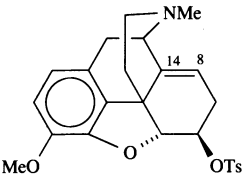
7

Access to (+)-naloxone has also enabled demonstration of the stereospecific blockade of nitrous oxide (N_2O) analgesia.[18] Using a modified hot-plate test in mice, (−)-naloxone antagonized the antinociceptive actions of N_2O in a dose-dependent (2.5–20 mg/kg) manner. In contrast, (+)-naloxone had no effect on N_2O analgesia at the highest dose tested (40 mg/kg). These data suggest that N_2O analgesia is mediated via opioid receptors either through the release of endogenous opioids or by physical perturbation of the receptors. There is evidence that nitrous oxide analgesia involves spinal and supraspinal κ-opioid receptors.[19]

Natural morphine brings about the release of beta-endorphin, met-enkephalin and other endogenous opioids into the circulation of dogs, while its (+)-antipode is ineffective in this respect.[276]

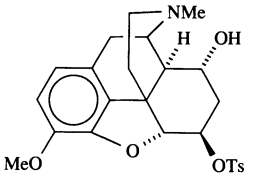
13.2.1. B/C *trans*-Morphine

Kugita *et al.*[20] have obtained several analogues of morphine in which the C-14 configuration (*R*) of the natural alkaloid has been inverted to provide a skeleton with a *trans*-B/C ring function. The synthesis starts from isoneopine, itself derived from thebaine. Addition of water across the 8–14 double bond of the 6-β-tosylate (**8**) by hydroboration (B_2H_6, H_2O_2) gave the 8-α-alcohol (**9**) with the desired B/C ring junction. The ditosylate of **9** underwent solvolysis and elimination with potassium acetate in boiling DMF to provide *trans*-codeine (**10**) directly.



8

isoneopine
6-tosylate



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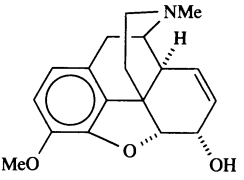


10

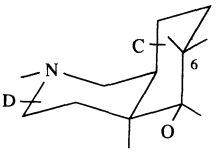
trans-codeine

O-Demethylation to *trans*-morphine was achieved by use of the diphenylphosphide anion. Hot-plate ED_{50} values in mice are shown in Table 13.1 for *trans*-morphine and related compounds. The iso-derivatives with 6-β-OH functions were obtained by inversion of the 6-tosylate of *trans*-codeine with 5% acetic acid.[21] *Trans*-morphine was one-tenth and *trans*-codeine less than one-half as active as their corresponding B/C *cis*-congeners, respectively. Inversion of the 6-OH function (α→β) elevated activity in all three cases examined while its removal (as in *trans*-dihydrodesoxymorphine) resulted in a compound superior in potency to morphine. In morphinan derivatives, which lack ring C substituents and are described later, the isomorphinan analogue with a *trans*-B/C ring junction invariably has the higher antinociceptive activity. A B/C *trans*-ring junction disrupts the T-shape of the morphine molecule because rings C and D are at right angles rather than approximately coplanar (**11**). Hence the hydroxyl function at C-6 is raised above

TABLE 13.1.
Hot Plate ED_{50} Values for B/C *trans*-Morphine and Related Compounds[20]

Compound	ED_{50} mg/kg sc (NIH data)
trans-Morphine	11.7
trans-Isomorphine	5.9
trans-Codeine	17.7
trans-Isocodeine	3.9
trans-Dihydrocodeine	15.5
trans-Dihydroisocodeine	6.7
trans-dihydrodesoxymorphine	0.8
Morphine	1.2
Codeine	7.5

the position it adopts in morphine, and a β-OH orientation, with OH directed toward ring D, is evidently preferred for uptake at opioid receptors.

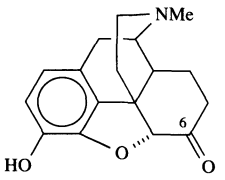


11

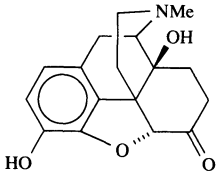
13.2.2. Ring C Diastereoisomers

Isomorphine, the 6-epimer of morphine, was first prepared by Small *et al.*[22] during the 1930s as part of the US Government's study of drug addiction. It was obtained by hydrolysis of α-chloromorphide, a product of reaction between morphine and PCl_3.[23] Although it has been stated that inversion of C-6 geometry generally enhances potency,[24] hot-plate ED_{50} data quoted by Mellett and Woods[25] indicate a contrary conclusion (ED_{50} mg/kg sc morphine 2.1, α-isomorphine 3.8; codeine 14.2, isocodeine 33.8).

Epimeric 6-hydroxy derivatives feature as metabolites of opiates of both agonist and antagonist nature. Both 6α- and 6β-epimers have been isolated from hydromorphone **12** with β- the major product; analgesic potencies were hydromorphone >6α-OH >6β-OH.[26] The antagonists naloxone **13**a and naltrexone **13**b also produce 6α- and/or 6β-hydroxymetabolites.[27] In chickens, the primary metabolite of naloxone is the 6α-epimer (isolated as the 3-glucuronide).[28]



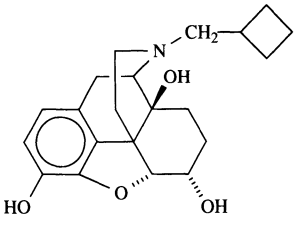
12



(a) R = $CH_2CH{=}CH_2$; (b) R = $-CH_2c\text{-}C_3H_5$

13

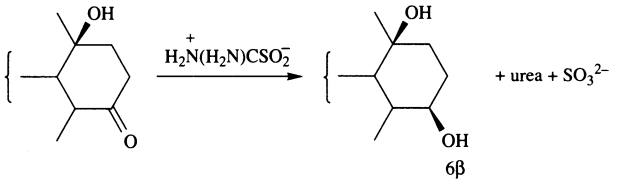
In man, naltrexone yields the 6β-epimer while chickens produce the α-form again. The relative order for analgesic antagonism is naloxone/naltrexone >6α >6β-OH metabolites. The 6β-epimers were devoid of agonist actions (i.e., are pure antagonists) while the 6α-epimers had dual properties. It is of interest that the 6α-hydroxy derivative nalbuphine **14** is also a dualist (0.25–0.5 × nalorphine, 1 ×



14 nalbuphine

morphine in man).[29,30] Attempts to prepare 6β-epimers by hydride reduction of the corresponding ketones led to the α-isomer only,[31] but the less usual reductant thiourea dioxide (formamidine sulfinic acid) gave β-products exclusively (see **15**).[32]

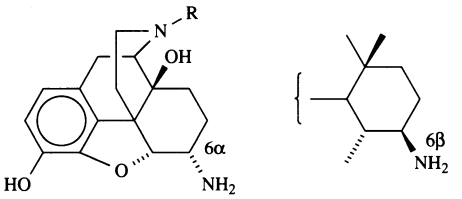
Evidence of configuration was provided by ^{1}H-NMR comparisons of 5-H and 6-H signals with those of dihydroisocodeine and dihydrocodeine.[33]



15

13.2.3. 6-Amino Derivatives: Affinity Ligands

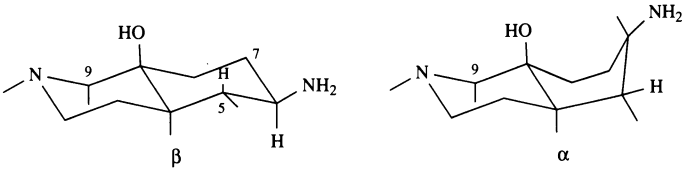
6-Amino analogues of dihydromorphines are of special interest, notably on account of their use in the design of affinity labels. Most work has been directed at opioid antagonists related to naloxone and naltrexone. Reductive amination of these C-6 ketones with $NaCNBH_3$ in the presence of ammonium acetate gave epimeric mixtures **16** (2α:1β) separable by fractional crystallization of the HCl salts.[34] Later stereospecific syntheses were devised as shown in Scheme 13.2.



(a) R = C_3H_5; (b) R = $-CH_2c-C_3H_5$

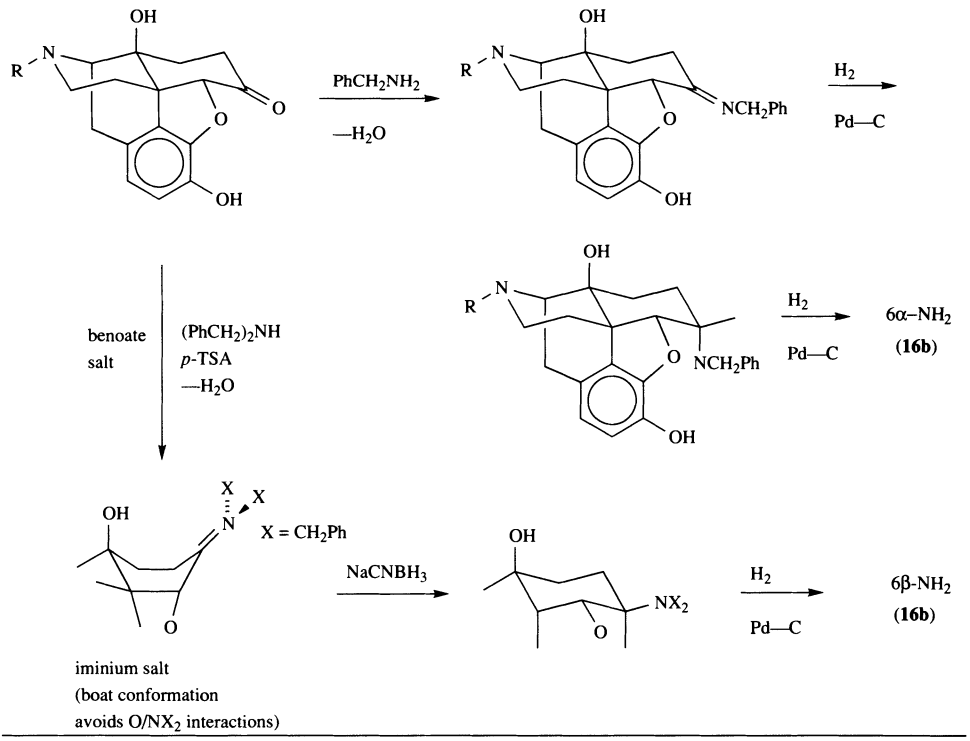
16

The configurations of the products were established from the $^3J_{5\beta\text{-}6}$ coupling constants: values for 6β-epimers (6.8–7.6Hz) were larger than those of 6α-isomers (3.2–4 Hz), evidence for a diaxal 5-H/6-H relationship in the β-derivative (see **17**).



17 (partial structures)

The conclusions were confirmed in a 360-MHz study.[36] Additionally, however, resolution of the two $^3J_{6,7}$ couplings gave evidence of the conformation of ring C. Since *both* included large and small values (6α, 13.1 and 4.1 Hz; 6β, 12.0 and 4.0 Hz) a near-*trans*-diaxal 6-H/7-H relationship must obtain in each epimer—this requires that ring C is a chair in the β-isomer and a skew-boat in the α-isomer as



Scheme 13.2. Stereospecific synthesis of 6α- and 6β-naltrexamine (R = CH_2c—C_3H_5 in formulas shown.[5]

shown. An X-ray analysis established that the twist-boat conformation is also preferred for α-oxymorphamine in the solid state.[37] Receptor affinities for rat brain membrane sites, reported in the same paper, showed both epimers to be μ-selective [displacement of ^{125}I-labeled Tyr-D-Ala-Gly-NMePhe-Met(0)ol (μ) and D-Ala^2-Leu^5 enkephalin (δ)] with β-affinities exceeding α-affinities by small factors [IC_{50} (nM) values μ: α 13, β 9; δ α 250, β 110]. Amination reduced the abilities of

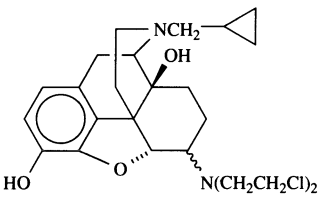
TABLE 13.2.
Tail-Flick ED_{50} Values of Morphine in Mice: Influence of Naloxone, Naltrexone, and Related 6-Amino Analogues[34]

Additive	Dose (mg/kg)	Morphine ED_{50} (mg/kg)[a]	IC_{50}[b]
None	—	5.3	—
Naloxone (**13a**)	0.04	12.3	5.5×10^{-9}
	0.16	44.4	
6β-NH_2 (**16a**)	1.6	16.0	8.6×10^{-8}
6α-NH_2 (**16b**)	1.6	4.9	1.4×10^{-7}
Naltrexone (**13b**)	0.08	134	5.5×10^{-10}
6β-NH_2 (**16b**)	0.32	27.4	9.1×10^{-10}
6α-NH_2 (**16a**)	0.32	8.6	2.9×10^{-9}

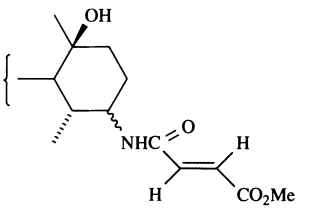
[a] The greater the morphine ED_{50}, the more effective the antagonist.
[b] Displacement of [^{3}H]naloxone (NaCl absent from incubate mixture).

naloxone and naltrexone to antagonize morphine in mice by the tail-flick assay, the 6β-epimer being the more potent of the two epimers (N-$CH_2cC_3H_5$ isomers were more effective than the N-allyl compounds, as was also found for the parent ketones); see Table 13.2. Binding data correlated with the *in vivo* results.

The incorporation of alkylating functions into opioid ligands with the object of obtaining molecules which will bond covalently (and hence irreversibly) to receptors is an important aspect of the detection and isolation of opioid receptors.[1] Portoghese's group has utilized the epimeric 6-amino analogues of naltrexone **16b** and oxymorphone (**16**, R = Me) to provide such ligands. Agents developed were the chlornaltrexamine **18** (α,β-CNA) nitrogen mustards and the fumaramate esters **19**



18 α,β-CNA

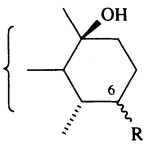


19 α,β-FNA

(α,β-FNA); the latter engage in Michael additions involving thiol functions of the receptor protein. The nitrogen mustards were made by reductive amination of the appropriate 6-amino epimer with diethanolamine and $NaCNBH_3$ followed by triphenylphosphine and CCl_4 to replace terminal OH by chlorine.[38,39] β-CNA had no analgesic activity in mice after icv injection, and produced long-lasting (3–6 days) narcotic antagonism in mice—its effects were blocked by pretreatment with naloxone. There is evidence that β-CNA irreversibly alkylates μ-, δ- and κ-subtypes of opioid receptors. The α-isomer also produced irreversible blockade of the same three receptor subtypes but was distinctive in having an irreversible agonist action on GPI (22 times that of morphine's reversible effect), although it lacked such action on the mouse vas deferens (MVD).[40] β-COA, the N-methyl analogue of β-CNA derived from oxymorphone, bound covalently to opioid receptors[41] *in vitro* and *in vivo* behaving as an agonist rather than as an antagonist. β-FNA (**19**) also has irreversible blocking properties but, with its less reactive alkylating function, is selective in regard to sites blocked.[42,43]

Pure μ-agonists were blocked but little effect was seen against κ-agonists and dualists such as nalorphine, and the compound is now employed for selective blockade of μ-sites.[44,45] Both β-FNA and its 6α-isomer behaved as *reversible* agonists at GPI sites (α, 7×; β, 5× morphine) but the α-isomer lacked alkylating properties in this tissue.[46] In fact α-FNA protected the GPI against alkylation by the β-epimer, suggesting that the two epimers bind to the same receptor. Epimeric 6-isothiocyanates (6-NCS) differed in similar ways (see below), and the action of affinity labels is presumed to involve: (1) a primary recognition step of forming a reversible ligand–receptor complex; followed by (2) a secondary step requiring proper alignment of the electrophile with a proximal nucleophile on the receptor. Data on epimers suggest that only the β-isomers provide the correct geometry for step (2). In the MVD, β-FNA but not the α-epimer irreversibly antagonized morphine, while neither isomer blocked the δ-agonist DADL (cf., however, results of Hayes *et al.*).[47] The reversible agonist activities of α- and β-FNA in β-FNA-

TABLE 13.3.
Agonist and Antagonist Activities of FNA Epimers and Related Compounds[46]



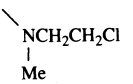
6-Substituent	Epimer	Agonist potency (morphine = 1)	Antagonist action	
			dose (nM)	IC_{50} ratio[a]
$NHCOCH=CHCO_2Me$[b]	α	7.3	20	1.2
trans			200	0.87
FNA (β-epimer)	β	5.0	20 (10^3)	6.0 (18.8)
$NHCOCH=CHCO_2Me$	α	0.8		
cis	β	1.1		
$NHCOCH=CH_2$	α	1.5		
	β	40% max at 1 μM		
$NHCOCH_2I$[b]	α	0.49		
	β	55% max at 1 μM		
NCS[b]	α	9.6	20 (500)	2.1 (2.3)
	β	1.9	20 (200)	6.9 (14.9)
NHCOEt	α	2.9		
	β	0.81		

[a] Agonist (morphine) IC_{50} value after 30 min incubation with test compound followed by washing, divided by control IC_{50}.
[b] All partial agonists of reversible nature except the β-$NHCOCH_2I$ derivative in the mouse vas deferens (MVD).

treated ileum (i.e., μ-sites blocked) were not significantly different from those exhibited in untreated tissue; hence these activities may be mediated through κ-receptors.

FNA epimers and their analogues were derived from the corresponding 6-amino derivatives **16**, e.g., reaction with the acid chloride of monomethyl fumarate gave an epimer of FNA while treatment with thiophosgene gave a 6-isothiocyanate.[46] Several epimeric pairs with reversible agonist properties in the GPI assay were identified (0.5–10 × morphine); α-epimers were the more potent while some of the less active β-isomers were partial agonists (Table 13.3). Only β-FNA and β-6-NCS derivatives irreversibly antagonized morphine. None of the derivatives blocked κ-selective agonists such as nalorphine. The sensitivity of α/β-FNA to the configuration of the alkenic unit is evident from the reduction of agonist potency that follows its replacement by a *cis*-linkage (see maleates of Table 13.3).

Subsequent papers of this series concentrated on variants of β-FNA.[48] It was discovered that *N*-methylation enhanced the reversible agonist properties of each epimer (β 600×, α 20× morphine, GPI) but removed the irreversible antagonist action of β-FNA.[49] The mono-functional nitrogen mustards **20** had similar phar-

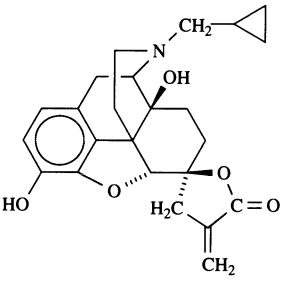


20 6-substituent of **18** (CNA)

macological profiles to α- and β-CNA (α 5.3×, β 159× morphine GPI); β at a dose of 20 nM gave IC_{50} ratios of 48.8 (*vs* morphine) and 17.3 (*vs* κ-agonist EKC) after washing—the influence of the α-epimer could not be determined because of its irreversible agonism.[50]

The solid state conformations of α- and β-FNA have been determined by X-ray diffraction. Like the parent 6-amino derivatives (page 437) they differ only in ring C conformation (chair for β-, twist-boat for α-). As a result the fumaramate moieties are near-equatorial in both structures and orthogonal to one another when the fused rings are superimposed; differences in the relative positions of the fumaramate double bonds are also evident and a model has been devised that accounts for step (2) of the dual recognition process being possible only for the β-isomer.[51]

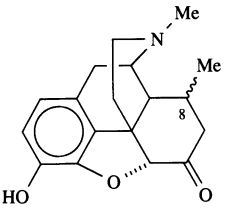
Affinity ligands with α-methylene lactone functions have also been investigated. The 6β-O-epimer **21** at a concentration of 5 nM caused a 50% inhibition of the binding of [^{3}H]naltrexone to brain tissue (30% irreversible—unaffected by washing); its epimer was less effective and had reversible action.[52]



21

6β-Azido analogues of dihydromorphine[53–55] and dihydronalorphine[56] have been reported, prepared by inversion of 6-tosylates by azide, but not their 6α-counterparts. The morphine derivative, azidomorphine, had notably high potencies (150–300× morphine in rats, 50× in man).

Epimeric 8-alkyl (Me etc.) dihydromorphinones (**22**) have been obtained by the conjugate addition of lithium dialkyl cuprates to codeinone.[57] Both 8-methyl

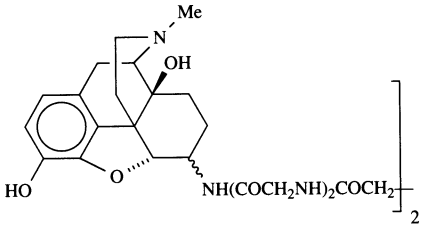


α-8Me (axial); β-8Me (eq)
22

epimers had greater affinities than dihydromorphinone for rat brain sites (vs. [^{3}H]etorphine) with that of α > β; α-**22** was twice as active and the β-epimer somewhat less active than the parent in the mouse tail-flick assay by the icv route.

13.2.4. Bivalent Ligands

Compounds of this type contain two pharmacophores joined by a connection unit (spacer). They have the potential of high selectivity for a single receptor subtype on the basis of simultaneous interaction (bridging) of both pharmacophores with vicinal receptors by use of a spacer of optimal length and the preference of bivalent over monovalent modes of binding.[58] Oxymorphamine (chiefly the α-epimer) and naltrexamine (chiefly the β-epimer) are the pharmacophores that have been employed for this work, since they may be linked via their 6-amino functions by a variety of spacer units. Some comparisons of bivalent ligands derived from 6α- and 6β-pharmacophores have been made. Thus the 6α-isomer **23** was 18.9 times, and the 6β-isomer 3.5 times more potent than the corresponding monomers as agonists in the GPI assay (monomers: **23** with 6 $\sim NH(COCH_2NH)_2COMe$).[59]



23

IC_{50} nM (GPI)
α 3.9
β 6.3

In binding experiments (GP brain vs. [^{3}H]naloxone), the β-derivative had a somewhat greater affinity than that of the isomer obtained from α-oxymorphamine (K_1 nM: β 10±0.3, α 14±3). It is clear that the C-6 geometry of bivalent ligands of this kind has only a minor influence on the activities of these highly flexible molecules.

Of special interest is the study of bivalent ligands which include combinations of (−)- and (+)-opiate pharmacophores and exploit the high enantioselectivity of opioid receptors (page 431).[60] Data for α-oxymorphamines linked by a succinyl–glycine chain are shown in Table 13.4. The superior potency of the (−)/(−) dimer over its partners supports the view that a pair of opioid receptor sites are occupied, while the enhanced activity of the (−)/(+) dimer over the (−)-monomer is considered due to the dimer receiving a nonopioid contribution to binding. Similar results were found for β-naltrexamine analogues (antagonists)

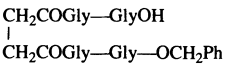
TABLE 13.4.
GPI Potency Data for α-Oxymorphamines Linked by a Succinyl–Glycine Chain[60]

α-Morphamine antipode employed		$CH_2COGlyGlyR$ | $CH_2COGlyGlyR'$	
R	R′	Potency ratio[a]	Relative potency
(−)	(−)	36.0±6.6	18.9
(−)	(+)	7.6±1.3	4.0
(−)	R′ = Me (monomer)	1.9±0.3	1.0

[a] IC_{50} of morphine divided by IC_{50} of morphamine derivative (GPI).

except that the nonspecific factor was less [relative potencies: (−)/(−) 33.5; (−)/(+) 1.4; monomer 1.0].

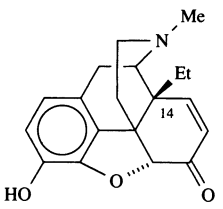
The dextro pharmacophores (+)-oxymorphamine and (+)-naltrexamine were derived from (+)-7-bromodihydrocodeinone dimethyl ketal (page 432). Levo oxmorphamine was coupled to the spacer (**24**) with the aid of HOBt/DCC in DMF, the product debenzylated to free the second carboxylate function, and itself coupled to either (−)- or (+)-oxymorphamine.



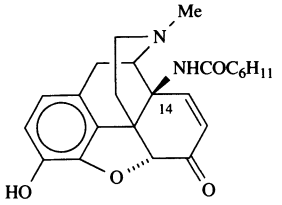
24

13.2.5. Substituents at C-14

Replacements of the axial C-14 hydrogen by a variety of substituents have been reported. Many of these derivatives have enhanced potencies over the parent compound, sometimes notably so, as found for the 14-ethyl-morphinone **25** (10,000× morphine in mice, tail-clip test sc)[61] and the amide **26** (analgesic ED_{50} 0.00053 mg/kg sc, morphine 0.60).[62] The potent and essentially pure (i.e., lack of



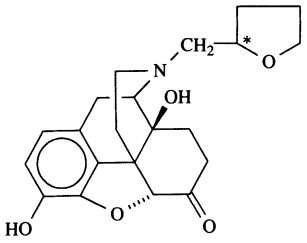
25



26

agonist action) opioid antagonists naloxone **13a** and naltrexone **13b** are both 14-hydroxy derivatives. Analogues of naltrexone with other small polar substituents at C-14 behaved as pure antagonist (Br, Cl) or dualists (SH, NO_2).[63] Apart from some 14-β-OH morphinans (page 451), epimers of the 14-*R*-morphines (possible only in the B/C *trans*-series, page 433) have not been described. Experience with such derivatives does show, however, that structural extensions positioned above the plan of the *trans*-fused C/D ring of an opiate generally enhance ligand–receptor binding to a significant degree.

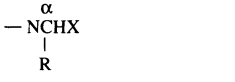
A few morphine analogues with chiral N-substituents have been reported. In the case of the 2-tetrahydrofurfuryl derivative **27**, the R^*-isomer was found to be



27

a potent agonist (mouse writhing sc ED_{50} 0.02 mg/kg, morphine 0.5 mg/kg) while the S^*-diastereoisomer was inactive—both isomers suppressed morphine analgesia, but at a potency level below that of nalorphine[64] (cf. related benzomorphans, page 459). Epimers were obtained by treating noroxymorphone with antipodal (+)-camphor 10-sulfonates of tetrahydrofurfuryl alcohol of known configuration.[65]

The comparative pharmacology of *N*-allyl, *N*-*n*-propyl, and *N*-cyclopropylmethyl normorphines and their α-methylated analogues have been reported. In the **28**a and **28**b series, α-methyl antipodes differed little in agonist tests (mice TF and writhing) and binding affinities to rat brain membranes (*vs* [^{3}H]naloxone), with no consistency of eutomer configuration.[66] The S-**28**b (R = Me) antipode was twice as effective as the *R*-form but 10 times less potent than **28**a (R = H) as an antagonist (inhibition of Straub tail response). The agonist (mice TF, sc) rank order of the *N*-cyclopropylmethyl series was **28**c (R = H) > *R* (R = Me) > *S* (R = Me); none antagonized morphine in the TF test. The more potent agonists had high affinities for κ-sites of GP cerebellum, and for μ- and δ- (less so) sites of rat brain.[67]



(a) X = CH=CH_2; (b) X = Et; (c) X = *c*-C_3H_5

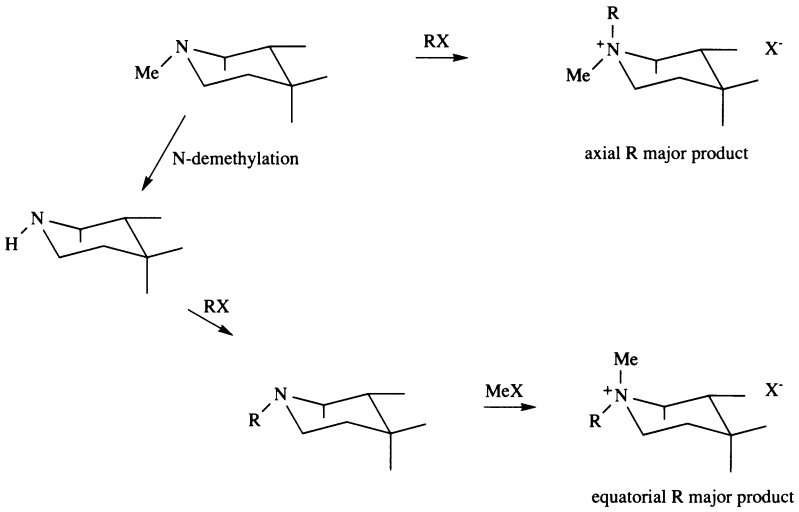
28

(N–R feature of normorphine)

Configurations were established by stereospecific syntheses of **28**b (R = Me) using norcodeine and *R*- or *S*-2-mesyloxybutane (with expected Walden inversion), interconversions of **28**a and **28**b (R = Me) by catalytic hydrogenation, and X-ray crystallography (**28**c, R = Me).

13.2.6. Quaternary Salts

Diastereoisomeric pairs of quaternary ammonium salts with chiral nitrogen centers may be obtained by reaction sequences shown in Scheme 13.3, and configurations established by knowledge of reactions chosen, NMR spectroscopy (relative chemical shifts of *N*-Me), and X-ray crystallography. Kobylecki *et al.*[68] found that the equatorial *N*-allyl isomer of the pair derived from nalorphine retained about 30% and the axial congener less than 2% of the ability of the parent to antagonize normorphine in the MVD assay. Iorio and Frigeni[69] likewise found the *N*-*eq*-allyl methiodide of naloxone to exceed the antagonist potency of its *N*-*eq*-Me epimer vs. morphine in a hot-plate test performed on mice (icv route to ensure penetration of CNS). The *N*-*ax*-allyl salt behaved as a weak agonist in the GPI assay (IC_{50} 4.5 μM, oxymorphone 9.7 nM). The Italian workers obtained similar results for a series of isomeric *N*-allyl, *N*-ethyl, and *N*-cyclopropylmethyl morphines.[70] In binding experiments (displacement of [^{3}H]-naltrexone from rat brain membranes) only the diastereoisomers obtained by methylation of nalorphine and naloxone bases (i.e., the *eq*-*N*-allyl isomer) proved effective.[71] Quaternary salts derived from levallorphan (page 450) were also examined. The *in vitro* affinity ranking of isomers correlated with those obtained from a peripheral *in vivo* test—



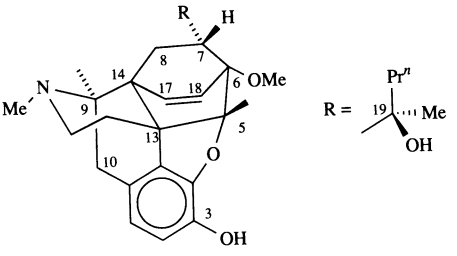
Scheme 13.3. Preparation of diastereoisomeric quaternary salts of opiates (partial structures shown centered around ring D).

namely, reversal of morphine's inhibitory effect on charcoal meal transit in the GI tract of mice (a test related to the well-known constipating side-effect of opiates).

13.2.7. Thebaine-Derived Opiates: Etorphine and Its Relatives

Diels-Alder adducts formed from thebaine (the diene) and various electrophilic alkenes have led to opiates of remarkably high potencies—several thousand times that of morphine in several cases.[8] The products are further examples of morphines whose structures have been built up by additions above the plane of the C/D rings.

Early NMR studies pointed to geometry of the kind **29** with the 6,14-etheno bridge "inside" (*endo*) the tetrahydrothebaine system and below the plane (α) of the C-7-C-8 feature. The substituent R was positioned at C-7 (no C-8 product was detected) in an α-orientation. A crystallographic study of 3-*O*-methyl **29** [R = C(OH)MePr^n] HBr salt confirmed this molecular shape[72] and established an *R*-configuration at C-19 by anomalous scattering.

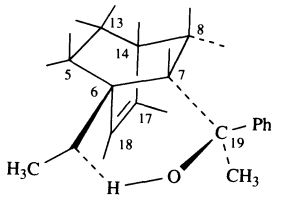


29

In the case of the product of addition of acrylonitrile to thebaine (**29**, R = CN, 3-OMe), the major product was assigned an α-configuration at C-7 on the basis of 60-MHz NMR evidence.[73] Long-range couplings were claimed to operate between H-18 and H-5β and H-18/H-7β—systems linked by four bonds which approximate to a letter "W" when the C-7 cyano substituent has an α-orientation. Such an arrangement satisfies requirements for long-range coupling provided all the bonds approximate to a single plane.[74] A Dreiding model reveals that both W-pathways diverge from planarity to a marked degree, and a spectrum recorded at 400 MHz failed to detect long-range splitting of the vinylic signals (Fig. 13.1). Chemical shift differences between the α- and β-diasterisomers, however, substantiate assigned configurations, e.g., the H-5β signal is about 0.5 ppm to lower field of the corresponding α-resonance as a result of falling within the deshielding zone of the cyano substituent when it has a β-orientation.

When the C-7 substituent in **29** is an asymmetric tertiary alcohol function (RR'COH) diastereoisomeric alcohols result and potency differences between isomers have been reported.[75] The 3-*O*-methyl ether of etorphine (**29**, R = CMePr^{n}OH) of *R*-configuration at C-19 was 89 times more active than morphine in rats (sc, tail pressure) while its C-19 diastereoisomer fell below (× 0.7) the potency of morphine.

Assignment of configuration to the diastereoisomeric phenylmethyl *t*-carbinols **30** and **31** was possible by NMR analysis.[73] The vinylic protons at C-18 and C-17 in one alcohol were upfield of corresponding signals due to the second isomer (Δδ 33Hz for H-17 and 55 Hz for H-18). These upfield shifts were attributed to aromatic screening (in **31** the vinylic protons lie close to and above the plane of the aromatic ring, H-18 being the closer proton and most affected). Hence the isomer with the higher-field vinylic signals must have phenyl placed as in **31** (*R*-configuration).



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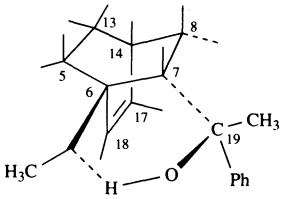
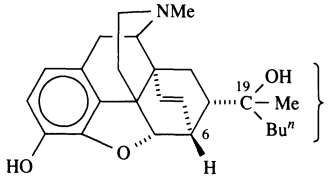


31

Rapoport has provided further isomeric examples of this kind. The first pair relates to the 6-desmethoxy analogues **32**.[76] Configurations at C-19 were based on the correspondence of the chemical shift of the carbinol methyl group with that of the 3-*O*-methyl ether of *R*-propylthebinol **33**.[73]



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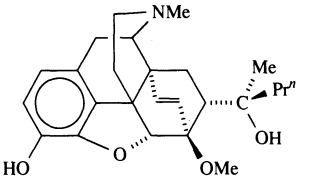

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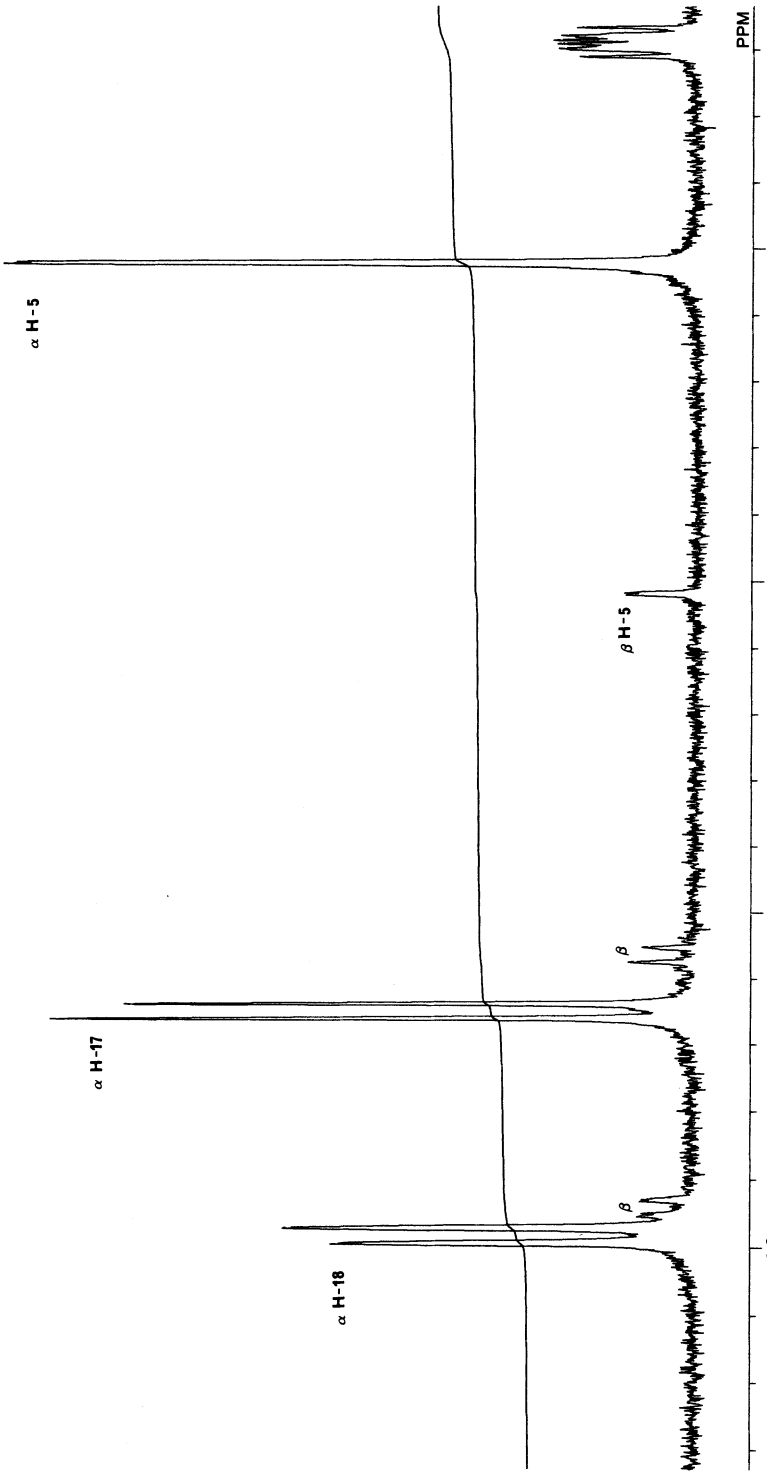
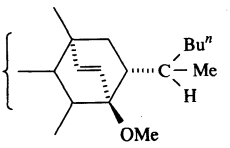


FIGURE 13.1. Part of the 400-MHz ^{1}H-NMR spectrum of the thebaine–acrylonitrile adduct (**29**, R = CN, 3-OMe) mixture in $CDCl_3$. Resonances due to the major (α) and minor (β) components are annotated: H-18 and H-17 are the vinylic protons, and H-5 that attached to C-5 (see **29**).

R-**32** was somewhat more potent than etorphine in the rat tail-flick assay (a result which discounted the need for an intramolecular bond between 6-OMe and the C-19 hydroxyl in active compounds), while the corresponding *R*-methyl ether and *S*-phenol were far less effective (Table 13.5). 19-Deoxy analogues **34** were obtained by reducing corresponding hydroxymethyl mesylates **35** with lithium triethylborohydride.[77] The configurations at C-19 of the precursor alcohols were



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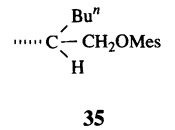
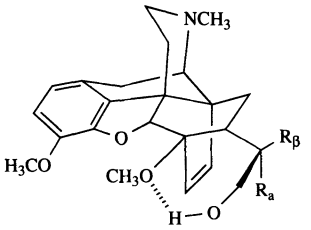


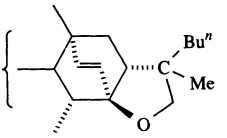
35

based on comparative chemical shifts of *n*-butyl (methyl in related epimers) and C-17/18 vinylic protons in isomeric pairs (**36**) (cf. **30** and **31**). Mesylate reduction also resulted in the epoxides **37**.



36

Intramolecular hydrogen-bonded conformation: the proximity of the R_α group and the etheno bridge results in shielding and an upfield NMR shift of the R_α proton resonance.



37

Antinociceptive potency data (Table 13.5) reveal that that an *R*-configuration at C-19 provides the eutomer in four out of the five pairs examined. The authors argue that these results support the idea that the C-19 *n*-butyl group must be directed toward liphophilic regions of the receptor for optimal interaction and that this arrangement is only possible in the *R*-isomer. The same pattern obtains in the *R*-20-OH derivative. However, in the epoxides the position of the butyl group is locked and its interaction with the lipophilic region is best achieved by an *S*-configuration.

Further stereochemical evidence of opioid ligands of this class is available from X-ray and theoretical studies of buprenorphine HCl (dualist in clinical use as analgesic)[272] and NMR (2D ^{1}H, ^{13}C) analyses of diprenorphine (antagonists);[273] both agents carry an *N*-cyclopropylmethyl substituent.

TABLE 13.5.
Antinociceptive Activities in Rats (Tail-Flick) of C-19 Diastereoisomers of Analogues of Etorphine[76, 77]

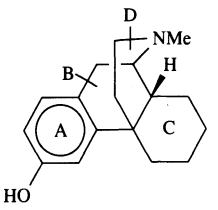
	ED_{50} (μM/kg)	Relative activity (morphine = 1)
R-**32** (6-deoxy)	0.0035 (0.2)[a]	477
S-**32**	0.15	11
R-**34** (19-deoxy)	0.0087	187
S-**34**	0.387	4
R-(20-OH precursor of **35**)	0.0021	773
S	0.0386	4
R-**37** (epoxide)	0.09	19
S-**37**	0.0081	206
R-**32** (6-Me)[b]	0.001	2200
S	0.034	65
Etorphine (*R*)	0.0045	371
Morphine	1.67	1

[a] 3-*O*-Methyl ether.
[b] 6-Methyl derivatives.[78]

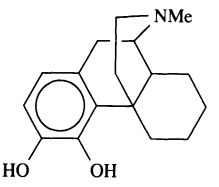
13.3. Morphinans

This group of opioids differs from morphine merely in the absence of the 4,5-oxy bridge and ring C functionalities. The relative ring fusions of rings B and C (*cis*) and C and D (*trans*) are the same as those of morphine. Since most morphinans examined have been made by synthesis from racemic materials, e.g., by the Grewe route, antipodal mixtures are commonly available and may be resoled to provide pairs of enantiomorphs.[7,8] The first example studied was racemorphan **38**.[79]

Relative geometries were established when a synthesis that led to racemorphan **38** produced *rac*-tetrahydrodeoxycodeine **39** when suitably modified (use of 3,4-dimethoxybenzaldehyde rather than anisaldehyde).[80] Benson *et al.*[81] found that



38
(levo antipode shown)



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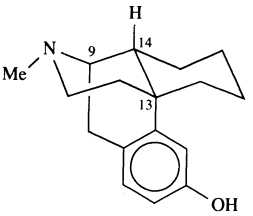
(−)-**38** and its methyl ether were twice as potent as the *rac*-materials, while corresponding dextro isomers had negligible activity (rats, radiant heat method). The respiratory actions of racemorphan were due to the (−)-isomer. All forms were inhibitors of the cough reflex—dextro antipodes (particularly that of the methyl ether, dextromethorphan) had significant antitussive activity without causing

cerebral depression. Dextromethorphan (Romilar) is in clinical use as an antitussive[82] (see also below). In man, Isbell and Fraser[83] reported that (+)-**38** could not substitute for morphine in addicts and that the analgesic effects of racemorphan resided wholly in the levo isomer (Dromoran). Hot plate ED_{50} values (mg/kg sc) in mice for **38** quoted by Mellett and Woods[25] were: *rac* 0.9, levorphanol 0.5, dextrorphan 48.2; values for 3-*O*-methyl ethers were *rac* 8.1 (−)-3.0, (+)-75.

The absolute configurations of levorphanol at C-9, C-13, and C-14 are identical with those of corresponding chiral centers of natural morphine. These results follow from chemical degradation of levorphanol **40** to the (−)-acid **41**, identical to that obtained from thebaine[5] and abietic acid.[85] In addition, sinomenine (**4**) has been transformed into (+)-3-methoxy-*N*-methylmorphinan, and dihydrothebaine into the corresponding levo 3-*O*-methyl ether of levorphanol.[86] Physical correlations (ORD and CD) between morphinans and morphine have also been established.[87–89]



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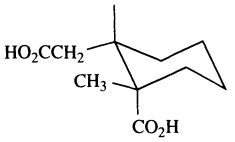


41

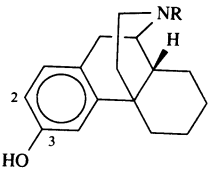
N-Alkylation of antipodal 3-hydroxymorphinans has provided a variety of isomeric pairs which span the pharmacological spectral range of opioid ligands (Table 13.6). In all cases levo isomers (of absolute configuration **40**) far exceed the activities of corresponding dextro antipodes (compound No. 9 pair is an exception—these are 2-hydroxy derivatives, however). Levo antipodes of Nos. 7 (levallorphan)[94] and 8 (cyclorphan) are dualists, and carry N-substituents associated with antagonism in the morphine group. The 3-furylmethyl analogue of compound No. 6 was also an antagonist [AD_{50} *rac* 0.8, (−) 0.3 mg/kg] but methylation (at C-3 for compound No. 6, and C-2 for the 3-furylmethyl derivative) gave agonists with levo forms 2.5–3 times as potent as racemates.[91]

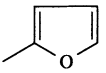
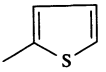
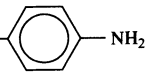
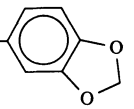
One of the first demonstrations of the principle of stereospecific binding (SSB) developed by Avram Goldstein utilized levorphanol and its antipode dextrorphan.[95] Although the original system[96] did not prove succesful since less than 2% of the total binding to rat brain homogenates was SSB, the work paved the way to the succesful use of [^{3}H]-naloxone (a ligand of high specific radioactivity) by Pert and Snyder.[97,98]

The N-methyl quaternary salt of levorphanol produced a dose-dependent inhibition of GPI contractions, but its potency relative to levorphanol itself (1) was only 0.068.[99] *N*-Methyldextrorphan was only weakly depressant at 4×10^{-5} M, while binding IC_{50} values *vs* [^{3}H]-etorphine, GP brain, were 0.008 μM for levorphanol and 0.52 μM for the methosalt—*N*-methyldextrorphan was a feeble competitor even at 10 μM.

High- and low-affinity sites have been detected for the binding of [^{3}H]dex-

TABLE 13.6.
Pharmacological Data for Antipodal Pairs of 3-Hydroxy-N-Substituted Morphinan Derivatives



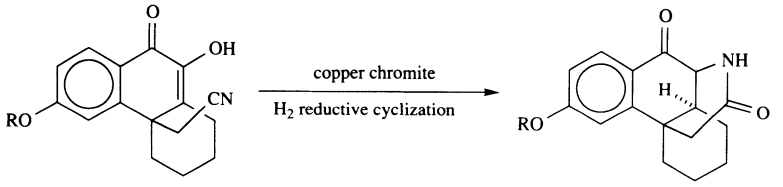
No.	R	Isomer	Analgesic activity ED_{50} (mg/kg sc)[a]	Antagonist activity AD_{50} (mg/kg sc)[b]	Reference
1.	$(CH_2)_2Ph$	(−)	0.11	—	90
		(+)	100		
2.	$(CH_2)_2$ 	(−)	0.01	—	90
		(+)	100		
3.	$(CH_2)_2$ 	(−)	0.02	—	90
		(+)	100		
4.	$(CH_2)_2$ 	(−)	0.02 (*rac* 0.6)	—	90
		(+)	100		
5.	$(CH_2)_2$ 	(−)	0.07	—	90
		(+)	100		
6.	CH_2 	*rac*	—	0.8	91
		(−)	—	0.5	
		(+)	—	8.0	
7.	C_3H_5 (allyl)	(−)[c]	0.29	0.39	92
		(+)	55	inactive (> 32)	
8.	CH_2c-C_3H_5 (CPM)	(−)	0.036	0.25	92
		(+)	34	inactive (> 32)	
9.	CH_2c-C_3H_5 (2-OH)	(−)	1.12	9	92
		(+)	13	7	
10.	$(CH_2)_2CN$[d]	(−)	0.07 μM/kg	—	93
		(+)	inactive		

[a] Nos. 1–9, writhing test in mice using phenylquinone.
[b] Nos. 7–9, tail-flick test in mice *vs* morphine; No. 6, suppression of morphine-induced analgesia in mice.
[c] Levallorphan (Lorfan).
[d] Cf. levorphanol 0.49 μM/kg.

tromethorphan (DM) to guinea-pig brain.[100] These sites are distinct from opioid receptors and DM binding is reversible, saturable, and stereoselective; IC_{50} values (nM) *vs* [^{3}H]DM were: dextromethorphan 25, levomethorphan 500, dextrorphan 2500, levorphanol 10,000 (high-affinity sites). σ-Site ligands such as (+)-cyclazocine (page 461) and (+)-3PPP (page 183) had high affinities for [^{3}H]DM sites, but phencyclidine (PCP) and other PCP agents were weak competitors.[101] Since DM and other central antitussives such as carbetapentane and caramiphen are devoid of the psychotomimetic side effects of σ-ligands, it is concluded that DM sites can mediate only the nonpsychotomimetic effects of such ligands.[102] Of further interest is that DM and related antitussives all protected rats against electroshock seizures and DM enhanced the anticonvulsant effects of phenytoin.[103] Relative abilities to displace [^{3}H]3-PPP from rat brain σ-sites were: (+)-cyclazocine 100, (+)-*N*-allyl-3-hydroxymorphinan 72, (−)-antipode of N-allyl derivative 6, DM 15, and levorphanol <1.[104]

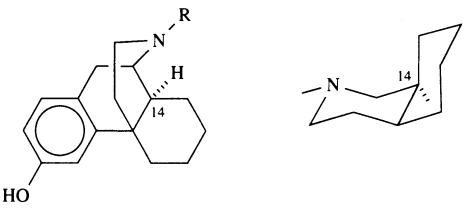
13.3.1. B/C *trans* Series: Isomorphinans and Other Analogues

Although Grewe's cyclization route to morphinans yields small amounts of isomorphinans, studies of these isomers with a B/C *trans*-ring fusion is based on material prepared by Gates and derived either from the intermediate **42**[105] or



42

trans-dihydrothebainone[106] (the latter route correlates the configuration of levo isomorphinans and natural opiates). In the rat tail-flick test (−)-**43**a was 8–10 times more potent than morphine while the dextro antipode was ineffective up to 32 mg/kg; the levo 7-ene analogue was slightly less active than (−)-**43**a while the dextro 7-ene was about one-quarter as active as morphine. These results are in contrast with findings for morphine and its much less active B/C *trans*-counterpart (page 434). Likewise the (−)-*iso*-*N*-cyclopropylmethyl analogue **43**b proved more



(a) R = Me; (b) R = CH_2c–C_3H_5 (isocyclophan)

43

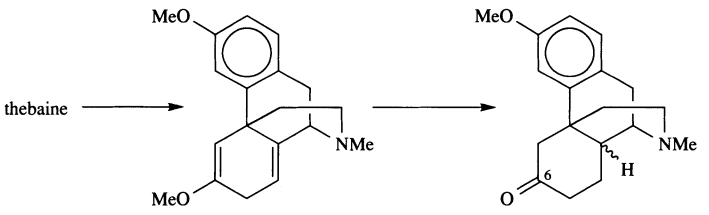
effective (7 × nalorphine) than cyclorphan (4 × nalorphine) as an antagonist of pethidine in mice by the tail-flick test.[106] More recent comparisons of cyclorphan

and isocyclorphan reverse the potency order and show both to be dualists (cyclorphan a potent, isocyclorphan a weak agonist)—see data below.[112]

	MWR[a]	OMST[b]	RTF[c]
Cyclorphan (14-β-H)	0.031	0.32	0.032
Isocyclorphan (14-α-H) (levo isomers)	8.7	0.67	0.057

[a] Agonist ED_{50} (mg/kg), mouse writhing.
[b] Antagonist ED_{50} (mg/kg), prevention of oxymorphone—including Straub tails in mice.
[c] Antagonist ED_{50} (mg/kg), rat tail-flick *vs* morphine.

Chemists working at Miles Laboratories gained access to several *cis*- and *trans*-B/C-fused morphinan-6-ones when they discovered that acid hydrolysis of 4-deoxydihydrothebaine (**44**) gave a mixture of isomers (**45**).[107] In most cases *cis*- to *trans*-B/C ring inversion depressed antinociceptive activity in the mouse writhing test but elevated it in the rat tail-flick assay (one example); see Table 13.7.



44 **45**

N-Cyclopropylmethyl congeners were dualists (*trans* 5 × *cis* as antagonists) while their N-cyclobutymethyl analogues had only agonist activities. Pairs of compounds alkylated at C-7,C-8 and at both these positions were also examined[107,108] but influences of such substitution were not well defined; two examples are included in Table 13.7.

Belleau and a Bristol Laboratories group developed a novel morphinan synthesis which led to 14-hydroxy derivatives in both *cis*-B/C and *trans* (iso) form.[109,110] Isomers of this kind may be differentiated by the presence (14-β-OH) or absence (14-α-OH) of an intramolecularly H-bonded OH band in the IR spectra of the bases (see page 463, benzomorphan examples).

The β-14-OH derivative **46** (butorphanol) proved to be a dualist (agonist 10 × morphine, antagonist 0.25–1 × naloxone); when the 14-OH function was moved to

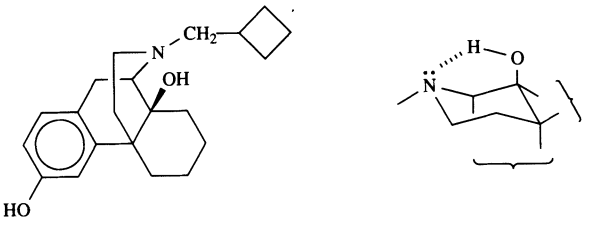


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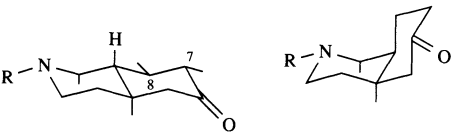
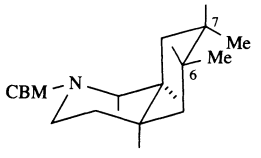
TABLE 13.7.
Biological Activities of Some B/C *cis* and *trans* Pairs of Morphinan-6-ones[107, 108]



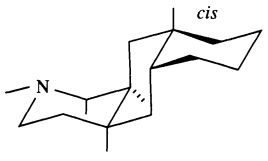
N–R	3-R	B/C fusion	ED_{50} (μM/kg) mouse writhing sc	rat tail-flick sc	rat tail *vs* morphine
Me	OMe	*c*	1.72	2.32	—
Me	OMe	*t*	3.54	70	—
Me	OH	*c*	0.52	70	—
Me	OH	*t*	0.75	1.33	—
CH_2c-C_3H_5[a]	OMe	*c*	0.66	—	14.9
CH_2c-C_3H_5	OMe	*t*	>25	—	3.6
CH_2c-C_4H_7[b]	OMe	*c*	0.58	—	inactive (25)
CH_2c-C_4H_7	OMe	*t*	20.3	—	inactive (30)
CH_2c-C_4H_7	OH	*c*	0.54	—	0.82
8-Et		*t*	24	—	11.6
CH_2c-C_3H_5	OH	*c*	23	—	0.35
t-7,8-di-Me		*t*	4.5	—	1.02
Morphine			2.1	19.3	—
Nalorphine			3.51	—	2.47

[a] Cyclopropylmethyl
[b] Cyclobutylmethyl

the α-position, a >100-fold drop in agonist and a much smaller fall (3×) in antagonist potency resulted.[111,112] The 14-α-OH analogue of oxilorphan (**46** with N-CH_2c-C_3H_5) was only marginally less active than the parent in agonist and antagonist evaluations.[112] Agonist potency was partially restored in the 6,7-dimethyl analogue **47** of isobutorphanol in which the methyls extend into a region that overlaps C-7 of butorphanol, while *cis*-fusion of an extra cyclohexane ring to C-6 and C-7 of the iso compound **48** (MWR ED_{50} 25 mg/kg) was less detrimental than a *trans*-attachment (MWR ED_{50} 80 mg/kg).[112,113]

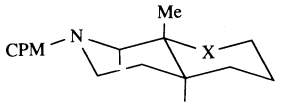


47
MWR ED_{50} 0.32 mg/kg
(*rac*-butorphanol 0.06 mg/kg)



48

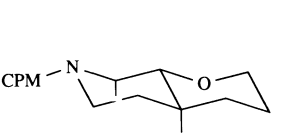
Incorporation of a 14-α-oxygen into the normal morphinan skeleton was achieved by synthesis of the tetrahydropyran **49** with a 14β-methyl group. This compound is a potent agonist (100× the deoxy analogue **49**, X=CH_2), evidence that the loss of analgesic potency of isobutorphanol is due principally to the change in ring C configuration.



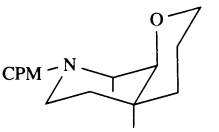
49

	ED_{50} mg/kg	
(X=O) 0.19 (ag)	0.6 (antag)
($X=CH_2$) 20.0	0.16
oxilorphan 8.7	0.03–0.3

Similar patterns of activity were seen for 8-oxy analogues of cyclorphan **50** and isocyclorphan **51**.[112,114] Levo isomers far exceeded the potencies of corresponding dextro antipodes in all tests.[114] It is of interest that the agonist potency



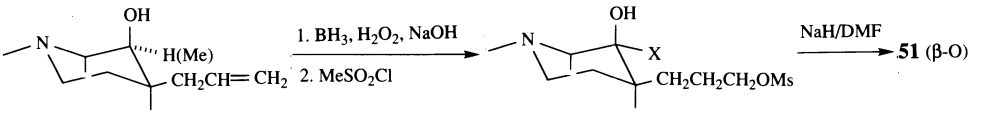
50



51

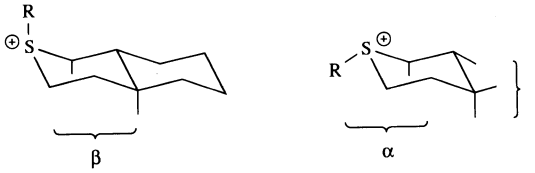
		ED_{50} (mg/kg)		
		agonist	agonist	
		mouse writhing	OMST	RTF
50	α-O	0.029	0.32	0.22
51	β-O	4.8	1.25	

of oxilorphan is depressed by the presence of the 14-methyl group (**49**, $X=CH_2$) since certain 14-methylmorphines have very high potencies (page 442). The 8-oxa compounds were made by stereospecific cyclization of 9β-**52** and 9α-5-allylbenzomorphans, configurations of which were known on the basis of IR evidence.[115]



52

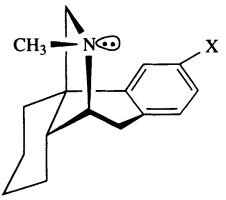
Lemaire and Belleau[116] examined both axial (β) **53** and equatorial (α) **54** conformers of *S*-methyl and *S*-allyl sulfonium analogues of racemorphan. They found that antagonist properties were confined to the α-derivatives (*vs* morphine GPI), while only the α-*S*-Me derivative had potent agonist properties in the ileum test (93% that of levorphanol, μ-selectivity).



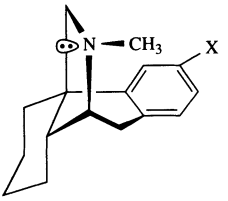
53 54

The sulfur isostere of isolevorphanol (**43**a) has also been studied (S-Me directed toward the aromatic ring, X-ray evidence).[117] In preliminary tests, the compound proved to be a potent agonist in the CNS and an antagonist in the GPI.

In the case of rigid opioids there is good evidence that a specific stereochemical orientation of the nitrogen feature in respect to the rest of the molecule (notably the aromatic group) is important for a productive interaction of the ligand with its receptor. Belleau and his colleagues first drew attention to this aspect when they discovered that the five-membered ring D analogue of racemorphan (**55**) was devoid of analgesic agonist or antagonist activities.[118] The compound was made by a general procedure described for ring-D-contracted congeners of morphinans.[119] X-ray analysis of the HBr salt revealed that its *N*-methyl substituent is directed away from the aromatic ring, while the $^{+}$N-H (and hence the lone-pair orbital in the corresponding base) points toward this structure. In morphinan analgesics with a six-membered D-ring (see **56**), and morphine and benzomorphan derivatives, the reverse stereochemistry obtains—at least in the solid



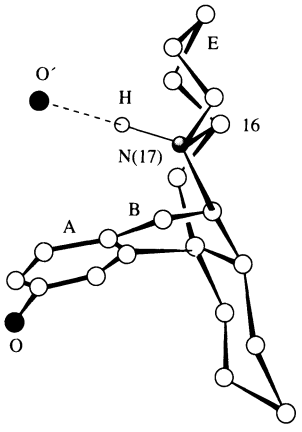
55



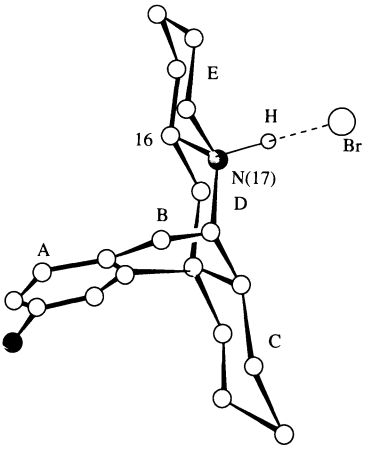
56

(one antipode shown X = OH)

state and probably in the solute condition. Belleau, in fact, attributed the inactivity of **55** to its inappropriate nitrogen lone-pair orbital orientation, but incorrect $^{+}$NH and/or $^{+}$NMe positioning could equally well be the key factor involved. A similarly ring-contracted analogue of a benzomorphan analgesic proved to be of low potency as well, but the example lacked a phenolic hydroxyl.[120] The 16,27-butanomorphinans **57** and **58** present examples of isomers of even greater rigidity



57
syn, boat ring D

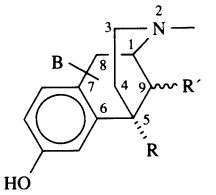


58
anti, chair ring D

that differ in their $^{+}$NH orientations; of these only the *anti*-$^{+}$NH/Ar isomer **58** had analgesic properties in mice (WR ED_{50} 3 ± 0.5 mg/kg, pentazocine ED_{50} 5.0 mg/kg.[113,121,122]

13.4. 6,7-Benzomorphans

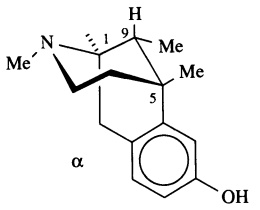
This series represents a further simplification of the morphine skeleton in which ring C is reduced to alkyl fragments (methyl and other hydrocarbon radicals).[7,8] The ring numbering employed for the group shown in **59** is that of May,



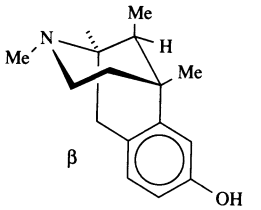
α 5-*R*, 9-*R cis*
β 5-R, 9- *R trans*

59

who first prepared these compounds[123]: C-9 is C-11 and C-5, C-6 in the *Chemical Abstracts* numbering system. Synthetic procedures lead to racemic mixtures of α- and β-diastereoisomers in which the C-5 and C-9 substituents are *cis* and *trans*, respectively, in relation to the hydroaromatic ring B. A large number of such diastereoisomers have been examined as opioid ligands together with corresponding antipodal pairs. Relative geometrics were first elucidated for the 5,9-dimethyl pair (α- and β-metazocine, N-Me **59**, R = R′ = Me); α- was assigned the *cis*-**60** and β- the *trans*-**61** configuration shown on the basis of rates of quaternization with methyl iodide (α- faster since reaction in β-isomer is hindered by axial 9-Me), and ^{1}H-NMR evidence (α-9-Me shielded by aromatic group, β-9-Me deshielded by N-lone pair).[124,125] An X-ray analysis of the *rac*-α-N-allyl derivative (**60**, N-Me replaced by C_3H_5) confirmed these assignments,[126] as did a similar examination of cyclazocine (see below).

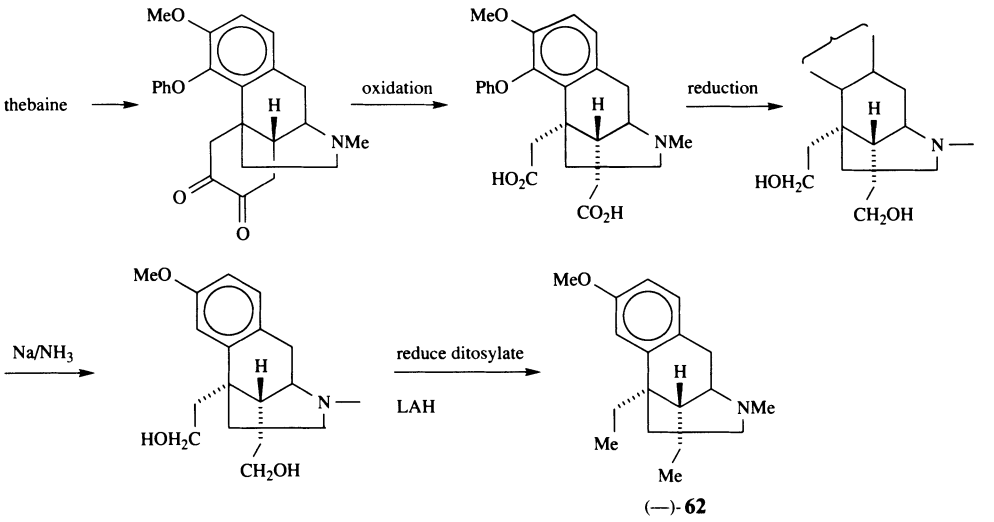


1*R*, 9*R*, 5*R*
60



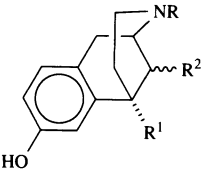
1*R*, 9*S*, 5*R*
61

The absolute configurations of (−)-α-metazocine (1*R*, 9*R*, 5*R*) and (−)-β-metazocine (1*R*, 9*S*, 5*R*) were assigned from comparisons of the ORD curves of the bases with that of levorphanol of known configuration.[127] These isomers are all eutomers (see below) which correspond in absolute geometry to related chiral centers of (−)-morphine. Prior to this work Sawa established the absolute confi-



Scheme 13.4. Conversion of natural thebaine to a 5,9-diethylbenzomorphan. [128]

TABLE 13.8.
Antinociceptive Potencies of Some Racemic 5,9-Dialkyl-N-methylbenzomorphans in Mice by the Hot-Plate Method



α R^1/R^2 *cis*

β R^1/R^2 *trans*

5-R^1	9-R^2	Isomer	ED_{50} (mg/kg)		Reference
			sc	oral	
Me	Me	α	3.0	23.9	123, 130, 131
		β	0.44	8.2	
Et	Me	α	4.9	31.7	124, 131
		β	0.07	1.1	
Me	Et	α	1.5	14.8	123, 124
		β	0.47	17.2	
Et	Et	α	4.2	—	130
		β	0.28	6.5	
Pr^n	Pr^n	α	71.2	—	124
		β	0.87	—	
Pr^n	Me[a, c]	α	2.9	72.1	124, 132
		β	0.12	4.5	
Me[a, b]	isopentyl	α	14.1	—	133
		β	1.5	—	
Morphine			2.1	3.7	

[a] More sensitive mice employed in these examples with morphine ED_{50} 1.2 mg/kg sc.

[b] K_i for displacement of [^{3}H]DAGO from rat brain slices: α 112, β 15, morphine 2.

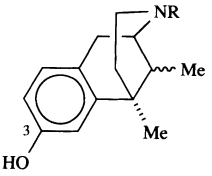
[c] Nonphenolic analogues had ED_{50} values (sc) of 11.5 (α) and 4.8 (β).[134, 135]

gurations of an antipodal pair of 5,9-diethylbenzomorphans by synthesis of the levo isomer (**62**) from thebaine, and the dextro isomer from sinomenine (Scheme 13.4).[128]

The issue of absolute geometry has been clinched by an X-ray determination of the absolute configuration of levo cyclazocine HBr (**60**, NMe replaced by CH_2c-C_3H_5) by the method of anomalous dispersion.[129]

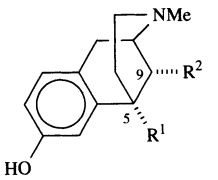
Without exception, β-diastereoisomers are substantially more potent than the α-forms as antinociceptive agents in mice by the hot-plate test (cf. *cis*- and *trans*-B/C morphines and morphinans, pages 434 and 451). Data for a series of racemic agonists are shown in Table 13.8. Differences are most pronounced when the 5-R′ group is Et or Pr^n, e.g., 5-Et, 9-Me, β 70× α; 5-Pr, 9-Pr β 82× α; they are modest for 5-Me derivatives, e.g., 5Me, 9Me (metazocines) β 6.8× α. Resolution of α- and β-metazocine by use of (+)-3-bromo-8-camphorsulfonic acid provided the N-methyl antipodes, which were converted to corresponding N-phenethyl derivatives of higher potency. Levo isomers, related in configuration to morphine and levorphanol (pages 430 and 449), were the eutomers in all cases (Table 13.9) with dextro antipodes either inactive or of low potency.[136] Most of the racemic α-diastereoisomers of Table 13.8 were subsequently resolved. In hot-plate tests performed on mice, levo antipodes exceeded dextro in potency as anticipated. However, some of the (+)-antipodes had codeine-like potencies with intermediate physical dependence capacities (PDCs)—a surprising result, since the eutomers were judged to have low liabilities in that respect. A few examples will suffice here: hot-plate ED_{50} mg/kg values: **63**a *rac* 2.1, (−) 1.2, (+) 7.9; **63**b (−) 0.8, (+) 21.8; α-**64** *rac* 0.26, (−) 0.11, (+) inactive (levo **64** differed widely from its antipode in

TABLE 13.9.
Antipodal α- and β-Metazocines and Related Compounds[136]



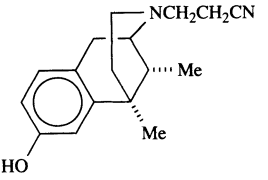
Isomer		R	ED_{50} mice hot-plate sc (mg/kg)
α-	(−)	Me	1.69
	(+)	Me	inactive
	(−) [3-OMe]	Me	8.7
	(+) [3-OMe]	Me	inactive
	(−)[b]	$(CH_2)_2Ph$	0.11 (3.9)[a]
	(+)	$(CH_2)_2Ph$	6.6 (12.9)
	(−) [3-OMe]	$(CH_2)_2Ph$	1.83
	(+) [3-OMe]	$(CH_2)_2Ph$	inactive
β-	(−)	Me	0.39
	(+)	Me	15.8

[a] Oral route.
[b] Levo phenazocine.



(a) 5,9-di-Et; (b) 5-Pr^n,9-Me

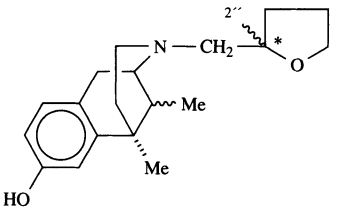
α-**63**



α-**64**

its ability to bind to rat brain opioid sites and did not substitute for morphine in monkeys addicted to this alkaloid.[93]

Merz *et al.* linked α- and β-normetazocine to the tetrahydrofurfuryl group by means of antipodal tetrahydrofurfuryl bromides of known configuration.[65,142] Among the eight stereoisomers of **65** examined, eutomers all had 1*R*,5*R* geometry while deviation from the chirality of levo α- and levo β-metazocine gave inactive products (mice tail-clip and hot-plate tests). 9*S* (β) isomers exceeded the potency of their 9*R* (α) diastereoisomers, and isomers with side chians of 2″-*S* chirality were severalfold more potent than 2″-*R* analogues (2″-configurations were revised in the 1979 paper of the series).[142] Some compounds were several times more potent than morphine, e.g., the 2″-*S*-analogue of (−)-β-normetazocine **65**, 120× morphine in the hot-plate test. Many of these compounds failed to elicit Straub tails and did not substitute for morphine in dependent monkeys—compounds of this kind were subsequently characterized as κ-ligands.[143]



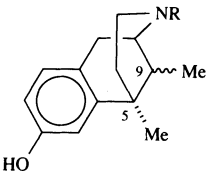
65

Binding studies of 1*R*,5*R*,9*R*,2″*S*-**65** (MR-2034) and its 2″*R*-epimer later established the greater affinity of the 2″*S*-isomer for κ-sites (vs. [^{3}H] bremazocine in the presence of DAGO and DPDPE to block μ- and δ-receptors).[274] 2″*S*-Geometry was likewise preferred in this respect for corresponding α,β-unsaturated γ-lactones and saturated γ-lactones (synthesized from intermediates of known configuration).

As in morphine and the morphinans, replacement of N-methyl of the metazocines by allyl, cyclopropylmethyl, and related groups led to antagonists and dualists. The data of Table 13.10 provide several examples and include antipodal pairs. In general, potency differences between *rac*-diastereoisomers acting as antagonists are less pronounced than those found in agonist tests, while large differences between antipodal pairs are still observed.

When Martin first proposed a subdivision of opioid receptors based on differing behavioral and pharmacological effects of opioids in the chronic spinal dog,[149] he designated *rac*-*N*-allylnormetazocine (**60**, NMe replaced by N-C_3H_5, SKF

TABLE 13.10.
Biological Data for Stereoisomeric Dualists[144, 145, 148]



α: *cis*-5,9-di-Me

β: *trans*-5,9-di-Me

R	Form	Antagonist activity[a] AD_{50} (mg/kg)	Writhing test (mice) ED_{50} (mg/kg)	GPI[d] IC_{50} (nM)
$CH_2CH=CMe_2$	α-*rac*[b]	3.9	3.1	1370
	(−)	0.9	1.4	526
	(+)	14.0	inactive (32)	25000
	β-*rac*	3.3	1.9	
	(−)	0.55	—	
	(+)	13	—	
$CH_2CH=CMe_2$	α-*rac*	10.9	4.1	
5-Et,9-Me	(−)	3.1	—	
	(+)	19.5	—	
	β-*rac*	equivocal	0.32	
CH_2c-C_3H_5 (CPM)	α-*rac*[c]	0.019	0.1	11[e]
	(−)	0.006	0.05	17
	(+)	2.5	inactive at 1	37000
	β-*rac*	0.014	> 2	
	(−)	0.005	—	
	(+)	19	—	
CH_2c-C_4H_7 (CBM)	α-*rac*[c]	0.37	0.08	
	β-*rac*	0.06	0.61	
$CH_2CH=CHCl$	α-*rac*	0.018	peak activity at 3	
cis	β-*rac*	0.047	—	
Nalorphine		0.13	0.54	

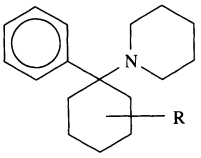
		AD_{50}, ED_{50} (mg/kg sc) in mice[145] tail-flick *vs* morphine	writhing	tail-flick	hot-plate
$(CH_2)_3CHMe_2$	α-*rac*	inactive	5.1	20.6	8.3
(isohexyl)	(−)	inactive	0.9	10.8	3.1
	(+)	inactive	16.3	inactive	70% at 100
$CH_2CH=CHCl$ *cis*	α-(−)	—	1.7	inactive	20% at 20
(also above)	α-(+)	—	55% at 30	inactive	30% at 50
$CH_2C\equiv CH$	α-(−)	0.03	0.2	inactive	50% at 20
	α-(+)	inactive	inactive	inactive	10% at 20
Morphine		inactive	0.2	5.8	1.0
Nalorphine		2.6	0.6	inactive	9.9

[a] Tail-flick *vs* morphine, mice.
[b] Pentazocine (Fortral).
[c] Cyclazocine.
[d] Inhibition of coaxially stimulated guinea-pig ileum.
[e] Kosterlitz[147] has also reported GPI ID_{50} (nM) values for cyclazocines: α-(−) 1.96, α-(+) infinite; β-(−) 12.9, β-(+) infinite. Note that the α-(−)-isomer is more potent (×6) than the β-(−)-form in this test.

10,047) as the prototype sigma (σ) ligand. This compound produced delerium in the dog and psychotomimetic effects in man that included dysphoria and hallucination.[150] Cyclazocine and pentazocine had simialr properties to SKF 10,047 (see Table 13.10 for formulas). The fact that psychotomimetic opioids elicit their opioid and psychic actions at different sites has been established by observations that the psychic effects of SKF 10,047 reside in the *dextro* isomer as judged by behavioral effects of the two antipodes in squirrel monkeys and rats.[151,152]

Dextro SKF 10.047 (48), pentazocine (18) and cyclazocine (36) all effectively displaced (+)-[^{3}H] SKF 10,047 from binding sites in guinea-pig brain and striatal membranes, while levo SKF 10,047 (1800) was only a feeble competitor ($K_1 \times 10^9$ M values in parentheses).[150,153]

The ability of σ-agonists to bind to sites labeled by tritiated 3-PPP (Chap. 6, page 185), which has nM affinity for σ-sites[153,154] and phencyclidine (**66**,



66 PCP (R = H)

PCP),[155–157] exacerbates the problem of identifying their sites of action (see also work on DM, page 451). IC_{50} (nM) values for the displacement of (+)-[^{3}H]3PPP from rat brain membranes were: cyclazocine *rac* 395, (+) 365, (−) 1440—these show levo forms to be the least effective.[154]

Some data for displacement of sites labeled by (+)-[^{3}H]pentazocine are shown below.[154]

Ligand	IC_{50} (nM)
(+)-Pentazocine	5.0
(−)-Pentazocine	136
(+)-SKF 10,047	97
(+)-3-PPP	48
PCP	2871
Haloperidol	5.3

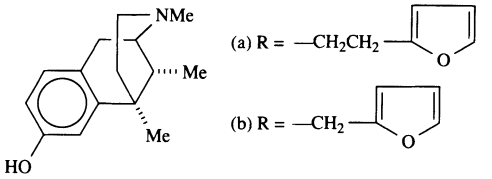
Sensitivity of the (+)-pentazocine site to haloperidol characterizes it as a σ-site[158]—PCP sites have low affinities for neuroleptic agents of this kind.

Dissymetric forms of PCP have been obtained by methylation of its cyclohexane ring and differences in the binding affinities of 2-Me and 4-Me *cis*/*trans* pairs recorded.[156] The dextro antipode of the chiral 3-methyl piperidine derivative retained 23%, and the levo form 4.1% of the affinity of PCP for sites labeled by [^{3}H] **66**.[157]

This work has recently been extended to 2-methylcyclohexane analogues; in binding assays vs. [^{3}H] TCP the 1*S*,2*R*-(-)-*trans*-derivative was five times and the (+)-antipode half as effective as PCP, while *cis*-forms had very low affinities.[277]

Merz and others followed up their work on *N*-tetrahydrofurfuryl agonists (page 459) by examining corresponding aromatic (2-furfuryl) derivatives.[143] The

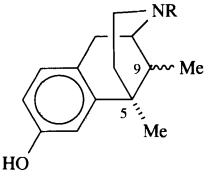
2-(2-furyl)ethyl derivative **67**a is a potent analgesi (30× morphine)[160] but the lower homologue **67**b is an antagonist as potent as nalorphine.[91] The activity profile of **67**b may be varied by simple structural change (Table 13.11). In all cases the levo isomer is the eutomeric form. When racemic α/β pairs were compared, α-diastereoisomers exceeded their β-partners for both agonist and antagonists (with one case of equipotency; see footnote *a* to Table 13.11).



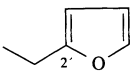
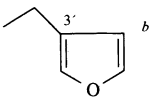
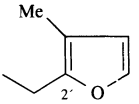
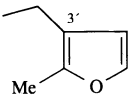
α-**67**

Although methyl is generally regarded as the N-substituent most commonly associated with pure agonism, unusual antagonist properties have been reported for

TABLE 13.11.
Some N-2- and N-3-Furfuryl Benzomorphans[162]

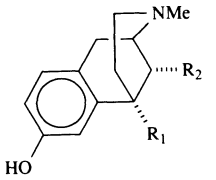


α-isomers (β-data in parentheses)

R	Form	Analgesic ED_{50} (mg/kg sc) mouse writhing test	Antagonist AD_{50} (mg/kg sc) *vs* morphine tail-clip test
	*rac*	18	1.0 (3.0)[a]
	(−)	—	0.5
	(+)	30	30
 [b]	*rac*	—	1.0 (1.0)
	(−)	—	0.1
	(+)	—	30
	*rac*	0.6 (3.2)	—
	(−)	0.35	—
	(+)	—	10
	*rac*	1.8 (11.0)	20
	(−)	0.7	—
	(+)	—	—

[a] Values for β-racemic mixtures in parentheses.
[b] The levo antipode of the 5,9-diethyl analogue of this compound is the potent antagonist Mr2266.

certain *N*-methylbenzomorphans. Thus, levo isomers of the benzomorphans **68**a–d, although displaying antinociceptive actions in mice (hot-plate test), failed to substitute for morphine in dependent monkeys and gave evidence of antagonist behavior in precipitating abstinence syndromes.[137–139] In the case of **68**e, the racemic mixture and antipodal forms all showed antagonist activity of this kind while corresponding forms of **68**f did not, although none could substitute for morphine.

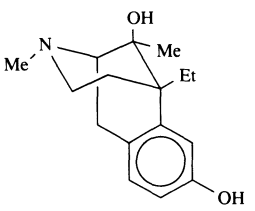


68 (–)-isomer

	a	b	c	d	e	f
R_1	Et	Me	Et	Pr″	H	Me
R_2	Et	H	H	Me	H	Pr″

13.4.1. 9-Hydroxy Derivatives

Prior to interest in naloxone and related 14-hydroxymorphines, May investigated a series of 9-hydroxybenzomorphans (C-14 of the former ≡ C-9 of the latter group.[163] Pairs of compounds diastereoisomeric about C-9 were obtained by stereoselective reactions of 9-keto precursors, and configurations were based on chemical transformations and IR evidence (spectra of 9β-OH derivatives displayed a band due to intramolecularly H-bonded OH; see page 452). Potentiation of antinociceptive activity was not achieved by hydroxylation at C-9. The most active member of the series was the 9β-OH derivative **68a** (mice hot plate ED_{50} mg/kg sc 1.7, morphine 2); the 9α-OH analogue was less effective (ED_{50} 6,7 mg/kg).[163b] Members of the α/β pair of the 5,9-dimethyl analogue of **68a** (5-Et) showed the same potency order (ED_{50} mg/kg 9α-OH 6.91, 9β-OH 6.03) but in a smaller degree.

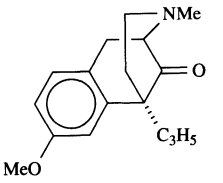


68a

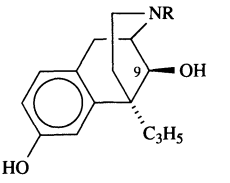
Albertson[164] examined some 9-OH benzomorphan pairs with antagonist properties. Substitution with 9β-OH/9α-Me raised the activities of both pentazocine (× 3) and cyclazocine (× 10) as antagonists of pethidine, while 9α-OH had depressant influences in this respect. MS evidence was used to supplement assignments of configuration.[165]

Some potent dualists have been derived from the 5-allyl-9-oxobenzomorphan **69** by selective reduction of the carbonyl function which introduces a β-OH at C-9. The *N*-cyclopropylmethyl and *N*-cyclobutylmethyl analogues **70** were effective agonists in mice (writhing test) and antagonists of oxymorphone with **70**a the more

potent in both respects.[166] A 9α-methyl group abolished the analgesic properties of **70a** but enhanced its antagonist action which proved to be highly stereoselective (levo >500× dextro). The 9α-methyl derivative of **70b**, in contrast, was somewhat more potent than its parent both as agonist and antagonist.



69

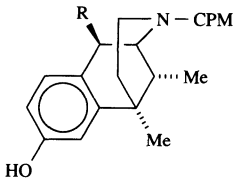


(a) R = CH_2c-C_3H_5; (b) R = CH_2c-C_4H_7

70

In a subsequent paper 9α-hydroxy analogues were reported[114]—these proved 3 to 7 times less active than the 9β-OH epimers, e.g. **70a** ED_{50} mg/kg mice writhing α 0.23, β 0.08; vs. oxymorphone, Straub tails α 1.9, β 0.5.

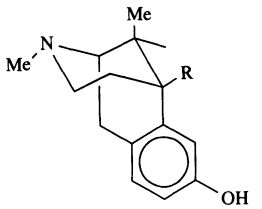
Benzylic hydroxylation at C-8 of the benzomorphan nucleus has been reported but only biological data on 8β-OH derivatives (**71**) presented[168]—these generally fell well below the potencies of parent compounds. The 8β-OH configuration was established by an intramolecular transesterification reaction between 8-OH and NCO_2Et of a related derivative.



71

	mg/kg	
	ED50 (writhing)	AD50 *vs* pethidine
R = H (cyclazocine)	0.15	0.02
R = β-OH	10	11

Clarke *et al.*[169,170] modified the much used Grewe-type synthesis to produce the 9-methyl-5-phenyl derivative **72**; only one diastereoisomer resulted and this had the absolute configuration shown (X-ray crystallography of levo *O-p*-bromobenzoate). Its hot-plate potency in mice approached that of β-metazocine and most of



72

R-Isomer	ED_{50} (mg/lg sc) mice hot-plate
Me *rac*[a]	0.44
Ph *rac*	0.5
(−)[b]	0.18
(+)	3.4
morphine	1.2

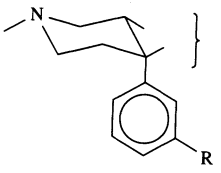
[a] *rac*-β-metazocine.
[b] GPA1657.

its activity lay in the levo antipode. The dextro isomer partially antagonized morphine analgesia in mice and precipitated an abstinence syndrome in morphine-dependent monkeys (cf. other N-Me derivatives, page 463). Analogues of levo-**72**

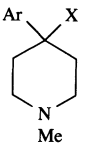
(R = Ph) with typical antagonist N-substituents (CH_2c–C_3H_5 etc.) all blocked morphine analgesia and behaved as weak to moderately potent agonists in mice by tail-flick and writhing tests, except the N-propargyl (N-$CH_2C{\equiv}CH$) member which proved to be a pure antagonist in guinea-pig ileum (it also showed no ability to inhibit adenylate cyclase in hybrid cells).[171] Several more antipodal pairs related to **72** were later reported[172]—levo isomers were the eutomeric forms in all tests applied.

13.5. 4-Arylpiperidines

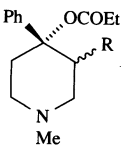
Compounds which include the 4-arylpiperidine fragment **73** of morphine (**3**) form a major group of opioid ligands and provide much data of stereochemical interest. The group as a whole may be subdivided into derivatives with carbon substituents at C-4 such as pethidine **74**a (meperidine) and ketobemidone **74**b, and those with oxygen substituents such as the reversed ester of pethidine **75** (R = H)



73



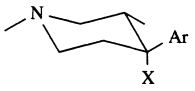
74



75

(a) Ar = Ph, X = CO_2Et; (b) Ar = *m*-OH C_6H_4 X = COEt; (c) Ar = *m*-OH C_6H_4 X = Me

and the prodines **75** (R = Me). Antinociceptive activity of the former group is enhanced by the presence of a *m*-phenolic substituent in the 4-aryl group[173] while that of the oxygenated derivatives **75** is depressed.[174,175] This unusual difference in structure–activity relationships may be explained by postulating that the active conformation of pethidine and its C-4 carbon analogues is an axial 4-aryl chair **73** (see above) thereby mimicking the 4-arylpiperidine fragment of morphine (**3**), while that of the C-4 oxygen group is an equatorial 4-arylchair **76**. From an energetic point



76

of view these proposals are supported by the computational studies of Froimowitz,[176] who showed that the axial 4-aryl chair conformers of pethidine and ketobemidone free bases differ by only 0.6–0.7 kcal mol^{-1}, while those of the reversed esters **75** have substantially higher energies (1.9–3.4 kcal mol^{-1}) than conformers with equatorial aryl substituents. Physical evidence of the conformational equilibra of hydrochloride salts of pethidine, ketobemidone, and related reversed esters is available from analyses of their ^1H- and ^{13}C-NMR spectra.[177] The data show that, apart from the reversed esters, *both* N-protonated epimers are significantly populated when the salts are dissolved in D_2O. The *eq* 4-arylchair con-

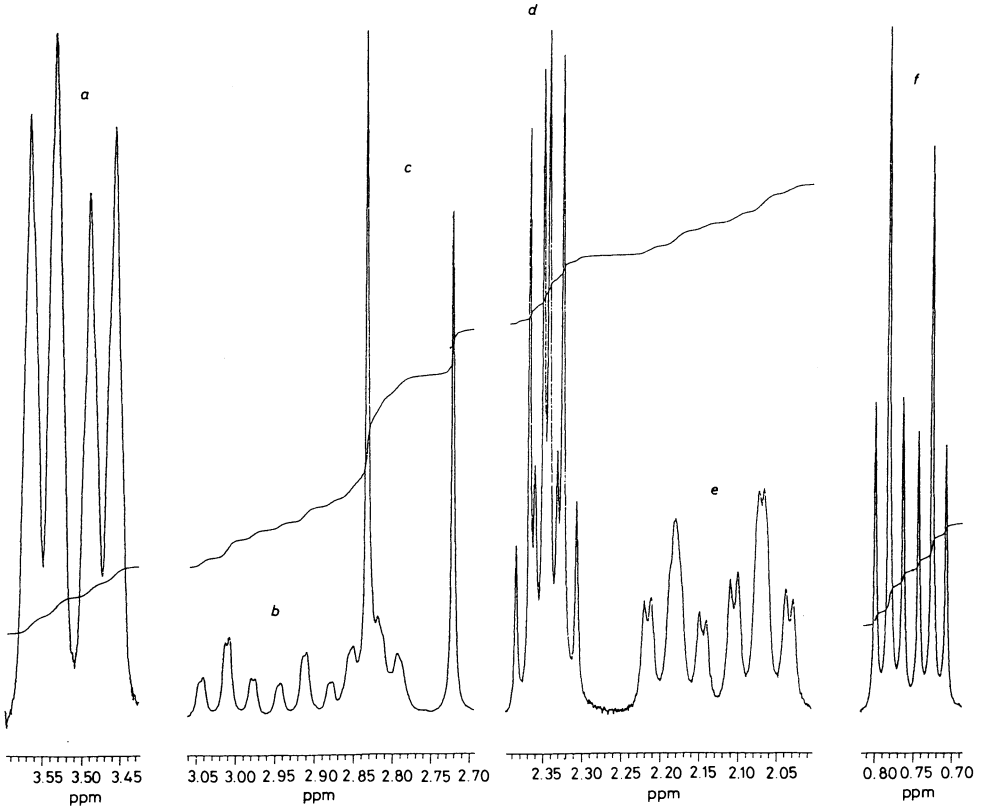
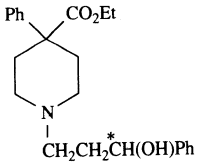


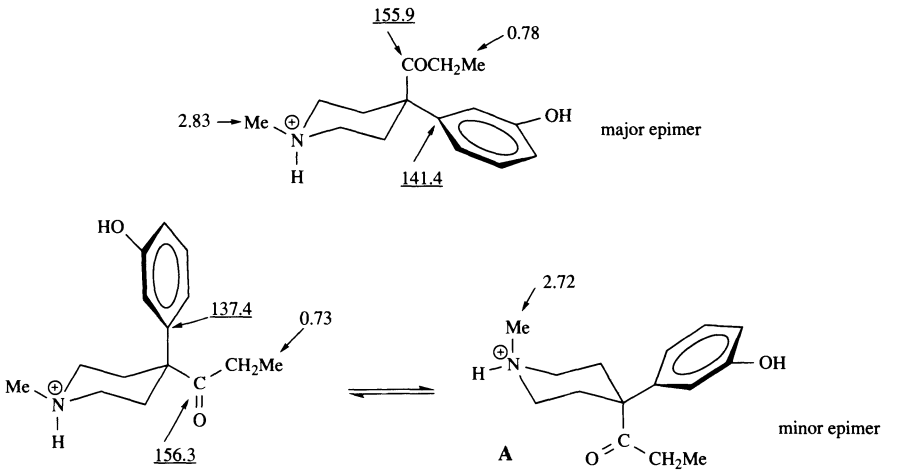
FIGURE 13.2. Parts of the 400-MHz ^{1}H-NMR spectrum of ketobemidone HCl (**74b**) in D_2O showing epimeric signals due to (a) eq 2,6-H; (b) ax 2,6-H; (c) N–Me; (d) OCH_2Me; (e) ax 3,5-H; (f) OCH_2Me (after Casy *et al.*).[177] Differing degrees of expansion have been used.

former is the major epimer of pethidine and ketobemidone, but the minor form in the case of the 1,4-dimethyl derivative **74c**. Some of the duplicate resonances seen in the spectrum of ketobemidone HCl are shown in Fig. 13.2. A few assignments to major and minor epimer signals are presented in Scheme 13.5; thus the higher-field N-Me signal of the minor epimer is attributed to the contribution of invertomer **A** which carries an axial N-methyl substituent (in general, axial N-methyl protons resonate to high field of related equatorial protons in piperidine derivatives),[178] while an axial carbon has a higher-field chemical shift than its equatorial counterpart as a result of steric polarization.[179] In the solid state, pethidine HCl and HBr salts adopt the equatorial 1-methyl-4-phenyl chair conformation.[180,181]

In contrast to reversed esters, not many chiral variants of pethidine itself have been described. Phenoperidine **77** (Operidine), an analogue in clinical use with a



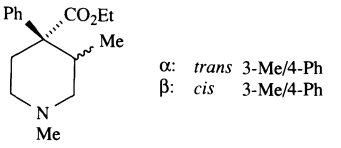
77



Scheme 13.5. N-Protonated epimers of ketobemidone: ^{1}H- and ^{13}C-NMR assignment (^{13}C values underlined).

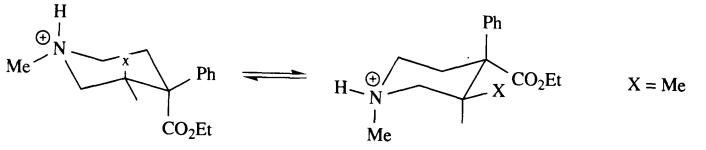
chiral N-substituent, has been resolved and its antipodes compared.[182] There was a modest (fourfold) activity difference between *R*-(+)- and *S*-(−)-enantiomers that probably reflects pharmacokinetic rather than receptor events, since both isomers possess high levels of potency (the weaker dextro antipode was seven times as active as morphine in mice).

3-Methyl analogues of pethidine **78** have been isolated in *rac*-α- and β-diastereoisomeric forms.[183] In the mouse hot-plate test, ED_{50} values (mg/kg sc) were: pethidine 4.7, α-**78** 3.6; β-**78** 0.42. These results were in accord with activity



78

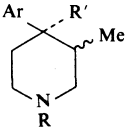
rankings among the reversed ester of pethidine and its 3-methyl derivatives, discussed later (page 478). Relative configurational assignments were based on differences in chemical shifts between diastereoisomeric proton groups (aromatic shielding of $COCH_2Me$, 3-Me deshielding by protonated nitrogen)—more direct evidence from ring proton signals was unavailable at the time of this work; cf. 400-MHz analyses of pethidine and ketobemidone described above. It should be noted that 3-methyl *cis* to 4-phenyl should raise the population of the axial 4-arylpiperidine conformer (**79**).



79

Following McElvain and Clemens's demonstration of antinociceptive activity among 4-arylpiperidines carrying a 4-alkyl substituent (a *m*-phenolic substituent was an essential feature),[184] Lilly workers examined a range of such compounds made chiral by the presence of a 3-methyl substituent.[185] Their data are shown in Table 13.12. Of special interest was the fact that the presence of 3-methyl *cis*-orientated to 4-aryl resulted in an opioid antagonist rather than agonist. Thus the β-(*cis*)-3,4-dimethyl derivative (Table 13.12, entry 1) was half (mice) or twice (rats) as active as nalorphine and lacked agonist properties, while the corresponding α-(*trans*)-isomer behaved as a low-potency dualist (entry 2). Results for 4-propyl diastereoisomers were less clear-cut; the α-form was primarily an agonist (like the 3-desmethyl derivative, although less potent) and the β-form was a significant antagonist in rats but a weak agonist in mice (entries 3–5). N-Substituents associated with morphine blockade in polycyclic opioids like allyl and cyclopropylmethyl depressed the antagonist potency of the β-1,3,4-trimethyl derivative somewhat in rats, while 2-phenethyl and 2-benzoylethyl raised activity (to the level of naxolone in the last case) with some evidence of receptor preference for the dextro antipode (entries 6–9). Antagonist properties were also recorded for β-3-methylketobemidone and the phenolic analogue of betaprodine, 50 and 16 times less active than nalorphine, respectively.

TABLE 13.12.
Agonist and Antagonist Activities of Some 4-*m*-Hydroxyphenyl-4-alkylpiperidines[185]

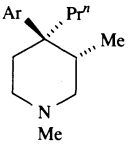


α: *trans* 3-Me, 4-Ar
β: *cis* 3-Me, 4-Ar
Ar = *m*-OH.C_6H_4

Entry	*N*-R	4-R′	Isomer	Antagonist AD_{50}[a]		Agonist measure	
				rats	mice	rats (ED_{2s})[b]	mice (ED_{50})[c]
1	Me	Me	β	0.24	1.0	> 50	> 50
2	Me	Me	α	13	45	33	15
3	Me	Pr	β	4.6	43	> 50	13
4	Me	Pr	α	Additive	45	2.0	2.4
5	Me	Pr	(des 3-Me)	Additive	—	0.89	0.85
6	C_3H_5	Me	β	0.47	0.98	—	—
7	CPM	Me	β	0.72	0.72	—	—
8	$(CH_2)_2Ph$	Me	β	0.11	0.14	—	—
9	$(CH_2)_2COPh$	Me	β(±)	0.056	0.049	—	—
			β(−)	0.05	0.14		
			β(+)	0.023	0.025		
Nalorphine				0.4	0.45	> 50	1.0
Naloxone				0.022	0.079	—	—
Morphine				—	—	1.8	0.97
Pentazocine				20	14	—	—

[a] Dose (mg/kg, sc) required for a 50% reduction in the response to morphine in rats (tail heat) and mice (Straub tail and locomotion).
[b] Dose required for a 2 s increase in reaction time in rat tail heat test.
[c] Dose required for a 50% reduction in the frequency of writhing.

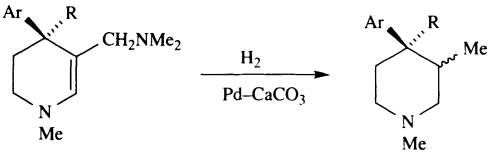
In further tests the α-1,3-dimethyl-4-propyl analogue **80** (LY 150720 picenadrol) behaved essentially as an agonist with about half the potency of morphine and its 3-desmethylparent with most of its activity residing in the dextro antipode, Surprisingly, the levo isomer (a weak agonist in mice) showed distinct antagonist properties with a potency between that of nalorphine and pentazocine.[186]



Ar = 3-OHC_6H_4 in this and subsequent formulas

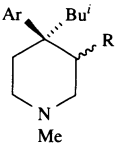
80

Picenadrol and its relatives also featured in a study carried out at Bath[187] which included evidence of relative stereochemistry and conformation. 3-Methyl diastereoisomers **82** were obtained by catalytic reduction of the Mannich bases **81** and separated by fractional crystallization of hydrochloride salts as either 3-methoxyphenyl or 3-hydroxyphenyl derivatives. (A stereospecific synthesis of the α-diastereoisomer, picenadrol, from 1,3-dimethyl-4-piperidone was recently reported).[188] NMR evidence of configuration is outlined below.



81 (a) R = Me; (b) R = Pr^n; (c) R = Bu^i **82**

In the tail-withdrawal test (rats) both the 4-isobutyl derivative **83**a and its 3α-methyl analogue proved as effective as the 4-*n*-propyl variant of **83**a (2–3 × morphine), while the 3β-methyl derivative **83**c was only a feeble agonist in this test. In mice, by the tail-flick procedure ED_{50} values (mg/kg) were: **83**a 0.8, **83**b(α) 2.3, **83**c(β) 17.6. Data obtained by *in vitro* methods complemented these findings (binding vs. [^{3}H]etorphine, and mouse vas deferens preparation). Attention was

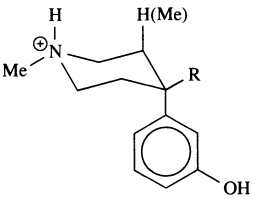


(a) R = H; (b) R = α-Me (*t* to 4-Ar); (c) R = β-Me (*c* to 4-Ar)

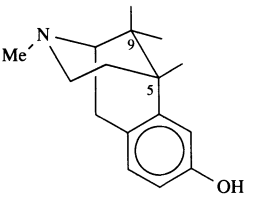
83

drawn to the fact that all potent 4-arylpiperidines of this class exhibit a preference (50% or above) for axial 4-aryl chair conformations **84** when protonated, as established from NMR evidence (see below). These results support the view that 4-arylpiperidine ligands bind to the opioid receptor in a manner similar to that of morphine and its congeners and mimic the geometry of the 4-arylpiperidine moiety

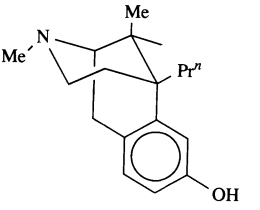
of polycyclic molecules. The most direct analogy is with benzomorphan analgesics **85** as pointed out by Loew *et al.*[191] Both 9-methyl-5-*n*-propyl diastereoisomers **86** (β-shown) were active (β 10×, α 0.5× morphine, mouse hot plate)[130,132] (page 457). The active 4-arylpiperidine α **84** (R=Pr^n) and **84** (R=Bu^{iso}) closely mimic the geometry of β-**86** in one of their preferred solute conformations in the protonated state. To support this interpretation it would be of interest to establish the absolute configuration of (+)-picenadrol (potency 10× levo isomer and equipotent to morphine)[186]—it should correlate with eutomeric benzomorphans.



84

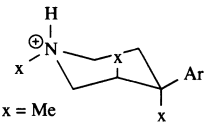


85



86

The only compound found to display prominent activity as an opioid *antagonist* was the β (*cis*) diastereoisomer **87**, which reversed all actions of fentanyl in rats at dose levels of 2.5 and 0.63 mg/kg and effectively countered respiratory depression at even lower dose levels (approaching the potency of naloxone). The compound was also characterized as an antagonist in the mouse vas deferens with a pA_2 of 6.91 and *vs* morphine in the tail flick test. The corresponding α (*trans*) 3,4-dimethyl diastereoisomer **84** (R=Me) was a much weaker antagonist *vs* fentanyl in rats, while neither isomer behaved as an agonist in the tail withdrawal test. Results for β-**87** confirm the earlier report of its antagonism of morphine in rats and mice.[185] Both β-**82** (4-Pr^n) and β-**82** (4-Bu^{iso}), steric analogues of β-**87** displayed weak antagonist action in the mouse vas deferens while the 4-isobutyl derivative reversed fentanyl-induced respiratory depression in one out of three rats at a dosage of 2.5 mg/kg.



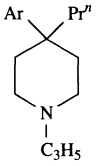
87

Following reports that naloxone decreased food and fluid intake in rats,[189] Zimmerman examined a large series of β (*cis* 4-Ar/3-Me) derivatives related to **87** as antagonists of κ-diuresis (induced by bremazocine) and in a food consumption test using obese rats (Zimmerman private communication). Some potent appetite suppresant agents were discovered, notably LY255582 [**87** with N-Me replaced by $CH_2CH_2CH(OH)C_6H_{11}$], which proved 28 times more effective than naloxone in the food consumption test, and was almost ten times the more potent antidiuretic. None of the series had measureable opioid agonists properties.

LY255582 is, in fact, a single antipodal form of one of 8 possible stereoisomers, obtained by a synthesis utilizing stereoselective steps.[190] X-ray

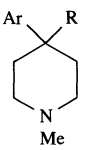
analysis confirmed its absolute geometry as 3*R*,4*R*,3′*S* (3′ refers to the chiral center of the N-substituent). In the mouse writhing assay it antagonized the analgesic effects of morphine (1.0 mg/kg sc) with an AD_{50} of 0.015 (cf. 0.13 mg/kg for naloxone).

Opioid test observations indicate that the binding modes of antagonists differ sharply from those of agonists in this series, a factor which overrides the influence of the N-substituent. Thus β-**87**, an N-methyl derivative, is an antagonist while the N-allyl analogue **88** (axial 4-aryl chair probably preferred) is half as active as morphine as an agonist and only a feeble antagonist.[191]

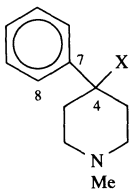


88

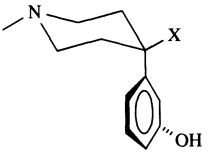
The stereochemistry of this series has been established by computational and NMR methods. Energy-conformational calculations[191] show that the derivatives **89** (R = Me, Pr^n, Bu^t) all favor axial-aryl chairs over equatorial-aryl chairs and all twist-boat forms—most pronounced for the 4-*t*-butyl member (in the solid-state this compound exists as the *ax*-aryl chair). The torsion angle τ ($C_8H_7C_4X_4$, see **90**) is close to 90° in low-energy conformers, indicating that the aromatic and piperidine rings are approximately orthogonal **91** when 4-aryl is axially disposed, i.e., distinct from the geometry of the 4-arylpiperidine moiety of morphine where τ ≅ 0° (see **92**). Addition of 3-methyl to the 4-*n*-propyl derivative favors the *ax*-aryl



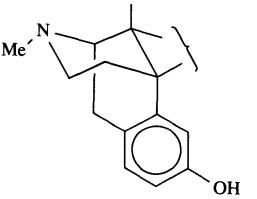
89



90

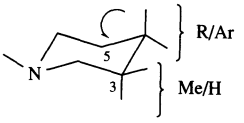


91
end-on view
of Ar ring



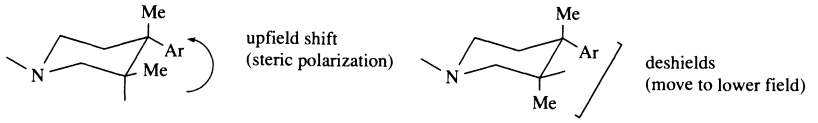
92 (partial)

chair by 0.7 kcal/mol in the case of the α-diastereoisomer, but the eq-Ar chair in the case of the β-diastereoisomer (Δ *E* 2.2 kcal/mol). NMR evidence for the conformational equilibria of the 3-desmethyl derivatives **89** (4-Me, Pr^n, Bu^{iso}) followed that already outlined for pethidine and ketobemidone.[192] In the case of 3-methyl diastereoisomers, evidence of 3-methyl orientation was obtained from comparative ^{13}C chemical shifts of C-5 of major and minor isomers; when 3-Me is axial the C-5 chemical shift moves upfield as a result of steric polarization (see **93**).[179]



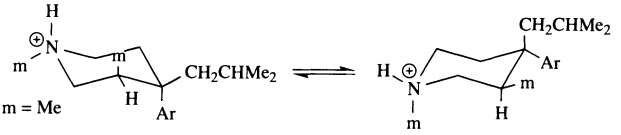
93

Assignment of the 4-alkyl/4-aryl conformational preferences of diastereoisomeric pairs was based on NMR features of Cq (C-1′) and 4-CH_2R signals, together with knowledge of the influence of vicinal *cis*-**94** and *trans*-**95** methyl on



94 **95**

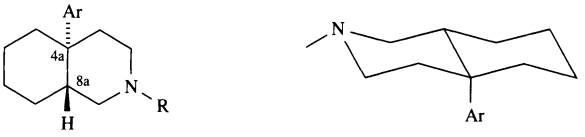
the ^{13}C chemical shift of axial 4-methyl.[193] Spectra of the α(*trans*)4-*n*-propyl and 4-*i*-butyl salts in D_2O displayed duplicate resonances characteristic of binary epimeric mixtures (equipopulated for 4- Pr^n, major and minor 2:1 for 4-Bu^i). Signal assignments to epimeric pairs were possible, aided by COSY spectra, and data analyzed in terms of *trans*-4-Ar/3-Me (α) conformations. For example, the principal epimer of the α-4-isobutyl compound was identified as **96** on the basis of its N-Me and H-3 chemical shifts (H-3 is deshielded by 4-Ar in **96** and has a notably low-field resonance) and ^{13}C-NMR features, especially those of C-5, 3-Me, and 4-CH_2CHMe$_2$. In **96** the 4-isobutyl protons fall withing the aromatic shielding zone as the group rotates about its bond to C-4, a consideration which accounts for the greater intensity of the higher-field pair of terminal methyl resonances in the spectrum (Fig. 13.3) The minor epimer is **97**.



96 major **97** minor

Results found for 2,5- and 3,6-dimethyl derivatives of **74c** (4-Me), disclosed in CPDD Proceeding Reports,[186] are summarized in a 1986 monograph.[8]

Trans-4a-Aryldecahydroisoquinolines **98** are conveniently mentioned here because they represent a 4-arylpiperidine fragment in which the aromatic group is forced to adopt an axial conformation **99** as a result of ring junction constraints.



98 4a*R*, 8a*R*-isomer shown **99** Ar = *m*-OHC_6H_4

Antinoceptive activity and receptor affinity data for two resolved examples are shown in Table 13.13. The absolute configuration of the eutomers dextro **98** (R = Me) (2.3 × morphine) and levo **98** (R = CH_2c-C_3H_5) (0.2–0.4 × morphine) was 4a*R*,8a*R* as shown, identical with that of corresponding chiral centers of morphine. The N-methyl derivative was μ- and the N-cyclopropylmethyl derivative κ-selective from binding data; only the latter compound antagonized the analgesic

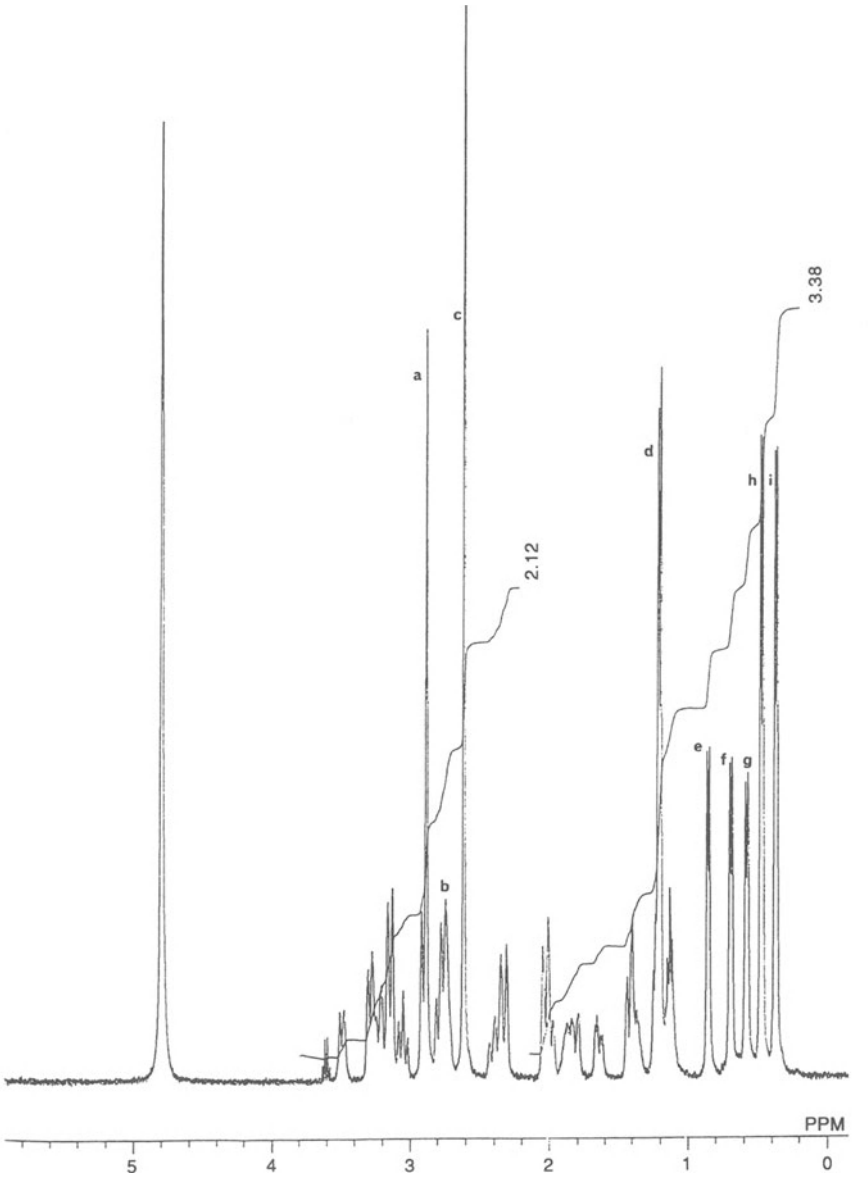


FIGURE 13.3. Part of the 400-MHz ^{1}H-NMR spectrum of *trans*-1,3-dimethyl-4-(3-hydroxyphenyl)-4-isobutylpiperidine (α-**83**b) hydrochloride in D_2O. Annotated epimeric resonances: (a, c) N−Me; (b) multiplet including major 3-H; (d) major 3-Me; (e, f) minor $CH_2CH\mathit{Me}_2$; (g) minor 3-Me; (h, i) major $CH_2CH\mathit{Me}_2$ (after Casy *et al.*).[192]

effects of morphine in the rat tail heat test—it was equipotent with nalorphine. The stereochemistry was established by X-ray crystallography of the D-mandelic acid salt of the precursor base **98** (Ar = $MeOC_6H_4$, R = H).

Although these derivatives mimic the 4-arylpiperidine moiety of morphine, the aromatic ring is unlocked from rings C and D by the absence of links to C-9 and C-5 (see **99**) and is free to rotate about its bond to the bicyclic nucleus. Potency

TABLE 13.13.
Biological Data for Some *trans*-4a-Aryldecahydroisoquinolines[194]

Compound	Form	Mouse writhing ED_{50} mg/kg sc	Rat tail heat ED_{50} mg/kg sc	Binding assays K_i (nM)[a]	
				vs [^{3}H]naloxone	*vs* [^{3}H]EKC
98 (R = Me)	*rac*	0.5	0.4	—	—
	(−)	2.1	1.6	8.9	433
	(+)	0.4	0.3	0.96	61
98 (R = CH_2c-C_3H_5)	*rac*	3.3	5.6	—	—
	(−)	2.2	3.0	4.2	2.5
	(+)	50	50	—	—
Morphine		0.9	0.7	—	—

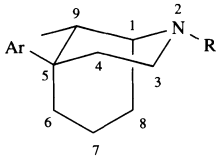
[a] Rat brain homogenates, EKC = ethylketocyclazocine.

losses are not seen, but a marked fall in receptor stereoselectivity is apparent from the eudismic ratio of just above 5 found for the N-methyl antipodes.

A Glaxo group has reported variants of **98** including a highly potent (μ-selective) 8-methyl-6-exocyclic methylene-*N*-cyclopropylmethyl derivative.[277]

13.6. Arylmorphans

These compounds may be regarded, like 6,7-benzomorphans, as sterically constrained 4-arylpiperidines except that the aromatic ring has an *equatorial* rather than *axial* conformation. The racemic mixture **100** (R = Me) was equiactive with



100
(+)-1*S*,5*R*-antipode shown

morphine in mice by the hot-plate test while, of its two antipodes, the dextro isomer was the more active (4 × *rac*-mixture). The weaker levo antipode was nevertheless an effective antinociceptive agent with a potency close to that of morphine. It also behaved as a nalorphine-like antagonist [as suggested by the (+): *rac* potency ratio >2] of modest potency (it caused precipitation of an abstinence syndrome in dependent monkeys at a dose of 8 mg/kg.[195,196] The configuration of the dextro eutomer (1*S*,5*R* by X-ray analysis of levo HBr salt)[197] shown in **100** indicates a relationship to 4-arylpiperidine analgesics such as α- and β-prodine (page 478) rather than (−)-metazocine **60** (and sterically related morphinans and morphines). This conclusion is supported by the fact that analogues of **100** with N-propyl, allyl, and cyclopropylmethyl substituents behaved as agonists rather than antagonists (*rac*-mixtures and dextro antipodes were examined).[198]

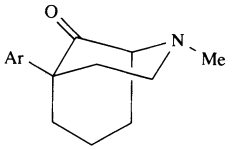
Insertion of methyl in the 9α-position of **100** (R = Me) gave a product which, in racemic mixture form, had about one-tenth the activity of morphine as agonist

(mouse hot-plate) and half that of nalorphine as antagonist; agonism was traced to the levo- and antagonism to the dextro-antipode[199a]; cf. antipodes of picenadrol (page 469).

The 9α-hydroxy analogue of *rac*-**100** (R = Me) was about twice (tail-flick), equi- (writhing), and half (hot-plate) as active as mophine in mice by the procedures indicated, while the 9β-OH isomer had low or nil potencies in these tests.[199b]

The (+)-9α-methyl derivative and the levo antipode corresponding to **100** (both antagonists) shared 1*R*,5*S* configurations (X-ray evidence).[278]

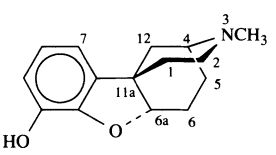
The 9α-methyl derivative was obtained from the keto intermediate **101** by



101

reduction of the Wittig product (9-methylene); NMR evidence of configuration was provided from aromatic and $Eu(fod)_3$ shift reagent shielding considerations. Catalytic reduction of the same intermediate in base form gave the 9α-OH product while use of the methiodide led to the 9β-OH epimer; there was also IR evidence of configuration—a band associated with the intramolecular N:...H-O bond was seen only in the spectrum of the 9β-epimer.

Preparation of a series of 5-arylmorphans in which the aromatic and piperidine rings are conformationally restrained is in hand by an NIH group. This work was prompted by the claimed importance of the torsion angle between these two rings being a factor affecting antipodal potency differences among chiral 4-phenylpiperidine analgesics.[203] So far, Ar of **100** has been linked via oxygen to position 6a (equatorial) and 9a (equatorial and axial) giving products **102–104**.[200–202]



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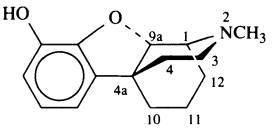
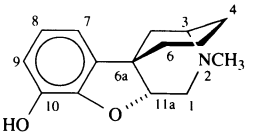


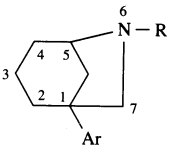
103
After Burke *et al.* [200 - 202]



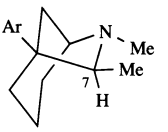
(11a ≡ 9a of **103**)
104

Torsion angles between the aromatic and piperidine ring plane (e.g., measured through atoms 1,11a, Cq, and 7; see **102**) were 86° for **102**, 8° for **103**, and 49° for **104** as determined by X-ray analysis. Compound **102** lacked *in vivo* agonist or antagonist activity while **103** had only a low affinity for rat brain opioid sites. Opioid receptor affinity for **104** was also low (IC_{50} 1000 nM). Evidently, ring orientations in all these restricted analogues are unsuitable for ligand uptake at opioid receptors—perhaps better success will result when other members of the series are examined.

Analogues of **100** with a five-membered heterocyclic ring (**105**) have also been examined.[204] Certain *rac*-6-methyl and 6,7-dimethyl derivatives displayed antinociceptive activity in mice which was attributed to the (+)-isomers **106** (levo



105



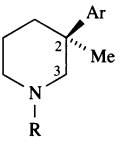
106

antipodes were antagonists). An X-ray analysis of (+)-**106** with a 7-methyl substituent (*endo*) has established a configurational relationship with (+)-**100** [arylmorphan]. There was no activity difference between 7-*endo*- and 7-*exo*-methyl derivatives.

13.7. 3-Arylpiperidines

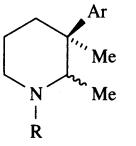
Discussion of this group follows logically from that of the N-arylpiperidines, since both possess alkyl substituents at the benzylic carbon center rather than acyloxy (OCOR) as present in reversed esters of pethidine.

All active derivatives of 3-aryl-3-methyl piperidines **107** are phenols (Ar= *m*-OHC_6H_4) and some behave as antagonists when carrying an N-allyl or

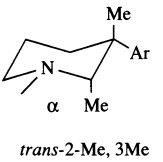


107 Ar = *m*-OHC_6H_4

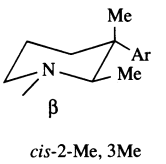
N-cyclopropylmethyl group—in this they resemble the morphine group but differ from 4-alkyl-4-aryl piperidines. Antinociceptive activity is low in N-methyl derivatives but substantial when N-arylalkyl substituents are present (p. 279 of Ref. 8). Higher potencies were also attained when a 2-methyl substituent was present; one diastereoisomer of the 2-Me, N-CH_2COPh derivative was half as active as morphine in mice by the hot-plate test while the N-allyl analogue lacked agonist action but antagonized morphine.[205,206] Further work established the stereochemistry of α- and β-2,3-dimethyl derivatives **108** (^{1}H-NMR evidence)[207] and provided further pharmacological data.[208] N-Phenethyl isomers (**108**) were



108
Ar = *m*-OHC_6H_4



trans-2-Me, 3Me

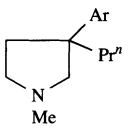


cis-2-Me, 3Me

significantly active as analgesics in mice (α, 0.7; β 0.3 to 0.4 times pethidine) and in the GPI test[209] while N-allyl and related N-substituted analogues were inactive. In rats, the N-allyl and N-cyclopropylmethyl derivatives antagonized fentanyl-

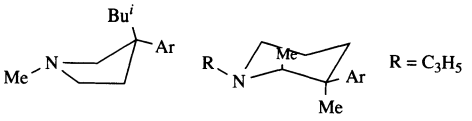
induced narcotic effects with potencies close to those of nalorphine; in both cases the β-isomer was the more potent by a factor of two or more (β-N-allyl 2 ×, β-N-$CH_2cC_3H_5$ 2–4 × nalorphine). There is ^{1}H- and ^{13}C-NMR evidence that equatorial 3-aryl chairs are the favored conformations of both α- and β-diastereoisomers[193] and X-ray evidence of stereochemistry in the case of α-**108** (R = allyl).[211] Some antipodal comparisons have been made. Cheng *et al.*[210] examined enantiomers of **107** with N = methyl, allyl, cyclopropylmethyl, and phenethyl. Except for the N-phenethyl analogues, all levo isomers (of 3*S*-configuration) were the eutomeric forms in antinociceptive assays, although potency levels were low, e.g., (−)-N-allyl derivative 0.07 × morphine in the writhing test. Some correlation was found between the writhing test potencies and receptor binding IC_{50} values of the antipodes. In the case of the 2-methyl antagonist (**108**, R = allyl), the levo form (2*R*,3*S*) proved more effective than its antipode in reversing fentanyl-induced effects in rats.[211] In both studies absolute configurations were established by X-ray crystallography; eutomers had a common 3*S*-configuration.

The 3-arylpiperidines **107** are closely related to pyrrolidine derivatives such as profadol **109**. Although enantiomers of profadol differed little in agonist activity,[212,213] derivatives with antagonist properties displayed pronounced eudismic ratios with levo isomers the more potent.[214]



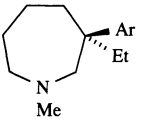
109
Ar = *m*-OHC_6H_4

The pyrrolidine–piperidine pair (**110**) of eutomers are related in configuration at the C-quaternary chiral center (X-ray analysis of HBr salts).[211]



110

Another chiral agent related to 3-arylpiperidines is the azacycloheptane **111**, marketed as the racemic mixture meptazinol. The levo antipode was about twice as effective as the dextro form but neither surpassed the racemic mixture in the mouse hot-plate [ED_{50} mg/kg *rac* 4.0; (−) 6.9; (+) 15.7 (NIH data)].[215] Its behavior in this and other respects is unusual (see p. 204 of Ref. 1).



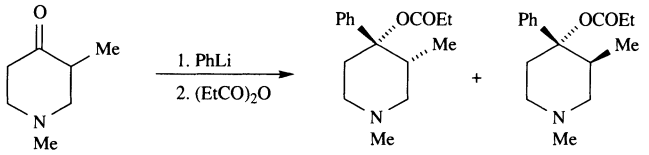
111
Ar = *m*-OHC_6H_4

13.8. 4-Phenylpiperidines with C-4 Oxygen Substituents

The bulk of studies of chiral 4-arylpiperidine opioid ligands relate to reversed esters of pethidine rather than pethidine itself, due probably to ease of synthetic access and the fact that replacement of 4-carbethoxy (CO_2Et) by 4-propionyloxy (OCOEt) usually produces a major increase in potency (up to 20-fold regardless of the nature of the N-substituent).[216] The effect of alkyl substitution in the piperidine ring of 4-arylpiperidine analgesics has attracted much interst ever since the 3-methyl analogues of the parent reversed ester were described by Roche workers in the late 1940s.[217] Since that time many 3-alkyl and all possible mono- and di-C-methyl derivatives of the reversed ester have been reported and much evidence of potency variation among stereoisomers disclosed.[218] In these derivatives the 4-aryl group is generally unsubstituted phenyl—insertion of *m*-hydroxy markedly depresses potency (see page 492).

13.8.1. α- and β-Prodine and Related Compounds

Ziering and Lee isolated a major (α) and minor (β) racemic ester, termed α- and β-prodine, respectively, from the product of reaction between 1,3-dimethyl-4-piperidone and PhLi/$(EtCO)_2O$[219] (**112**→**113**, **114**); relative configurations were

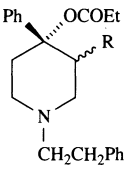


112

α-**113**
c-3-Me, *r*-4-OCOEt

β-**114**
t-3-Me

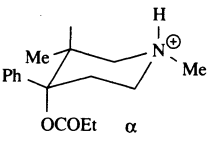
subsequently established as *c*-3-Me for α-prodine and *t*-3-Me for β-prodine (*r*-4-OCOEt). The α-racemic mixture was found to be as potent as morphine in animal tests, while β-prodine was almost five times as active as the standard agent.[217] Later studies confirmed these finding and showed, in addition, that a significant potency rise over that of the parent reversed ester (3-desmethylprodine) only occurred in the case of the β-isomer.[221,263] The same relationship was found for the N-phenethyl analogues (**115**), and for pethidine and its 3-methyl congeners (page 467).[222]



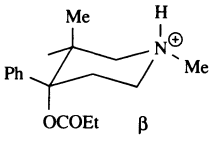
115

Relative activity in mice hot-plate test, morphine = 1 (Ref. 222)	
R = H	3.5
R = *c*−Me (α)	4.5
Rτ − Me (β)	22.0

The relative configurations of the prodines remained controversial for some years until the validity of arguments that reversed the original assignments[223] were substantiated by X-ray crystallographic analyses.[224,225] The solid state conformations of α- and β-prodine are shown in formulas **116** and **117**, respectively;



116



117

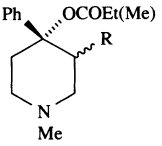
equatorial 4-phenyl chairs are preferred in each case. ^{1}H NMR data show that **116** and **117** are also the preferred solute conformations of the prodines in D_2O.[177,226] α-prodine is also active in man (40–60 mg ≡ 100 mg methidine)[227] and the compound (now withdrawn) was previously marketed as Nisentil.

When analogues of the prodines bearing 3-alkyl substituents larger than methyl were examined, it turned out that the α-isomer (*c*-3-R) was the more potent of diastereoisomeric pairs, i.e., the case of the 3-methyl pairs is unique[228] (Table 13.14). Receptor affinities measured by competition with [^{3}H]dihydromorphine at rat brain sites confirmed the higher affinity of β- over α- (**118**, R = Me), and α- over β- (**118**, R = Et, allyl, and *n*-hexyl).[229]

Activity variations among 4-phenylpiperidine opioids (structural and stereochemical variants) are considered to be little influenced by pharmacokinetic factors,[230–232] a viewpoint supported by a detailed study of the metabolism and distribution of prodine isomers in mice (using tritiated analogues).[233] In no case was the difference in analgesic potency between isomers fully accounted for by metabolism or distribution. At identical doses, the racemic prodine diastereoisomers and 3-desmethylprodine differed significantly in brain levels attained after 15 min, but the magnitudes of the difference were not large (β 12% above α; α 11% above 3-desmethyl). Uptake by plasma protein differed in extents by less than 10% among racemic mixtures and antipodal forms.

Hence, taking the unsubstituted esters (**118**, R = H) as standards, the drug–receptor interaction appears to be enhanced by α-ethyl and impeded by α-*n*-

TABLE 13.14.
Antinociceptive Activities (ED_{50} mg/kg, sc MHP) of Some Reversed Esters of Pethidine[a]

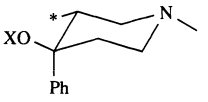


118

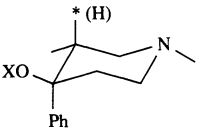
R	α (*t*-3R/4Ph)	β (*c*-3R/4Ph)
H	0.85 (3.62)	0.85 (3.62)
Me	0.92 (6.0)	0.18 (0.98)
Et	0.4 (2.1)	3.5 (15.9)
Pr^n	2.0 (10.4)	14.7 (23.4)
allyl	0.09	11.7
Bu^n	54.7 (29.3)	12.8 (26.5)
$C_6H^n_{13}$	inactive at 80	54.4

[a] From Ref. 228; acetate data in parentheses.

propyl (moderately) and α-*n*-butyl (severely) while all β-substituents except methyl have detrimental influences (the case of 3-allyl is discussed below). The prodines are exceptional in that the β-member is not only distinctly more potent than the α-form, but also exceeds the activity of the C-3 unsubstituted parent. If close correspondence between binding and preferred conformation is accepted, the influence of a β-3-methyl may be achieved *directly* through interaction with a binding site on the receptor specific for the axial methyl conformation (the γ-2,3-dimethyl analogue, also with a preferred axial 3-methyl conformation, is the most potent of this particular isomeric set; see later). Larger hydrocarbon groups of the same axial orientation are not accommodated at this site and act against ligand–receptor association. An alternative explanation, however, is that a β-3-methyl group has an *indirect* influence on ligand–receptor association by facilitating a rise in the population of reversed-ester conformations that bind more effectively than the equatorial 4-phenyl chairs favored for unsubstituted and 3-α-substituted derivatives. Thus the axial 4-phenyl conformer is more favored for the β-isomer **119** than either α-**120** or



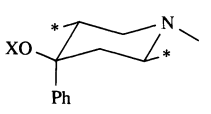
β-**119**



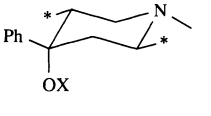
α- (and desmethyl) **120**

The asterisk (*) denotes Me, and X denotes COR (usually COEt); N-Me is omitted. See Table 13.15, note *b*.

the 3-desmethyl analogue, and such conformers may well have greater receptor affinity than *eq*-4-phenyl analogues (cf. arguments relating to C-4 carbon piperidines, page 469). α-Promedol **121**, for example, an isomer in which the axial 4-phenyl form may be preferred, is about 10 times more potent than the γ-isomer **122** (see later). A similar case may be advanced if flexible (boat) forms are conformations most favored by the receptor.



α-**121**

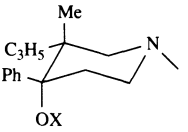


γ-**122**

As will be seen, the former (direct) role of β-3-methyl is considered the more probable because it does not require the proposal of a radical difference in the ligand–receptor interactions of the prodine and promedol diastereoisomers.

The case of the 3-allylprodine analogues of the parent reversed ester is of special interest. The stereochemistry of the allylprodines (**118**, $R = CH_2CH = CH_2$) was clarified by concurrent reports from two groups.[234,235] Assignments based on ^{1}H-NMR and chemical evidence (α-*c*-3-allyl, β-*t*-allyl, *r*-4-OCOEt) have been confirmed by X-ray crystallography of *rac*-HCl salts.[203] In mice (hot plate test), α-allylprodine was about 13 times and the β-isomer about one-tenth as active as morphine. Thus the allylprodines concur with the SAR pattern of 3-alkyl reversed esters (α-isomers the more potent, except for 3-methyl). Bell and Hyne[237] conceived the idea of combining optimal 3-methyl geometry in betaprodine with that

of 3-allyl in allylprodine and made the gratifying discovery that the more active antipode of this type was **123**, a compound more potent in mice than either (3*S*,4*S*) - betaprodine (ED_{50} 0.25 mg/kg) or (3*R*,4*S*)-allylprodine (ED_{50} 0.03 mg/kg). Stereochemical assignments were based on ^{1}H-NMR data (chemical shift of α-3-$CH_2CH=CH_2$ protons little changed after N-protonation) and CD evidence.

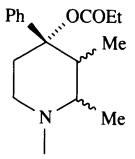


123

	ED_{50} (mg/kg MHP sc)
3*R*,4*R*	0.01
3*S*,4*S*	25

Over the past 20 years or so mono- and di-C-methyl analogues of the reversed ester of pethidine have been reported in addition to 3-alkyl derivatives of the prodine type; these include 2-methyl, 2,6-, 2,5- (promedols), 2,3-, 3,3-, and 3,5-dimethylpiperidines (summarized in Table 13.15). In each case stereochemical problems arise that were not solved conclusively in most of the original reports, and since 1970 efforts have been made to isolate all possible variants, to compare their pharmacology, and to establish configurations. It turns out that wide-ranging potency variations are found not only among the various positional isomers but also within isomeric sets, and it is of great interest to discover whether activity variations may be accounted for by stereochemical demands of the receptor that are valid for the entire group and whether the effects of methyl in its different positions and orientations about the piperidine ring are additive. To this end knowledge is required of (1) the relative configurations of the piperidine ring substituents, (2) the conformational equilibria of each isomer, preferably in the protonated state, and (3) separation of chiral diastereoisomers into antipodal forms followed by establishment of the absolute configuration and opioid potency of each member of enantiomorphic pairs. Items (1) and (2) have been established by NMR (^{1}H and ^{13}C) studies for solutes and X-ray crystallography for solids. Most data on optically active forms are due to the elegant work of Portoghese and his colleagues, by chemical interconversions and X-ray analysis.

The interpretations of stereochemical structure–activity data that follow are based almost entirely on ED_{50} values of the hot-plate assay in mice after subcutaneous injection of the test substance carried out at the National Institutes of Health (Drs. E. L. May and A. E. Jacobson). Where *in vitro* assay data on isomeric sets are available, correlations with *in vivo* potency rankings are found even though 4-phenylpiperidines generally have rather low orders of activity/affinity in these tests. Results for a triad of 2,3-dimethyl analogues of the reversed ester of pethidine with mouse hot-plate ranking γ > α > β illustrate this point (see **124** and data).[258]

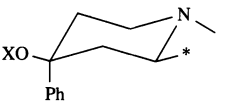
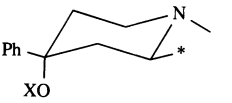
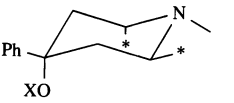
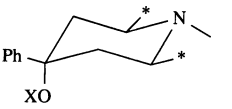
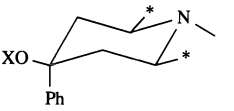
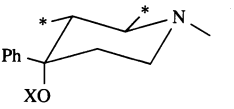
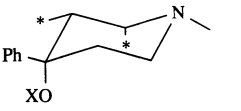
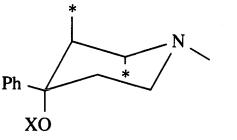
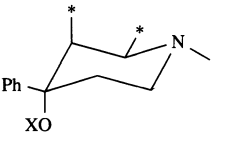


124

	IC_{50}, nM(GPI)	IC_{50}, nM (MVD)	K_i (nM)[a]
γ	196 ± 46	234 ± 24	19.5 ± 3.8
α	1750 ± 470	234 ± 24	186 ± 39
β	30–35% inhibition at 10,000	Inactive at 10,000	1903 ± 422

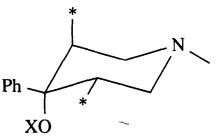
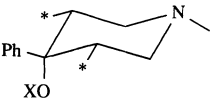
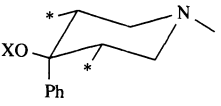
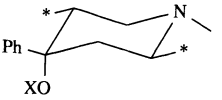
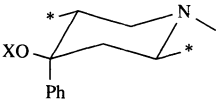
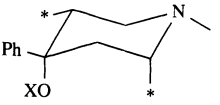
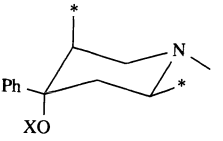
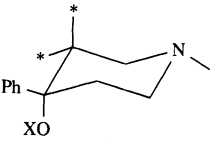
[a] Displacement of μ-ligand [^{3}H-D-Ala^2, MePhe^4, Gly-ol^5]enkephalin.

TABLE 13.15.
Stereochemical Data on *C*-Methyl Derivatives of Reversed Esters of Pethidine

Substituent	Isomer designation or characteristic	Configuration[a, b]	Ref.
2-Me	α		179, 238, 239
	β		
2,6-di-Me	mp 198–199° C (HCl)		240, 241
	mp 203-204° C (HCl)		
	mp 145–154° C (acid succinate)		
2,3-di-Me	β		242–244
	α		
	γ		
	δ		

(table continued)

TABLE 13.15. (*Continued*)
Stereochemical Data on *C*-Methyl Derivatives of Reversed Esters of Pethidine

Substituent	Isomer designation or characteristic	Configuration[a, b]	Ref.
3,5-di-Me	γ		245–247
	meso		
	meso		
2,5-di-Me	γ		179, 248, 249[c]
	α		
	β		
	δ[d]		
3,3-di-Me			257[e]

[a] Depicted in most cases as the preferred chair conformation; only one enantiomorphic form is shown for chiral molecules.

[b] To simplify these and subsequent formulas, asterisks are used to denote the position and orientation (axial or equatorial) of C-methyl groups (nitrogen carries methyl in all cases), while X denotes acyl (COEt or COMe).

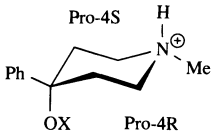
[c] In some of the references α and β are interchanged as a result of an error in an abstract of the original paper[248]; in this book the original designations of Nazarov are employed. Initial interpretations of some of the ^{1}H-NMR data in terms of configuration were incorrect.[249, 250]

[d] Configuration originally assigned incorrectly,[256] now established by elimination.

[e] Absolute configurational study.

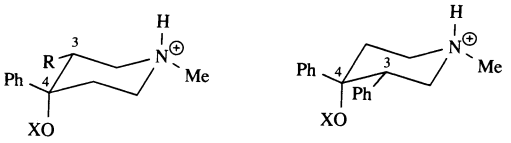
13.8.2. Analysis of Stereostructure–Activity Relationships in 4-Phenylpiperidine Reversed Esters

In this analysis Portoghese's treatment of the reversed ester of pethidine in its preferred 4-phenyl chair conformation is followed.[259] Adapting biochemical nomenclature,[260] the two sides of the achiral reversed ester **125** may be differentiated as prochiral-4*S* and prochiral-4*R*. If an alkyl group is inserted in the Pro-4*S* side or edge of the molecule, the formerly symmetric C-4 atom becomes asymmetric and acquires an *S*-configuration in terms of the Cahn–Ingold–Prelog convention; insertion of alkyl in the Pro-4*R* side gives C-4 an *R*-configuration.



125

The question to be posed is: can the opioid receptor discriminate between the two enantiotopic edges of the molecule in the same sense as enzymes differentiate chemically alike paired groups of substrates of the Caabc type such as citrate (Ogston effect, see Chap. 1, page 6). Data on the antipodal forms of 3-alkyl derivatives of the prodine type clearly provide an affirmative answer. Thus, it is found that antipodes of α-3-methyl (α-prodine), α-3-ethyl, and α-3-propylpiperidines differ in antinociceptive potency (mouse hot plate) and that eutomers all have the same configuration; **126** depicts the more (3*R*,4*S*) and **127** the less (3*S*,4*R*) potent enantiomer.

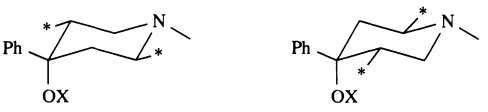


126 X = COEt **127**

R	3*R*,4*S*	3*S*,4*R*
Me	0.91	22.4 (cf. R = H, 1.3)[a]
Et	0.9	25 (cf. R = H, 0.85)[b]
n-Pr	1.0	25.2[b]
C_3H_5 (allyl)	0.03	7.8[b]

ED_{50} mg/kg sc mouse hot plate.
[a] Ref. 259. [b] Ref. 261.

The same configurational pattern is displayed by the α-3-allylprodines[261] and (+)- and (-)-γ-promedol **128**.[262] One interpretation of these findings[218,263] is that the pethidine reversed ester presents the Pro-4*R* rather than Pro-4*S* side of the molecule to the receptor. Substituents positional in the 4*S*-side are remote from the receptor surface and so do not hinder approach of the ligand to receptor binding sites—the similar orders of potency of α-4*S* (**126**, R = Me, Et, and *n*-Pr) and the unsubstituted ester support this proposal. In the α-4*R* antipodes the ring alkyl



(+)-2*S*,4*S*,5*R*
ED_{50} 0.94 mg/kg

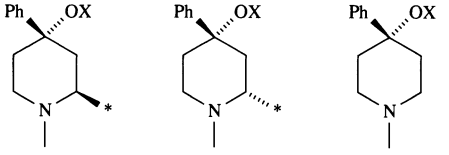
(–)
ED_{50} 8.8 mg/kg

128
(+)/(–)-γ-promedol

substituent (certainly when equatorial and adjacent to C-4) prevents effective drug–receptor association because it is now immediately adjacent to the receptor surface. These arguments are given further weight by the fact that the *cis*-3,5-dimethyl analogue **129** lacks analgesic properties.[246] The 3-allylprodines are accommodated by the same proposals, except that in this case the 3-alkyl substituent plays a significant positive role in enhancing drug–receptor binding present within the 4*S*-edge.

129

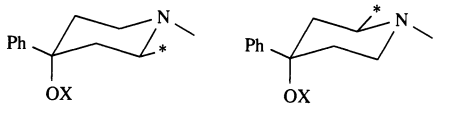
Turning now to derivatives with substituents adjacent to nitrogen, the racemic α- and β-2-methyl reversed esters prove to have similar activities (unlike prodine diastereoisomers) and are somewhat less potent than the parent desmethyl compound (see **130**).[238]



rac-β	**130** α	desMe
ED_{50} (mg/kg) 1.4 (4.9) MHP	1.3 (2.4)	0.85 (4.7)

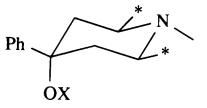
(acetate data in parentheses)

The β-2-methylpropionate, with a preferred equatorial 4-phenyl chair conformation, has been resolved and a tenfold potency difference between antipodes revealed.[232] The eutomer was shown to have the 2*S*,4*R*-configuration by X-ray analysis (see **131**). It is noteworthy that receptor stereoselectivity toward equatorial ring-methyl groups α to nitrogen is the reverse of that toward those placed adjacent to the 4-phenyl substituent in reversed esters. α-*eq*-Methyl within the 4*R*-enantiotopic edge has little influence on activity as judged by concurrent assay data on



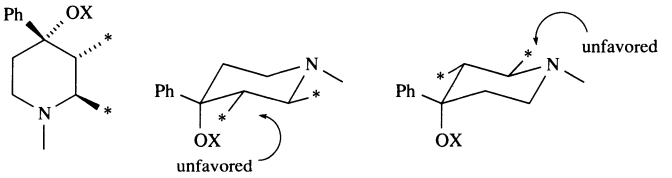
(–)-2*S*,4*R* **131** (+)-2*R*,4*S*

the desmethyl parent (ED_{50} 0.4–0.6 mg/kg) (Jacobson, private communication), while a similarly oriented group in the 4*S*-edge significantly impedes drug–receptor binding as confirmed by IC_{50} values of 1.2 μM for the levo and 11.0 μM for the *dextro* isomer (displacement of [^{3}H]etorphine from guinea-pig brain homogenate).[232] The inactivity of the *cis*-2,6-dimethyl analogue **132** conforms to this interpretation.[240]



132

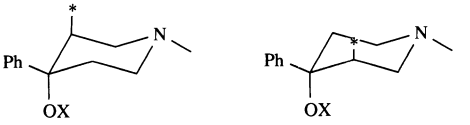
Reversed esters with equatorial methyls adjacent to both 4-phenyl (as in α-prodine) and nitrogen (as in **130**) are considered next. The activity of the racemic γ-2,5-dimethyl derivative (promedol) is close to that of the desmethyl parent and resides chiefly in the (+)-isomer of 2*S*,4*S*,5*R*-configuration (see **128**).[262] Note that the methyls are placed in favorable and unfavorable enantiotopic edges in (+)- and (-)-isomers, respectively, as judged by results on the 2- and 3-monomethyl derivatives. The corresponding 2,3-dimethyl isomer (β-**133**) has a very low potency (ED_{50} 30.7 mg/kg in mice by hot plate test)[244] in accord with the fact that a favorable placement of both methyls is impossible in either isomer.



β-**133**

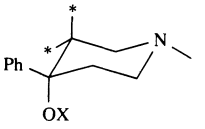
Thus in 2- and 3-monomethyl, and 2,5- and 2,3-dimethyl derivatives with preferred equatorial hydrocarbon constituents, consistent and additive stereochemical structure–activity relationships obtain. Furthermore, eutomeric forms have potencies close to that of the desmethyl parent in support of an essentially passive role for methyl substituents of this type in the drug–receptor interactions.

β-Prodine presents a case where the 3-methyl substituent has a preferred axial orientation, and the (+)-3*S*,4*S*-isomer has been shown to be the more potent antipode.[259] Hence, in terms of equatorial 4-phenyl chairs, the Pro-4*S* edge is still the preferential substitution site (as in α-prodine). Receptor stereoselectivity toward axial 3-methyl (13-fold) is less than that in regard to equatorial groups (25-fold) (see **134**). In addition, axial 3-methyl placed along the Pro-4*S* edge raises potency (×4), so its role is not passive as is a similarly placed equatorial group.



β-**134**	(+)-3*S*,4*S*	(–)-3*R*,4*R*
ED_{50} (mg/kg) MHP sc	0.25	3.3

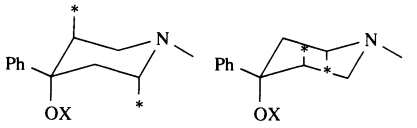
As anticipated, the more active antipode of 3-methylprodine proved to be the 4*R*-form **135** with 3- substituents placed as in the eutomers of α- and β-prodine (X-ray analysis of the 4-piperidol precursor as HBr).[257]



135

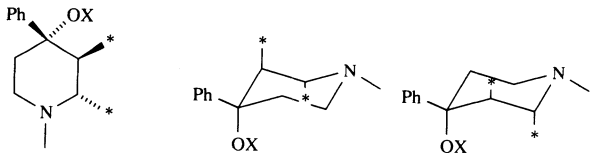
ED_{50} mg/kg (rat tail withdrawal iv)
R-(+) 0.5
S-(−) inactive at 10

A correlation between eutomeric forms of β-prodine and the α-2,5-dimethyl derivative (α-promedol) requires the latter to adopt an equatorial 4-phenyl chair conformation at the receptor (see **136**).[265]



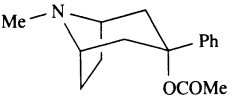
α-**136** (+) 0.06 (−) inactive at 50
ED_{50} (mg/kg)
MHP

The precursor α-4-piperidinol base prefers an axial 4-phenyl chair conformation in both the solid (X-ray evidence)[254] and solute state (^{1}H-NMR evidence).[281] However, there is evidence from energy calculations that the equatorial 4-phenyl chair has the lower energy context when 4-OH is acylated (base)[266] while the ^{1}H-NMR spectrum of α-promedol HCl in D_2O is typical of a binary mixture of protonated epimers of the *eq*-4-phenyl–*ax*-4-phenyl chairs in equilibrium.[281] An equatorial 4-phenyl chair is also favored for the γ-2,3-dimethyl analogue of promedol (**137**) and the corresponding 4-piperidinol hydrochloride.[244] Racemic γ-



γ-**137**

137 is about four times as potent as the reversed ester of pethidine and 100 times that of the corresponding β-isomer **133**. It is likely that the 4*S*-isomer will prove the more active antipode, and it follows that the receptor can accommodate axial methyl adjacent to nitrogen within either enantiotopic edge; cf. 2-Me configuration in (+)-α-promedol **136** and 4*S*-**137** (γ-2,3). The last point is supported by the retention of activity in the tropane analogue of the acetoxy reversed ester of pethidine **138**.[267]

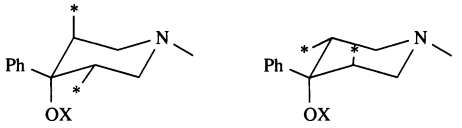


138

ED_{50} (mg/kg) 3.4
MHP
(cf. 4.7 for reversed acetoxylester of pethidine)

Differences in receptor sensitivity toward axial and equatorial α-methyl substituents are nicely illustrated in the case of isomeric 2,6-dimethyl piperidines, described shortly.

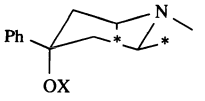
A detailed study of 3,5-dimethyl analogues has been made.[245] Of the three diastereoisomers only the γ-isomer (*t*-3-Me, *c*-5-Me, *r*-4-OCOEt) was active (at least twice as potent as pethidine in mice); the (+)-eutomer has an axial 3-methyl group advantageously placed and an equatorial 3-methyl group disadvantageously placed (see **139**). The potency raising action of *ax*-3-Me [placed as in (+)-β-prodine] appears to offset the adverse influence of *eq*-5-Me (placed as in the distomer of α-prodine). The 2,6-dimethyl triad of diastereoisomeric esters presents a



(+)-3*S*,5*S* (−)-3*R*,5*R*
4.1 25.01

139 ED_{50} mg/kg (MHP), pethidine 28.8

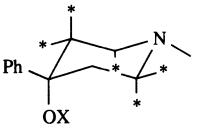
case similar to the 3,5-dimethyl derivatives. The sole active member was the *trans*-2,6-dimethyl-*eq*-4-phenyl chair (examined as acetate ester).[240] Later, the precursor 4-piperidinol was resolved and antipodal propionates evaluated in mice by the hot-plate test (see **140**). The eutomer proved to be the 2*S*,6*S*-isomer (X-ray analysis of 4-piperidinol HBr), a result supporting speculations of receptor sensitivity to α-2-methyl orientations.[268]



Mouse, hot plate
ED_{50} (mg/kg)
2*S*,6*S* 1.1
2*R*,6*R* inactive at 5 and 20

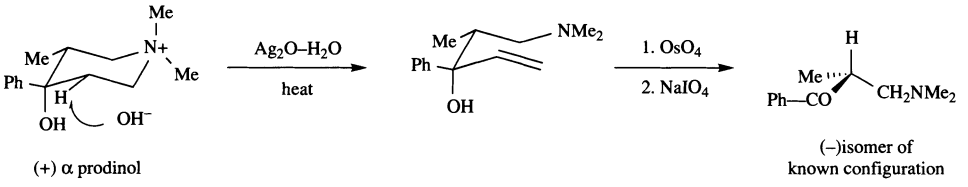
140

From this analysis the absolute orientations of methyl substitution that favor or have minor influence on the ligand–receptor interactions of the reversed ester of pethidine may be summarized in terms of the equatorial 4-phenyl chair **141** (see Ref. 8, p. 265) for consideration of further methylated reversed esters in terms of **141**.



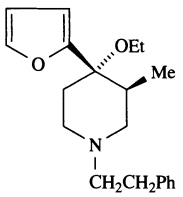
141

Reversed ester analogues with 4-OCOR replaced by an alkoxy (ether) function are rare. One notable example is the 4-(2-furyl)-3-methyl derivative **142**, which is 1.2–2.5 times more active than morphine in mice.[269] The stereochemistry of its for-



Scheme 13.6. Configuration sequence for α-(+)-prodinol.

mation by alkyl–oxygen fission of the corresponding 4-propionate was established as *t*-3-Me, *r*-4-OEt by a ^{13}C-NMR analysis.[270]

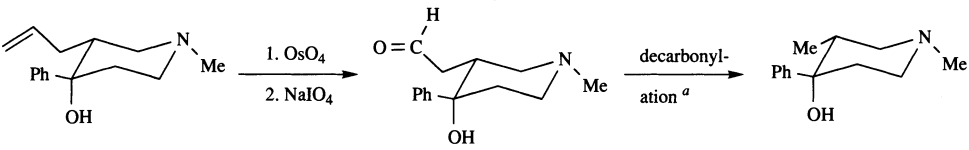


142
t-3-Me, *r*-4-OEt

13.8.3. Stereochemical Methods

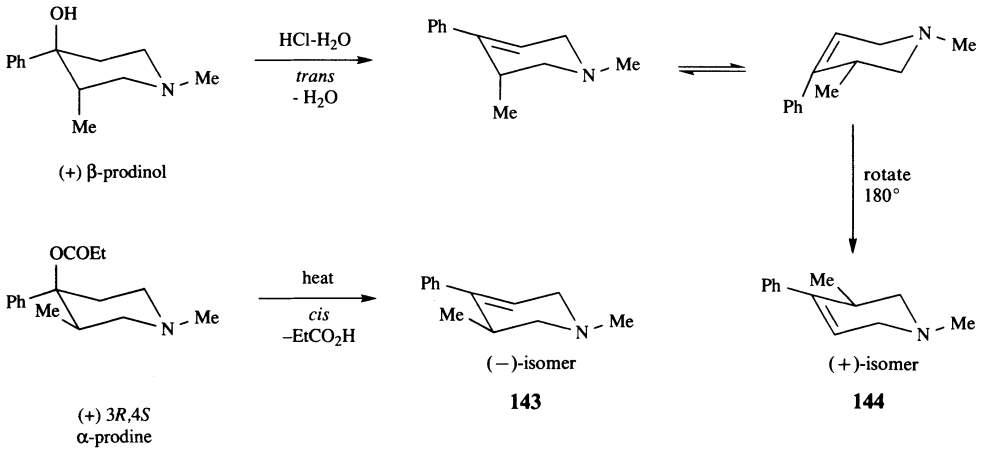
These methods are summarized elsewhere.[8] Questions of solid-state conformation (often of precursor 4-piperidinols) and absolute configurations have been solved for many examples in this series by X-ray methods, as noted. Knowledge of absolute geometry has also been aided by chemical transformations. A sequence in which a metho salt of (+)-α-prodinol was converted to a phenyl ketone of known configuration is shown (Scheme 13.6).[259]

(+)-γ-Promedol alcohol was likewise degraded to the same *levo* ketone, hence the two *dextro* 4-piperidinols have identical C-3(5) configurations.[262] The chirality of the antipodal 3-allylprodines was determined by their conversion to corresponding prodinols, now of established configuration (Scheme 13.7).[261] The eutomeric forms of α- and β-prodine (*cis* steric pathway) gave the (−)-tetrahydropyridine **143** with preservation of C-3 chirality, while dehydration of (+)-β-prodinol (*trans* steric course leaving C-3 geometry intact)[271] gave the (+)-alkene (**144**). Manipula-



[a] Reagent: tris(Ph_3P) rhodium(I)chloride.

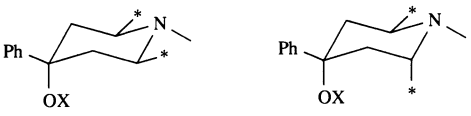
Scheme 13.7. Configurational sequence for 3-allylprodines.



Scheme 13.8. Sequence linking α- and β-prodines.

tion of structures as shown reveals that (+)-α- and (+)-β-prodine must therefore have opposite configurations at C-3 (Scheme 13.8).[259]

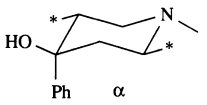
NMR (^1H, ^{13}C) data have provided much information about relative configurations and solute conformations, and a few examples are included here. Chiral and achiral (*meso*) diastereoisomers of 3,5- and 2,6-dimethyl-4-phenylpiperidines are readily differentiated by their ^1H-NMR spectra because the two methyls of *meso* forms have identical environments and give rise to the same resonance, while chiral isomers involve an axial–equatorial pair that results in two separate signals (see **145**). Coupling constant data of vicinal protons often yield direct evidence of



cis **145** *trans*

2,6-di-Me δ 1.58	δ 1.2, 1.07
^1H NMR signal doublet, HCl in $CDCl_3$	pair of doublets
(data refer to acetoxy esters)	base in $CDCl_3$

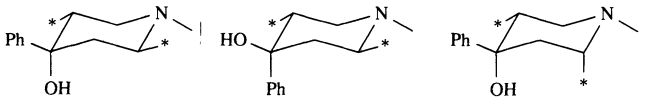
stereochemistry as a result of the relationship between ^3J magnitudes and dihedral angle between coupled protons (see Chap. 2, page 23). The ^1H-NMR spectrum of the alcohol precursor of α-promedol, for example, is fully resolved in $CDCl_3$; spectral analysis supports the axial 4-phenyl chair shown, **146**.[281]



146
α-2,5-dimethyl

Turning to some ^{13}C-NMR examples, chemical shifts of *ax*- and *eq*-2-methyl carbons of the 2,6-dimethyl derivatives **145** may be differentiated. Of the two

C-2(6)-methyl resonances seen in the spectrum of the *trans*-isomer, the higher field (near 13 ppm) is assigned to the axial group and the lower field (near 20 ppm) to equatorial methyl on the principle of the more sterically compressed carbon having the higher-field position.[179] The ^{13}C-NMR chemical shift comparisons between the three promedol alcohol isomers **147** then show that 2-methyl has an *eq*-conformation in the γ- and α-isomers and an *ax*-orientation in the β-isomer. The similar C-q

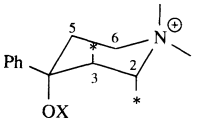


147

^{13}C chemical shift [a]	γ	α	β
C-2-Me	20.1	20.6	13.0
C-5-Me	12.0	14.5	15.0
C-q	147.2	144.9	147.4

[a] ppm from TMS.

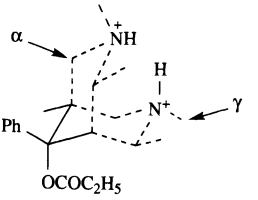
(quaternary aromatic carbon) chemical shifts of the γ- and β-isomers and the higher-field C-q resonance of the α-isomer enable 4-phenyl conformational assignments to be made as shown.[252] The same approach was taken to assign the relative configurations of 2,3-dimethyl analogues.[244] The γ-ester hydrochloride of this series is of special interest; its diaxial methyl conformation **148** is supported by the marked upfield shifts (5–6 ppm) of both the C-5 and C-6 carbons compared with corresponding shifts of the unsubstituted reversed ester (diagnostic of steric compression due to opposed axial methyls),[84] and the unusually low field position of the C-methyl signals due to mutual deshielding.



148

13.8.4. Receptor Speculations

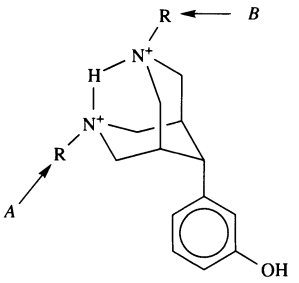
If it is assumed that 4-arylpiperidines with C-4 carbon and C-4 oxygen substituents share at least one common site at the opioid receptor, then the model **149** proposed by Fries and Portoghese[265] which involves a common aromatic and two distinct anionic binding sites demonstrates how ligands with preferred axial and



The more potent axial phenyl (α) and equatorial phenyl (γ) diastereoisomers superimposed on one another. Portions that are not superimposed are shown by dashed lines.

149

equatorial 4-arylpiperidine chairs may associate with the receptor. This model employed α-promedol as the axial 4-phenyl chair—now known to be an unlikely binding conformation (see above). The modified model **150** relates C-4 carbon aryl

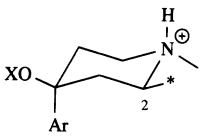


150

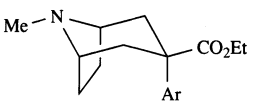
A denotes an agonist–antagonist site
B denotes an agonist site only

piperidines (axial 4-aryl) and C-4-oxygen arylpiperidines (equatorial 4-aryl).[8] Since the N-R substituent projects to different regions of the receptor in the two modes, the model explains why N-allyl (and like groupings) derivatives of the axial 4-arylpiperidine type induce antagonism or have dualist actions (zone *A* triggered), while those of the equatorial 4-aryl type behave only as agonists (zone *B* triggered).

In terms of this model, a *meta*-placed phenolic hydroxyl should enhance activity in both *ax*- and *eq*-phenyl modes of uptake. This is true for simple 4-arylpiperidines with C-4 carbon substituents, such as ketobemidone and picenadrol (see pages 465 and 469) which have significant *ax*-aryl populations. However, phenolic insertion in 4-phenylpiperidines with preferred *eq*-phenyl conformations virtually abolishes activity in reversed esters of pethidine.[174,175] This fact counts against arguments based on model **150**, but may be explained by a difference in the orientation of the aryl (made nonsymmetrical by *m*-substitution) and piperidine rings in the two chair conformation. Thus the arrangement that places the phenolic OH close to the receptor subsite appropriate to the rigid morphine model may be favored in axial-aryl but not in equatorial-aryl chair conformations of the flexible piperidines. Portoghese *et al.*[174] proposed an alternative model with a pair of well-separated aromatic subsites. However, in the piperidine **151** and



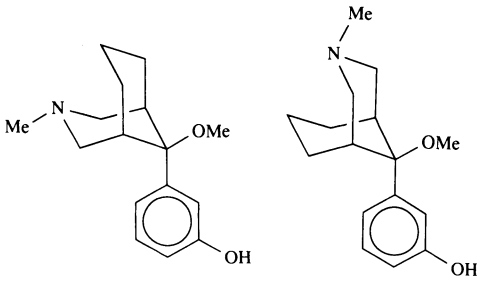
151



152

tropane **152**, both of which show preference for an axial-aryl conformation, phenolic analogues were less potent than the unsubstituted phenyl derivative.[140,280] The α-2-methylpiperidine **151** (major epimer shown) exists as an mixture of N-protonated epimers in D_2O (NMR evidence).

The azacyclanes **153**, formally constructed by bridging the 3,5-positions of 4-arylpiperidine ligands with trimethylene, provide both axial and equatorial



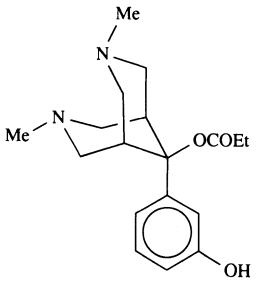
α **153** β

		IC_{50} (nM)[a]	ED_{50} (nM/kg)[b]
α-Isomer	nonphenolic	560	2700
	phenolic	14	2.8
β-Isomer	nonphenolic	590	4000
	phenolic	12	6.1
Morphine		160	3400

[a] Binding assay *vs* [^{3}H]etorphine.
[b] Mouse writhing (acetic acid-induced).

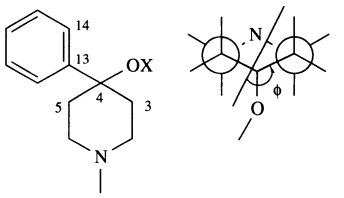
species.[141] When Ar is phenyl, both isomers have morphine-like potencies in mice (writhing test) and have similar binding affinities (less than that of morphine). Activities of both members rise markedly after insertion of *m*-OH in the phenyl ring.[161] Both isomers may be accomodated by model **150**, but are exceptional in being C-4-oxygen piperidines where phenolic analogues exceed the potency of non-phenolic examples (no simple 4-alkoxy piperidines with phenolic functions have yet been described).

Isomeric 4-hydroxyazacyclanes were obtained by treating corresponding 3,5-propano-4-piperidones with aryl Grignards. In the N-methyl case, diastereoisomers were differentiated by the fact that the N-Me ^{1}H chemical shift of the α-isomer was upfield to the β-signal by about 0.2 ppm, ascribed to aromatic shielding in the *ax*-4-aryl case (α, **153**). The 3,7-diazabicyclanes **154** also proved to be effective antinociceptive agents (mice writhing test: 3–5 × morphine after central administration).[162] 9-Phenyl derivatives were inactive.



154

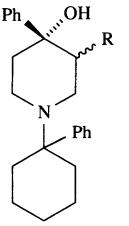
Portoghese[167] has interpreted potency differences between C-methyl pethidine-type antipodes on the basis of the sign and magnitude of C(14)-C(13)-C(4)-C(5) torsion angles (see **155**, all fall in the range -128 to 167° for the more active antipodes of α- and β-prodine, 5-methylprodine, α- and γ-promedol, and



155

α-allylprodine), and steric hindrance between the C-alkyl substituent and the receptor when the ligand is in a pharmacophoric conformation. The significance of the orientation of the phenyl and piperidine rings in *absolute terms* is doubtful, however, since, as has been pointed out,[203] the corresponding value of the relatively weak and nonstereoselective analgesic (+)-β-allylprodine is also negative and within the range found for potent agents. Furthermore, activity differences between antipodal forms of the β-2-methyl reversed ester cannot be accounted for in this manner because the C-methyl substituent is too far removed from C-4 to influence the orientation of the aromatic substituent.[232]

Following the report that analogues of PCP which incorporate elements of the reversed ester of pethidine, such as **156**a, exhibit antinociceptive actions and displace [^{3}H]morphine from binding sites,[236] 3-methyl analogues **156**b and c were



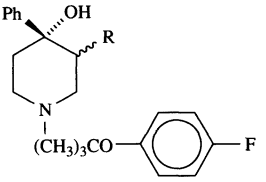
(a) R = H; (b) R = α-Me*; (c) R = β-Me

156

*ED_{50} (mg/kg sc) mice tail-flick test:
rac 15.3; 3*R*,4*S* 8.7; 3*S*,4*R* > 30

examined.[264] Stereostructure–activity relationships were found to mirror those of α-prodine in regard to C-3 chirality (3-*R*,4-*S* eutomer in both series) but diverged in respect of relative geometry at C-3 and C-4 (α > β for PCP derivatives, β < α for prodines). It was concluded that these hybrid PCP/4-aryl piperidines associate with opioid (of μ-subtype from binding evidence) rather than PCP receptors; *rac*-**156**b failed to displace the selective ligand [^{3}H]TCP[220] (2-thienyl analogue of PCP) from rat brain membranes at a concentration of 1000 nM (cf. PCP, K_1 60 nM).

Certain analogues of 4-aryl-4-piperidinols are active as antinociceptive agents provided they include appropriate N-arylalkyl substituents.[8] Potent derivatives with chiral N-substituents have been reported, but examined only in racemic mixture form. Iorio *et al.*[279] prepared antipodal forms of the haloperidol (DA antagonist) analogue **157** which owed dissymmetry to chiral centers within the piperidine ring. In both antinociceptive tests and binding assays vs. [^{3}H]



157

tail-flick ED_{50} (μmol/kg sc)
3*R*,4*S*-(+) 0.8
3*S*,4*R*-(–) >60.8

dihydromorphine and [^{3}H] spirperidone, the 3*R*,4*S*-(+)-antipode proved to be the eutomeric form—evidence of the common stereospecificity of opioid and dopamine receptors toward ligands of this kind.

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Opioid Ligands

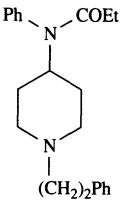
Part 2

14.1. Introduction

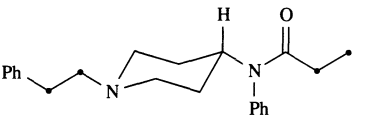
Chiral ligands discussed in this chapter have structures that bear little obvious relationship to morphine and many of the derivatives dealt with in Chapter 13, apart from the common presence of aromatic and basic features within the molecule. Groups described comprise (1) fentanyl, (2) methadone, (3) opioid peptides, and (4) a series of compounds which defy classification.

14.2. Fentanyl Group[1]

This group of analgesics is based, like pethidine and its congeners, on an N-substituted piperidine ring. It differs from 4-arylpiperidines, however, in failing to require a small carbon or oxygen substituent at C-4, and having an amido nitrogen function interposed between C-4 carbon and the aromatic group. The parent compound fentanyl (**1**, Sublimaze, Leptanol), now well-established as a potent clinical agent used in surgical analgesia (*Martindale* **29**, page 1304), is achiral. Its solid[2] and probable solute conformation (NMR evidence)[3] is the equatorial 4-anilido-chair **2**. Dissymmetric analogues have been obtained by creation of a chiral center either within the piperidino-N-substituent or the piperidine

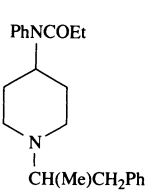


1

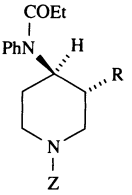


2

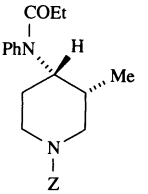
ring. The highly potent compound **3** (α-methylfentanyl) has achieved notoriety as the street drug "China White."[4] Isomeric potency comparisons have been made for certain 3-alkyl and 3-allyl analogues of fentanyl. In the case of the 3-methyl derivatives, the *trans*-isomer (**4**a) and fentanyl were almost equipotent, while the *cis*-isomer **4**a was six times more active than the parent in the rat tail withdrawal test.[5] The *cis*-compound had been isolated previously by Riley *et al.*[6] and shown to be ten times more effective than fentanyl in the rat tail-flick assay. Most of the activity of the *cis*-racemate resides in the *dextro* enantiomer [(+)-*cis*-**5** 19×,



3 China white



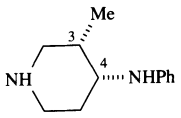
t-**4**
(a) R = Me



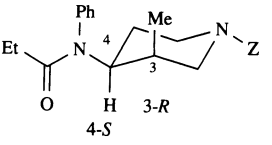
c-**5**
3*R*, 4*S* shown
(eutomer)

Z = $(CH_2)_2Ph$ for **4** and **5**

(−)-isomer which is itself 0.2× fentanyl]. The stereochemical structure–activity relationships thus mirror those of 3-methyl pethidines and the related prodines (page 478) at least in terms of relative 3Me/4-Ar,N(COEt)Ar geometry. Absolute configurations follow from an X-ray analysis of the L-(+)-tartrate salt of the *levo* secondary amine precursor **6** (3*S*,4*R*).[7] The eutomer *cis*-(+)-3-methylfentanyl is derived from (+)-**6** and hence has the 3*R*,4*S* configuration (**5** and **7**). It is evident from the comparisons of **7** with the eutomer of β-prodine (**8**) that the two antipodes differ in the absolute configuration of 3-methyl along the enantiotopic edge of the molecule. Binding IC_{50} (M) values for antipodes of *cis*-3-methylfentanyl (vs. [^{3}H] fentanyl, rat fore-brain) were (+) 2.1×10^{-10}, (−) 1.8×10^{-8}, and 1.6×10^{-9} for the *rac-trans*-diastereoisomers in accord with *in vivo* data.[8]



6
(–)-3*S*, 4*R*



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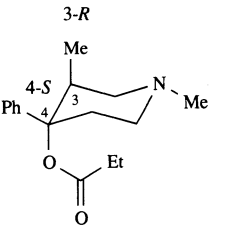
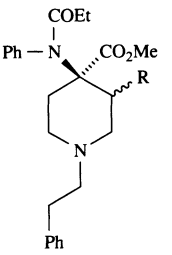


8 β-prodine

The potency of fentanyl is significantly enhanced when C-4 carries a carbon function, as in carfentanil **9**a (27× fentanyl in rats, tail withdrawal).[9] The *levo* antipode of the *cis*-3-methyl derivative **9**b, termed lofentanil, is five times as potent as fentanyl in man with a remarkably long duration of action.[10] Leysen and Laduron[8] quote rat tail-withdrawal ED_{50} values (mg/kg) as 0.00057 for (−)-*cis*-(**9**b), 2.2 for (+)-*cis*- (**9**b), and 0.008 for the *rac-trans*-compound (**9**c) (fentanyl 0.011); binding IC_{50} (M) vs. [^{3}H] fentanyl were: (−)-*cis* **9b** 2.6×10^{-10}, (+)-*cis*

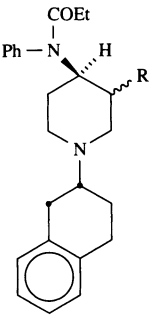
1.0×10^{-8}, *rac-trans* 1.6×10^{-9}, fentanyl 2.5×10^{-9}. Dextro *cis*-**9b** (the distomer) antagonized fentanyl-induced repiratory depression in rats with an ED_{50} value of 0.45 mg/kg (cf. naloxone 0.03 mg/kg), a property unique to the fentanyl group.[11] No evidence of lofentanil absolute stereochemistry has been published.

Similar stereochemical methods and results were obtained for *N*-tetrahydronaphthyl analogues **10** of 3-methylfentanyl (mouse tail-flick AD_{50} mg/kg: 3-demethyl 0.012; *cis*-3-Me 0.004; *trans*-3-Me 0.018).[12] In these derivatives a restraint is placed on rotation of the N-substituent.



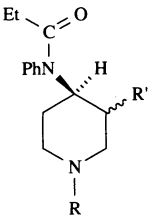
(a) R = H (carfentanil)
(b) R = Me, *cis* to PhNCOEt
(c) R = Me, *trans* to PhNCOEt

9



10

TABLE 14.1.
Antinociceptive Activities of Some 4-(*N*-Phenylpropionamido)piperidines in the Rat Tail-Withdrawal Test After iv Administration[a]

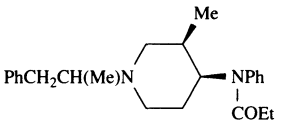


Entry	R	R′	Configuration	ED_{50} (mg/kg)
1	Me	H	—	inactive (100 mg/kg)[b]
2[c]	$(CH_2)_2Ph$	H	—	0.01[d]
3	Me	$CH_2CH{=}CH_2$	*cis*	10.0
4[e]	$(CH_2)_2Ph$	$CH_2CH{=}CH_2$	*cis*	0.08
5[f]	$(CH_2)_2Ph$	Pr^n	*cis*	0.02
6	$(CH_2)_2Ph$	Pr^n	*trans*	0.04

[a] From Refs. 5, 13, and 14.
[b] In mice by hot-plate test, sc.[14]
[c] Fentanyl.
[d] Ref. 5.
[e] Janssen (unpublished data) reports both *cis*- and *trans*-isomers, examined as oxalates, to have ED_{50} values near 0.04 mg/kg in the rat TW test.
[f] Compound entry 5 was more potent than compound 4 in displacing DAGO (μ), DADL (δ) and bremazocine (κ) from GP brain homogenates, and both compounds showed preference for μ-sites (H.W. Kosterlitz, private communication).

To probe further the relationship between fentanyl and 4-arylpiperidine opioids, isomeric 3-allyl and 3-propyl analogues of fentanyl were examined—a critical test since the effect on potency of such substituents in reversed esters of pethidine differs radically from that of methyl, e.g., potency raised over 10-fold by 3-allyl *trans* to 4-aryl and depressed by a *cis*-substituent (page 479). However, potency results for the 3-allyl and 3-propyl diastereoisomers (see Table 14.1) reveal a divergence of such relationships in that (1) the *cis*- and *trans*-3-Pr^n isomers have activities in the same range, with the *cis*-form the more potent by a factor of only 2, and (2) the reduced analogue of the *cis*-3-allyl derivative is more active than the alkenic parent (in reversed esters of pethidine, the 3-allyl derivative is by far the more potent derivative; Chap. 13, page 479). Although fentanyl and reversed esters of pethidine are both characterized as μ-agonists,[15] the stereochemical SA data discussed above indicate that they represent distinct classes of opioid ligand which differ in their modes of association with receptors.

All four diastereoisomers of α-methyl *cis*-3-methylfentanyl **11** have been obtained by synthesis using the (+) and (−) intermediates **6**.[5] The pair derived from (+)-**6** (3*R*,4*S*) had high potencies (10–15 × fentanyl, tail-withdrawal test in rats) while those from (−)-**6** (3*S*,4*R*) were both about one-fifth as active as the parent compound—results consistent with those of *cis*-3-methylfentanyl itself. It is evident that the presence of an α-methyl group has little influence on activity.



11

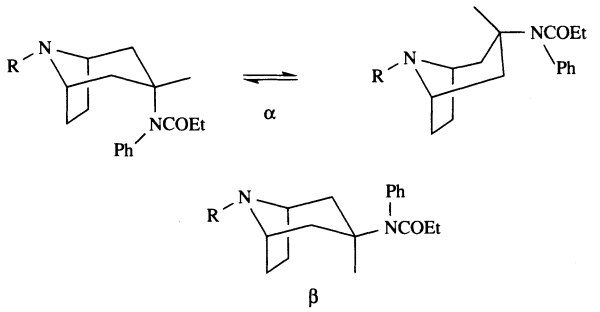
Chemistry. The relative configurations of the diastereoisomeric 3-methylfentanyls may readily be established from the dimensions of the 4-H ^{1}H-NMR signal (see **12**). Proton NMR and ^{13}C-NMR (notably the C-5 resonance) data likewise allowed the 3-allyl and 3-propyl congeners to be characterized stereochemically.[13]

12

4-H resonance 4.4 ppm doublet (12.5 Hz) of triplets (5 Hz) (i.e., one large, two small couplings)	4.53 ppm triplet (12.5 Hz) of doublets (4.5 Hz) (i.e., two large, one small coupling)

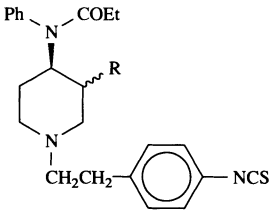
Bridged 2,6-bimethylene congeners of fentanyl have been made from 3-tropanone and provide further examples of stereoselectivity of action in this group.[16,17] ^{1}H-NMR data (similar to work on 3-methylfentanyl and the tropane analogue of pethidine)[18] gave evidence of configuration and preferred conformation (boat for α-isomers). α/β-Pairs with a variety of N-substituents were tested as antinociceptive agents in the mouse tail-flick test (Table 14.2). The β-forms of the

TABLE 14.2.
Antinociceptive Activities of Some Tropane Analogues of Fentanyl in Mice by the Tail-Flick Test[16]



R	Isomer	AD_{50} (mg/kg sc)
Me	α	> 100
Me	β	> 100
CH_2Ph	α	35.2
CH_2Ph	β	1.8
$(CH_2)_2Ph$	α	2.22
$(CH_2)_2Ph$	β	0.047
Fentanyl	—	0.024

N-benzyl and N-phenethyl derivatives were substantially more potent than the α-diastereoisomers, results which support the *eq*-NPhCOEt chair as the active conformation of fentanyl. The isothiocyanate analogue of fentanyl **13a** (FIT), developed as a ligand that would bind covalently to opioid receptors (affinity labeling, page 436), is effective in this regard but selective for δ-sites—a remarkable result, since the parent compound is a μ-agonist.[19] Extension of this work led to the synthesis of the *cis*-3-methyl analogue **13b**.[7] The *dextro* antipode (super-FIT)

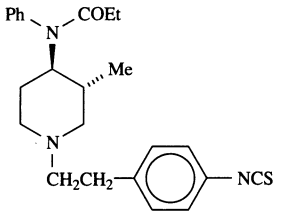


(a) R = H; (b) R = *cis*-Me
13

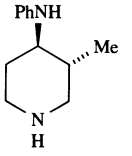
bound to rat brain membranes with ten times the potency of FIT, again with specificity of δ-sites (assays involved measuring the number of [^{3}H]DALAMID binding sites remaining after incubating the tissue with the affinity label: DALAMID is a δ-ligand). In NG 108-15 hybrid cell membranes (rich in δ-sites), it was about 50 times more effective than its *levo* antipode. In spite of its δ-selectivity in binding experiments, super-FIT proved about nine times more active than morphine and 15 times more active than FIT in the mouse hot-plate assay. Both

FIT and super-FIT behaved as partial agonists in regard to inhibition of δ-receptor coupled adenylated cyclase in NG 108-15 membranes (super-FIT was 5–10 × FIT and 100 × levo **13**b). Tritiated super-FIT is recommended for studies of δ-opioid receptors. The absolute configuration of super-FIT is 3*R*,4*S* (X-ray analysis of intermediate **6**), i.e., identical with that of the eutomer of *cis*-3-methylfentanyl. The configurational requirements of μ- and δ-opioid receptors toward this type of ligand are therefore the same, evidence of their similar nature. Antipodes of the *trans*-diastereoisomer **14** have also been reported.[20]

Dextro *trans*-**14** and (+)-*cis*-**13**b (SUPERFIT) were equipotent (rat brain membranes) or close in potency (NG108-15 membranes) in their ability to acylate [^{3}H]DADL (δ) binding sites—*levo* enantiomers were considerably less potent. Only (+)-*cis*-**13**b had antinociceptive activity *in vivo*. Dextro *trans*-**14** has the 3*S*,4*S* configuration (X-ray analysis of the 2,4,6-trinitrobenzesulfonic acid salt of its dextro precursor **15**), hence the *cis*- and *trans*-eutomeric forms share the 4*S*-configuration.



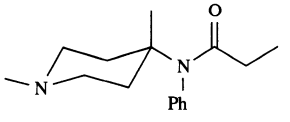
14



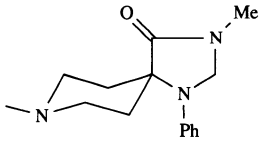
15

Computational studies of fentanyl and its analogues have been reported.[21,22] By combined use of X-ray crystallographic data and AIDA (Aid in Interactive Drug Analysis), a modular processing system designed for modeling of small drug molecules up to about 200 atoms, conformational energy maps of fentanyl, its axial 3-methyl analogue *c*-**12**, carfentanil **9**a, lofentanil (levo **9**b), and the equatorial 3-methyl analogue of lofentanil were computed. Fentanyl had the largest conformational flexibility of the group and this may be the reason why it is the least active member. In terms of collision theory, the activity-enhancing influence of axial 3-methyl may be explained by its ability to reduce the energetically allowed conformational domain (as does equatorial 3-methyl, hence axial 3-Me may also provide an additional binding contribution).

In the solid state the 4-NPh moiety of fentanyl adopts an equatorial conformation with respect to the piperidine ring possessing an α-oriented phenyl ring (**16**)[2,25]—an arrangement probably favored also in the solute state (^{1}H-NMR and computation evidence). Evidence that this geometry is required for receptor uptake is provided by the high potency of the spirane **17** (450 × pethidine) in which N-Ph

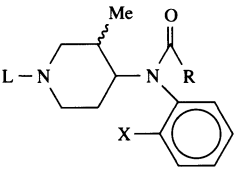


16



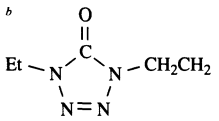
17

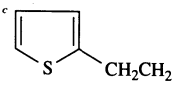
TABLE 14.3.
Antinociceptive Potencies of Some *cis–trans* Pairs of 3-Methylfentanyl Derivatives[27]

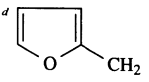


Isomer (*rac*)[a]	L	R	X	ED_{50} (mg/kg) mouse hot-plate	More potent isomer
c	2-phenethyl	CH_2OMe	H	0.0016	*c*, ×25.6
t				0.041	
c	2-phenethyl	CH_2OMe	F	0.0041	*t*, ×5.9
t				0.00069	
c	tetrazolylethyl[b]	CH_2OMe	F	0.098	*c*
t				inactive	
c	thienylethyl[c]	CH_2OMe	F	0.00056	*c*, ×4.8
t				0.0027	
c	2-phenethyl	CH_2OMe	Cl	0.078	*t*, ×16.3
t				0.0048	
c	2-phenethyl	CH(Me)OMe	H	0.035	*c*, ×16
t				0.575	
c	thienylethyl	CH(Me)OMe	H	0.0119	*c*, ×2
t				0.024	
c	thienylethyl	CH(Me)OMe	F	0.0057	*c*, ×4.2
t				0.024	
c	2-phenethyl	CH(Me)OMe	OMe	inactive (5)	*t*
t				0.651	
c	2-phenethyl	furoyl[d]	H	0.005	*c*, ×16
t				0.082	
c	tetrazolylethyl	furoyl	F	0.305	*c*
t				inactive (5)	
c	thienylethyl	furoyl	F	0.004	*c*, ×6.3
t				0.025	
c	2-phenethyl	furoyl	OMe	0.217	*t*, ×2
t				0.118	
c	2-phenethyl	furoyl	Cl	1.96	*t*, ×8
t				0.247	
Fentanyl				0.018	

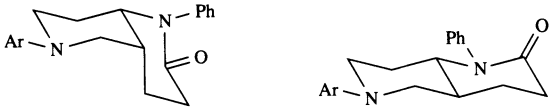
[a] Configurations established from evidence of *c*:*t* ratios obtained in Schiff base reductions, NMR, and order of elution of isomeric diamines on silica (*cis* less polar than *trans*).

[b]


[c]


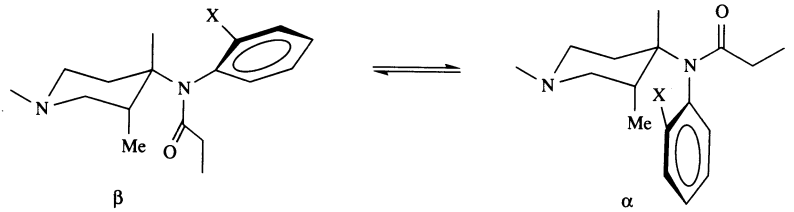
[d]


is locked into an α-disposition (**16** and **17**),[23,24] and the lack of activity in the β-phenyl bicyclo-analogues (**18**).[26]



18 Ar = $(CH_2)_2Ph$

Further clues derive from potency comparisons between a series of *cis–trans* 3-methylfentanyl pairs (Table 14.3).[27] While the anticipated superior potency of the *cis*- over the *trans*-member is generally observed, there are several cases in which the *trans*-derivative has the higher activity. In all such cases the anilido N-phenyl carries and *o*-placed substituent. One may argue that the α-aryl conformation **19** is unfavored in *cis*-derivatives as a result of nonbonded interactions



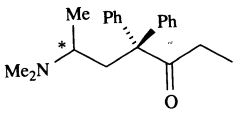
19

between axial 3-Me and the *ortho*-substituent X, whence the biologically less appropriate β-arrangement has the higher population. The α-aryl conformation of the corresponding *trans*-derivative is not so destabilized (equatorial 3-Me is well removed from the group X) and the *trans*-form may therefore bind to a receptor in this state more effectively than its *cis*-partners in spite of the less favorable disposition of its 3-methyl group.

A recent review by Anaquest authors on the evolution of the fentanyl group of analgesics includes a discussion of conformationally restrained analogues.[205]

14.3. Methadone and Related 3,3-Diphenylpropylamines[28]

This group constitutes the chief examples of acyclic opioid ligands (nonpeptide). The prototype member **20**, characterized as a μ-ligand,[15] was introduced into clinical practice in 1946. Today it is employed chiefly for the ambulatory maintenance of patients dependent on heroin that are associated with severe abstinence phenomena (*Martindale* **29**, page 1310).



20

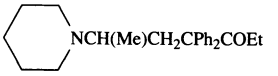
Methadone and most of its congeners have a single chiral center and provide a rich field of examples of stereochemical selectivity in analgesics, with potency and morphine-like side effects residing mainly in one antipode of each enantiomorphic pair. The methadone group is of historic importance in that it was the first analgesic class to be subjected to detailed stereochemical study and to consideration of how enantiomers might differ in their binding interactions with opioid receptors. Methadone was resolved in 1948,[29] and Thorp *et al.*[30] were the first to report the superior activity of the *levo*-form, a fact soon confirmed by others; in Janssen's compilation[31] the potency ranges 1.4–2.3 are given for (−)-methadone and 0.06–0.15 for (+)-methadone (*rac*-methadone = 1) in a variety of animal tests. Mouse hot-plate data obtained at the National Institutes of Health, Maryland [ED_{50} (mg/kg) were *rac*, 1.6; *levo*, 0.8; *dextro*, 25.7] showed the levo to be twice as active as the racemate with a somewhat modest eudismic ratio typical of many chiral molecules of noncyclic structure.[32] In man, 4–6 mg of (−)-methadone were found to be as effective as 7–9 mg of the racemic mixture against postoperative pain.[33] In binding experiments (displacement of [^{3}H]-naloxone or dihydromorphine from rat brain membranes), (−)-methadone was more effective than its antipode by factors of 10 to 50.[34–36] Terenius[37] employed antipodal forms of methadone to demonstrate the stereospecific binding (SSB) of [^{3}H]-dihydromorphine.

A number of authors have studied the comparative phamacology and pharmacokinetics of antipodal methadones. Misra and Mulé[38] challenged the generally accepted view of the potency difference between the two isomers being due to receptor events and claimed that the phenomenon may be attributed to (1) formation of an apparently active metabolite in rat brain from the *levo* but not from the *dextro* isomer, (2) a significant difference between the half-lives of the isomers in rat brain and plasma, and (3) differential stereospecific *N*-dealkylation (a major route for *levo* but not for *dextro* methadone). Sullivan and others[39] found, however, that at equal analgesic dosage, brain and plasma concentrations of (+)-methadone were at least 25 times greater than those of the *levo* isomer, while no qualitative differences were observed between isomers with respect to *in vivo* metabolic pattern or *in vitro* *N*-demethylation rates.

Studies of the binding of methadone antipodes to α_1-acid glycoprotein (AAG, AGP), the major binding protein for neutral and basic drugs, have been conducted.[40,41] In the 1990 work, free fractions of methadone in plasma (measured by a GC assay)[42] were *rac* 12.7, (+) 10.0, and (−) 14.2%. A significant correlation was obtained between the binding ratio (bound/free) for *rac*-drug and the total AAG concentration. Binding was traced to the orosomucoid 2A variant of AAG.

Enantiomer activity differences are also found for congeners of methadone which share similar chiral centers (Table 14.4). Of these compounds, phenadoxone was used clinically as the racemic mixture during the 1950s. The piperidine analogue (**21**, dipipanone) is still in use in the UK as a tablet formulation with the antiemetic cyclizine—there is no published information on its enantiomers.

Isomethadone (**22**), a structural isomer of methadone in which the chiral center

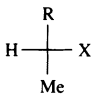


21 dipipanone

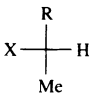
α β
$Me_2NCH_2CH(Me)CPh_2COEt$

22

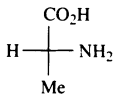
TABLE 14.4.
Activities in Mice and Configuration of Methadone and Thiambutene Analgesics[a]



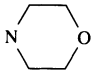
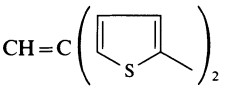
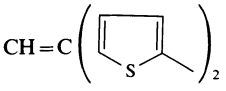
R-series



S-series



R-(−)-alanine

R	X	Isomer	Activity[b]	Ref.
CH_2CPh_2COEt (methadone)	NMe_2	*R*-(−)	180	30, 31
		S-(+)	10	
$CH_2CPh_2SO_2Et$	NMe_2	*R*-(−)	180	43
		S-(+)	10	
CH_2CPh_2COEt (phenadoxone)		*R*-(−)	195	44
		S-(+)	5	
	NMe_2	*S*-(−)	30	45
		R-(+)	170	
	NEt_2	*S*-(−)	50	58
		R-(+)	120	

[a] From Ref. 46.
[b] (±)-Methadone = 100.

is β instead of α to the basic nitrogen atom, has also been resolved[47] and its enantiomorphs found to differ sharply in antinociceptive potency (eudismic ratios are generally greater than those for methadone, a result which may reflect the greater conformational mobility of the latter compound).[48] In a clinical trial against postoperative pain, (−)- and *rac*-isomethadone were, respectively, equal to and one-third as potent as morphine.[49] As in the methadone group, congeners of isomethadone also display antinociceptive potency differences between enantiomers (Table 14.5). Two compounds of this group are in clinical use in the form of a specific isomer. The morpholino derivative moramide is of special interest because of its high eudismic potency ratio. The (+)-isomer is about 700 times more potent than the *levo* form in the mouse hot-plate test and is 18.5 and 40.5 times more active than morphine in mice and rats, respectively.[52] The *dextro* isomer, dextromoramide (Palfium), is the clinical use (*Martindale* **29**, page 1300). It is an analgesic of high oral potency, although less active in humans than in animals; one report of postoperative pain relief equates 5 mg of dextromoramide with 10 mg of morphine, with shorter duration than the opiate.[54] Its side effects are similar in degree to those of morphine.[28]

Dextropropoxyphene **23** is also in clinical use as a single stereoisomer. The corresponding α-racemic mixture (propoxyphene) is a variant of isomethadone in

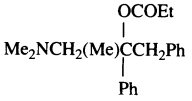
TABLE 14.5.
Antinociceptive Activities in Rodents and Configuration of Isomethadone and Analgesics with Related Chiral Centers

R-(−)-α-methyl-β-alanine

R	X	Isomer	ED_{50} (mg/kg)[a]	Ref.
CPh_2COEt (isomethadone)	NMe_2	*R*-(−)	1.2	32
		S-(+)	49.8	
$C(OCOEt)PhCH_2Ph$	NMe_2	α-(±)	25.4	49
(propoxyphene)		α-3*R*-(+)	7.5	
N(COEt)Ph	$N(CH_2)_5$	*R*-(−)	9.0	51
(phenampromide)		*S*-(+)	36.0	
$CPh_2CON(CH_2)_4$	$N(CH_2)_4O$	*R*-(+)	0.12	52
(moramide)		*S*-(−)	85	
2-Pyridyl analog of phenam-	$N(CH_2)_5$	*S*-(+)	13	53
promide (propiram)		*R*-(−)[b]	18	

[a] Mouse hot-plate test except for phenampromid antipodes, which were tested by the rat tail-flick procedure, and propiram isomers, examined by a radiant heat method in mice.
[b] Although the marginally weaker antipode, it showed the greater pharmacological resemblance to morphine.

which the two phenyl groups are situated on adjacent carbon atoms; in doses of 20 to 40 mg/kg it raised the pain threshold of rats to a similar degree as did 2 mg/kg methadone, while its (+)-enantiomer was twice as effective as the racemic form.[55] In the mouse hot-plate test, ED_{50} values were 27.3 and 8.3 mg/kg for *rac*- and (+)-**23**, respectively (cf. *rac*-methadone 1.6).[32] Clinically dextropropoxyphene

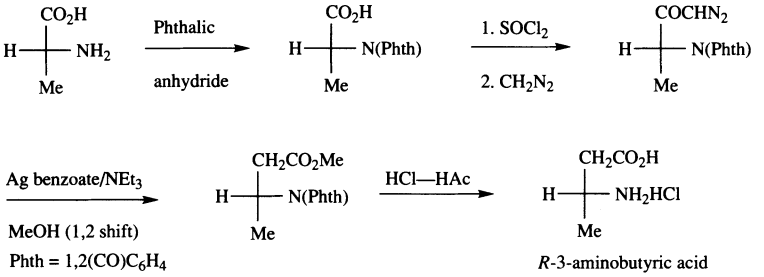


23

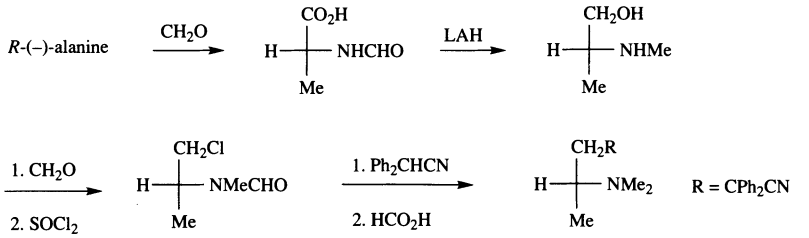
rates as an analgesic of codeine-like potency[56] and preparations containing it mixed with aspirin or paracematol have been widely prescribed for many years for the relief of mild to moderate chronic pain (*Martindale* **29**, page 1300).

In both the methadone and isomethadone groups, there is a relation between the absolute configuration of the eutomeric forms (Tables 14.4 and 14.5), a finding which has stimulated much speculation on ligand–receptor interactions.[28] Evidence of absolute configuration has been provided almost exclusively by chemical methods.

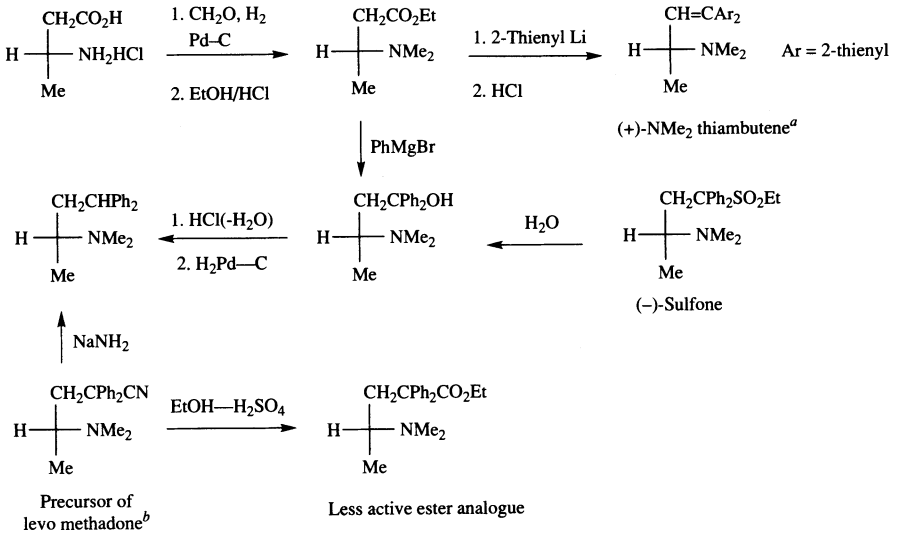
Chemistry. The key intermediate for the methadone studies was optically active 3-aminobutyric acid derived from *R*-(−)-alanine by the sequence of Scheme 14.1. Rearrangement of the diazoketone in the 1,2-shift step was subsequently shown to proceed with retention of configuration by synthesis of (−)-methadone nitrile from *R*-(−)alanine by reactions that did not touch the chiral center (Scheme 14.2).



Scheme 14.1. (46)



Scheme 14.2. (57)



[a] NEt_2 analogue obtained by replacing CH_2O by MeCHO in the reductive alkylation step.
[b] (−)-phenadoxone, the morpholino analogue off levo methadone, was 64 times as active as its (+)-antipode (electric grid assay); it was correlated with levo methadone by study of molecular rotations in various solvents.(44)

Scheme 14.3.

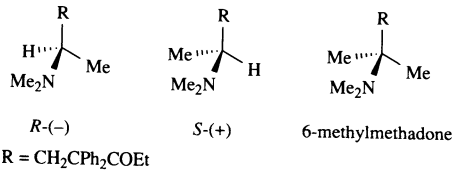
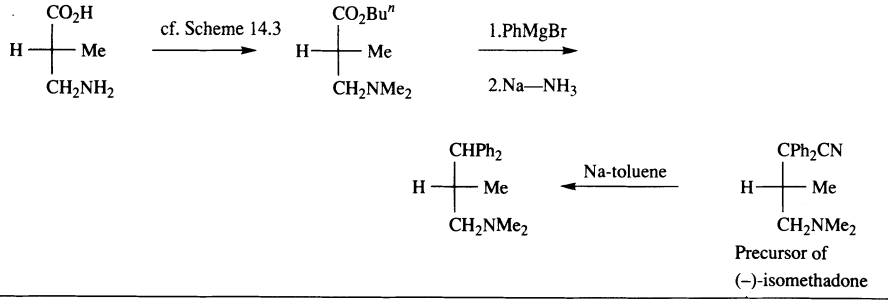


FIGURE 14.1. Tetrahedral representation of *R*-(−)-, *S*-(+)-methadone, and 6-methylmethadone.

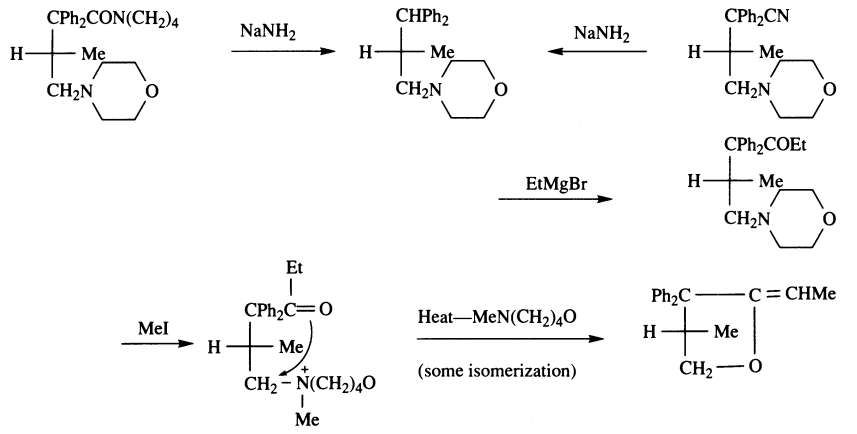
Scheme 14.3 summarizes the use of optically active *R*-3-aminobutyric acid in correlating the eutomers of methadone, its sulfone analogue, and the thiambutenes.

When these relationships were first established, the concept of a three-point fit of ligand molecule at the receptor surface provided a simple interpretation of the antipodal potency ratios seen for methadone and its congeners. In such a situation only one member of an antipodal pair will be able to present the required configuration to the receptor surface. Hence, if Me, NMe_2, and R are taken as the significant pharmacophoric groupings of methadone, chiral methyl is correctly oriented in the *R*-(−)-isomer and incorrectly in *S*-(−)-methadone (Fig. 14.1). To test this concept, 6-methylmethadone (which contains methyls oriented both as in *R*- and *S*-methadone) was examined and its inactivity (as agonist and antagonist) provided evidence that an incorrectly placed methyl prevents drug–receptor interaction.[58] The role of the α-methyl group in *R*-(−)-methadone was thus deduced as minor in terms of ligand binding, although it may have an advantageous influence on the attainment of the active conformation of the drug, since the desmethyl analogue, normethadone, is significantly less potent than methadone itself.

Similar configurational studies have been carried out in the isomethadone series (Table 14.5). A sequence involving *R*-(−)-α-methyl-β-alanine was used to establish the configuration of antipodal isomethadones (Scheme 14.4). The approach to the configurational assignment of dextromoramide was first to relate it to the corresponding ethyl ketone and then to show that both the ketone and (−)-isomethadone gave identical (−)-tetrahydrofuran derivatives on pyrolysis of the methiodides (Scheme 14.5); these assignments supported those of earlier work based on the evidence of molecular rotational changes with solvent polarity.[59,60] The eutomer of phenampromide also fits the configurational pattern of levo isomethadone and dextromoramide, relationships in sharp contrast with those of (+)-diampromide and (−)-methadone (see later). Portoghese's route starts from an antipode of *N*-phenylalanine of known configuration (Scheme 14.6).[61]



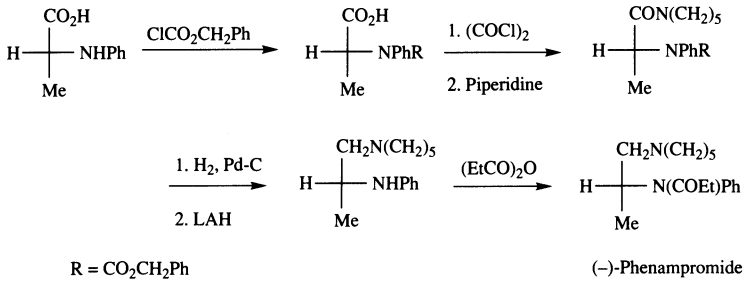
Scheme 14.4.



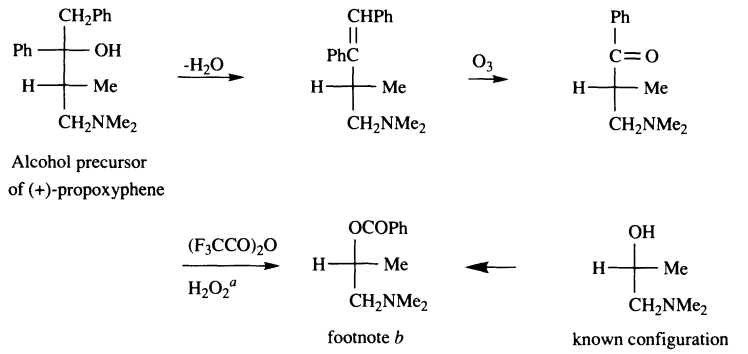
Scheme 14.5.

Propoxyphene has chiral centers at both C-2 and C-3, with the latter equivalent to that of isomethadone. Pohland and others[62] showed the C-3 centers of (+)-propoxyphene and (−)-isomethadone to be identical (3*R*) by a sequence that rests on retention of configuration during a Baeyer–Villiger oxidation step (Scheme 14.7). In subsequent work, this assumption was justified by a correlation that did not involve the C-3 center and employed a Mannich base intermediate.[63] The C-2 configuration of (+)-propoxyphene was established by a lengthy sequence in which the compound was degraded to (+)-2,3-diphenylpropane of known (*S*) configuration.

Configurational studies undertaken up to this point satisfactorily correlated the eutomeric forms of methadone and isomethadone type respectively. Evidence of deviations from configurational identity among analgesics with apparently close relationships to methadone was available, however, early in the postwar investigations of acyclic analgesics. Thus the eutomer of the ethyl ester analogue of methadone was formed from the nitrile precursor of the *less* active (+)-isomer of methadone (Scheme 14.3).[64] Several studies have confirmed these findings and established that the ester is a true morphine-like analgesic.[65] The α-methadols provide another example of analgesics that fail to correlate stereochemically with methadone, as will be discussed.



Scheme 14.6.



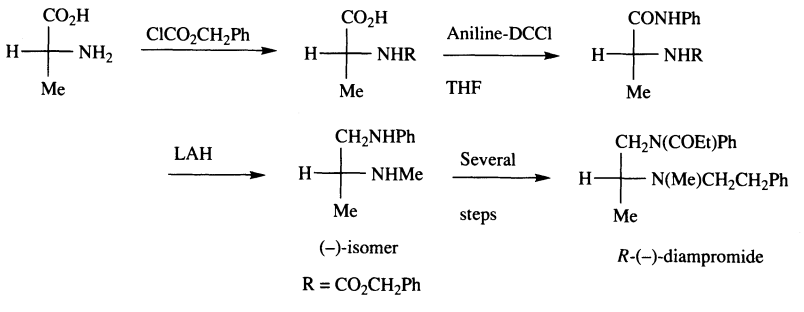
[a] Baeyer-Villiger step.

[b] Related to levo isomethadone. (Ref. 63)

Scheme 14.7.

Anomalies of this kind, although noted,[58] did not receive serious consideration until Portoghese provided yet another example in his stereochemical studies of diampromide. This compound is a member of a group designed as methadone analogues by the formal replacement of one phenyl group and its attached quaternary carbon by nitrogen[28]; it turned out, however, that the eutomer of diampromide is identical in configuration with *S*-(+)-alanine, and hence has an opposite chirality to that of *levo*-methadone.[66] The configurational sequence (Scheme 14.8) involves one-step amide to amine and *N*-carbobenzyloxy to *N*-methyl interconversion and links *R*-(−)-alanine to the distomer of diampromide. These stereochemical relationships extend to several analogues of diampromide (Table 14.6); it is to be noted, however, that configurational preferences of the opioid receptor for phenampromide (a basic anilide related to isomethadone) and the parent diphenylpropylamine analgesic are the same. In the data of Table 14.6, only the *N*-benzyl-*N*-methyl derivative shows a eutomer twice as potent as the racemic mixture, suggesting that distomers of the other examples potentiate the activity of their antipodes.

Configurational data on diampromide and its relatives therefore indicate that the assumed analogy with the methadone class in invalid and that the two classes probably differ in their binding modes at the receptor.[69] Probable conformations



Scheme 14.8.

of methadone and the *N*-benzymethylamino anilide of Table 14.6, based on spectroscopic and X-ray crystallography studies, have been proposed.[68,70]

Methadols and their acetates, derived by reduction of methadone to create a second chiral center, provide another set of stereochemical SAR data (Table 14.7). Evidence of the configuration of the C-3-OH center of the methadol has been obtained by ^{1}H-NMR analysis of derived 2-ethyl-3,3-diphenyl-5-methyltetrahydrofurans[75,76] and application of Prelog's rule.[77] Indirect evidence of C-3 stereochemistry is obtained from the fact that reaction of α- and β-methadol (the two *rac*-distereoisomers) with cyanogen bromide gives two different tetrahydrofurans, respectively, as are also produced on pyrolysis of corresponding methiodides. The configurations of the cyclic products (*c*- or *t*-2-Et-5Me) may be deduced from chemical shift differences between the α/β 4-CH_2 protons and the findings applied to the configurations of the precursor alcohols, making the reasonable assumption of the reactions proceeding with inversion at C-6 (see **24**).[75] Similar arguments

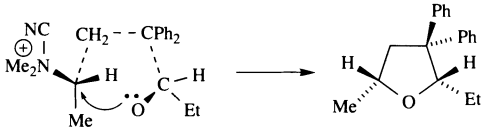


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TABLE 14.6.
Antinociceptive Activities of Enantiomorphs of Diampromide and Related Compounds

MeN.CHMe.CH_2.N.COEt
| |
R Ph

R	Form[a]	AD_{50} values (mg/kg)[b]	*S*/*RS* potency ratio	Ref.
$PhCH_2$	*RS*-(±)	8	1.86	51[c]
	S-(+)	4.3		
	R-(−)	inactive (50)		
$Ph(CH_2)_2$	*RS*-(±)[d]	3.7	1.0	51
	S-(+)	3.6		
	R-(−)	11.7		
$Ph(CH_2)_3$	*RS*-(±)	12.5	1.4	67
	S-(+)	8.9		
	R-(−)	11.9		
p-$MeC_6H_4.CH_2$	*RS*-(±)	1.6	1.14	67
	S-(+)	1.4		
	R-(−)	inactive (50)		
Me	*RS*-(±)	50[e]	1.7	68
	S-(+)	35		
	R-(−)	40		

[a] $[a]_D$ Sign of base given.
[b] Tail-flick method.
[c] Hot-plate ED_{50} values in mice are (±) 15, (+) 12 and (−) ≥ 40 (Ref. 68).
[d] Diampromide.
[e] Hot-plate ED_{50} values in mice.

TABLE 14.7.
Hot-Plate Activities in Mice of Some Methadols and Normethadols (sc Route)[a]

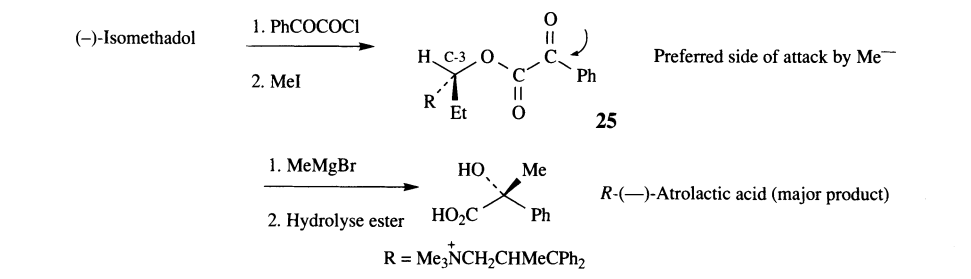
$Me_2N.CHR.CH_2.CPh_2.CHEt$
$|$
OR'

(methadols, R = Me, normethadols, R = H)

Precursor	Form	Configuration	ED_{50} values (mg/kg) Methadols (R′ = H)	ED_{50} values (mg/kg) Acetylmethadols (R′ = $COCH_3$)
R-(−)-Methadone	α-(+)	6*R*:3*R*	24.7	0.3
(0.8)[b]	β-(−)	6*R*:3*S*	7.6	0.4
S-(+)-Methadone	α-(−)	6*S*:3*S*	3.5	1.8
(25.7)[b]	β-(+)	6*S*:3*R*	63.7	4.1
(±)-Normethadol HCl		*RS*	9.88	—
(±)-Normethadol-(±)-tartrate		*RS*	10.3	—
(+)-Normethadol-(+)-tartrate[c]		*R*	17.7	—
(±)-Acetylnormethadol HCl		*RS*	—	4.44
(+)-Acetylnormethadol HCl		*R*	—	2.7

[a] From Refs. 73 and 74.
[b] ED_{50} value.
[c] α-(+)-Methadol and (+)-normethadol correlated sterically at C-3 by fact of their near-identical ORD curves.[74]

were advanced by Portoghese and Williams[77] and the *cis*-geometry of the tetrahydrofuran produced from α-methadol was subsequently confirmed by X-ray crystallography.[78] Inspection of chiral centers shows that reaction leading to a *cis*-product requires a 6*S*-3*S* configuration for α-(−)-methadol. Scheme 14.9 illustrates how Prelog's rule of asymmetric induction has been applied to establishing the C-3 configuration of the methadols and isomethadols.[77,79] The optically active secondary alcohol is first converted to a phenylglyoxylate ester by reaction with benzoylformyl chloride. This ester (after *N*-methylation) is assumed to favor a conformation in which the carbonyl groups are antiparallel and the smallest group of the alcoholic chiral center (C-3) is eclipsed by the ketone carbonyl. A methyl car-



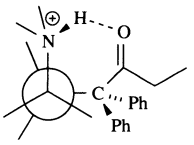
Scheme 14.9.

banion, from a Grignard reagent, will prefer to approach the ketone carbonyl from the side of the smaller of the two remaining groups attached to C-3 (i.e., Et in **25**). Hence *R*-(−)-atrolactic acid is the major product if the C-3 configuration is as shown (*R*), while the *S*-(+)-antipode results if opposite chirality applies.

The complete configurational relationships of the methadone, methadols, and acetyl methadols are shown in Table 14.7 together with mice hot-plate data. From these results it appears that the C-3 rather than C-6 configuraion is of prime importance concerning the activities of the methadols. Thus, the two more active isomers of α- and β-methadol both have the 3*S*-configuration, while the most active isomethadol, β-(+), belongs to the same C-3 steric series.[79,81] It follows that the *S*-member of methadol enantiomers lacking asymmetry at C-6, the normethadols, should be the more potent antipode, and this prediction has been confirmed (Table 14.7). The relative activities of (+)- and (−)-α-methadol are reversed when the alcohols are acetylated (Table 14.7), and the α-(+)-isomer derived from levo methadone is the more potent ester. This remarkable inversion of stereoselectivity may be interpreted in terms of the C-6 center asserting its dominating role. Alternatively, it may be considered due to esters requiring a *R*-C-3 center for optimal activity. The latter is more probable because the same steric reversal occurs in the case of normethadols and their acetate esters, with the less active *R*-alcohol yielding the more active acetate. Although the methadols and acetylmethadols are closely related in structure, their conformations at the receptor may well differ as a result, for example, of a hydrogen-bond donor group (OH) in one ligand being replaced by an acceptor group (OCOMe) in the other. Strong intramolecular hydrogen bonding has been demonstrated in diastereisomeric methadol bases (in CCl_4) and hydrochlorides (in $CHCl_3$), and preferred conformations have been proposed on this evidence.[82] This interpretation of differing stereoselectivities of structurally related analgesics in terms of differing binding models is akin to that proposed in the case of the more active enantiomorphic cholinergic agents (+)-muscarine and (−)-muscarone, which have opposite configurations at the C-5 center (Chap. 8, page 240).[83] It is interesting that α-(−)-acetylmethadol (6*S*,3*S*) is far less potent than the dextro isomer after intraventricular administration, and it has been suggested that the analgesic effects of the levo isomer are due to a metabolite rather than the intact drug itself.[84]

Sullivan *et al.*[85] found that α-(−)*N*-desmethylmethadol and (−)-methadone were similarly potent as analgesics; hence, the former represents an active metabolite of methadone (see page 511). It is possible that the *in vivo* formation of the methadol (and the *N*-desmethyl analogue) may account for the relatively better effectiveness of (+)-methadone as an analgesic when given orally rather than parenterally.[86]

Several papers dealing with the conformation of methadone and its relatives have been published in attempts to justify or refute hypotheses about the "active" forms adopted by the highly flexible molecules of this type at opioid receptors. Of the various techniques employed, X-ray crystallography provides information about solid-state conformation that may given clues to probable or possible molecular orientations in solution and at the receptor site. Conformations of methadone and its congeners that mimic morphine require close approach of the C-3 oxygen function and amino nitrogen atom. N/C_3-O proximity is only possible if the C(4)-C-(5)-C(6)-N torsion angle (τ) is less than 120° (see **26**). This parameter



26

Newman diagram,
molecule viewed along C(6)–C(5) bond
(also applies to **29** and **30**)

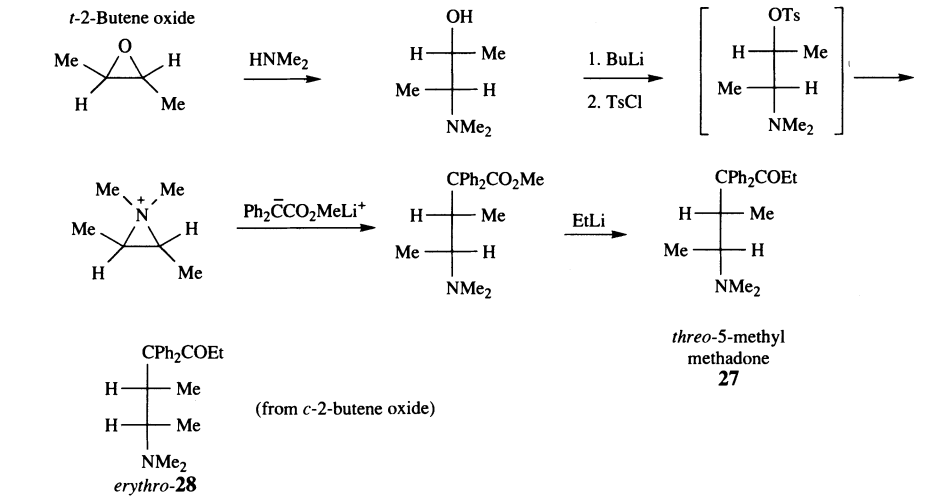
is therefore of special significance in detecting intramolecular nitrogen–oxygen interactions, and data on this point are summarized in Table 14.8.

Only in the case of methadone base (item 1) is the torsion angle τ of a value consistent with close N/C-O contact; the N to carbonyl carbon (C-3) distance of 291 pm is, in fact, almost 30 pm less than the sum of the van der Waals radii of carbon and nitrogen. This distance is only possible at the expense of introducing other interactions in the molecule that are repulsive and the conformation is probably stabilized by the electronic attraction between the nitrogen lone-pair of electrons and the carbonyl carbon atom (N: ··· C=O). The N to O(3) distance and torsion angle found for α-methadol hydrochloride (item 4) is also indicative of an interaction between nitrogen and oxygen functions and there is pK_a[77,80] and IR[82] evidence of intramolecular hydrogen bonding (N^+-H ··· O-C_3) in this salt. In all other cases, however, torsion angles have values that show that the C(4)-C(5)-C(6)-N chain is fully extended with oxygen and nitrogen functions well separated. There is now evidence, in fact, that an antiperiplanar-like disposition of Ph_2COEt and $^+NHMe_2$ groups is one of the pharmacophoric dispositions of methadone and

TABLE 14.8.
C(4)–C(5)–C(6)–N Torsion Angles (τ) and N to O(1) Distances of Certain Diphenylpropylamine Analgesics[a]

Item	Compound	τ°	N to O(1) in pm
1	(−)-Methadone (**1**) base	−68.5 −69.8	3.43
2	(−)-Methadone HBr	−146.3	3.81
3	(−)-Isomethadone[b] HCl	−152.5	3.69
4	α-Methadol[c] HCl	116.1	3.159
5	α-Methadyl acetate HCl	−146.2	4.88
6	Dextromoramide[d] acid tartrate	−166.5	not reported
7	Dextromoramide base	−159.4	not reported

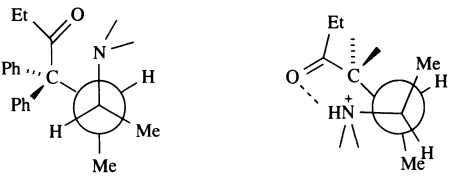
[a] From Refs. 87–89.
[b] $Me_2NCH_2CHMeCPh_2COEt$.
[c] $Me_2NCHMeCH_2CPh_2CH(OH)Et$.
[d] Table 14.5.



Scheme 14.10. (Only one antipode depicted.)

related analgesics. This conclusion was reached from studies of the *threo*-**27** and *erythro*-**28** 5-methylmethadone diastereoisomers prepared by stereospecific reaction sequences (Scheme 14.10).[90]

The authors of this work suggest that the striking difference in analgesic potency between the isomers (*erythro* 5.4 × methadone, *threo* inactive, MHP) might be traced to a difference in the conformational equilibria. As in previous investigations of acyclic diastereoisomers,[91] ^{1}H-NMR spectroscopy provided key data on this point. The magnitudes of the vicinal coupling constants between the C-5 and C-6 protons ($^3H_{5,6} \leqslant 1$ Hz for *threo*, 6–8.3 Hz for *erythro*) show that, while the contribution of the antiplanar form **29** must be small for the *threo*-isomer, it is probable that all three staggered or near-staggered conformers of the *erythro*-isomer are significantly populated. NMR and pK_a data suggest that the inactive *threo*-racemate (**27**) exists chiefly in a hydrogen-bonded conformation (**30**) that



29 **30**

does not possess the requisite geometry for association with the opioid receptor (this arrangement was not, however, found in the solid state).[92] In contrast, the *erythro*-isomer offers much greater latitude for effective binding because of its increased conformational flexibility. A circular dichroism and NMR investigation of methadone and isomethadone provided evidence of the greater conformational flexibility of the former analgesic, and this conclusion was advanced as a possible cause for the twofold greater enantiomeric potency ratio of (−):(+) isomethadone,

and also the observation of inversion of receptor stereoselectivity in the methadone series ($NCHMeCH_2C$) but not in the isomethadone (NCH_2CHMe) group of analgesics (see page 517).

The CD data shown in Fig. 14.2 illustrate the radical solvent-induced inversion of the sign of the Cotton effect of methadone, a phenomenon not seen for isomethadone base or salts of the two ketones, in demonstration of methadone's high degree of conformational mobility. Similar sign inversion are anticipated from optical rotatory dispersion (ORD) measurements, but available data are confined to a single solvent (dioxane).[93] The results of quantum chemical studies of methadone add further support to the experimental evidence that methadone has several low-energy and therefore readily interconvertible conformers[94,95] and to Portoghese's proposals about receptor interactions of methadone, 5-methylmethadone, and isomethadone.[96]

It is instructive to note that the inactive *threo*-isomer of 5-methylmethadone contains the 5*S*,6*R* stereoisomer, which combines the configurations found in the more active enantiomers of methadone and isomethadone.[90] This observation indicates that the chiral centers do not behave as independent units, but interact with other groups to afford a conformational population that strongly influences the potency of the diastereoisomers. In other words, conformational factors have greater weight than those of absolute configuration in governing drug–receptor binding in diphenylpropylamine analgesics. The antipodal forms of the *erythro*-isomer **28** show an isomeric potency ratio of about 6 in the MHP procedure (relative potencies: levo, 11.3; dextro, 1.8; morphine, 1) and the more active levo isomer has a 5*S*,6*S* configuration (X-ray evidence).[96] More pronounced activity differences were seen after *in vitro* tests while GPI:MVD potency ratios indicated that (−)-**28** was primarily an μ-agonist in contrast to (+)-**28** and levo isomers of methadone and isomethadone, which interacted with both μ- and δ-sites.

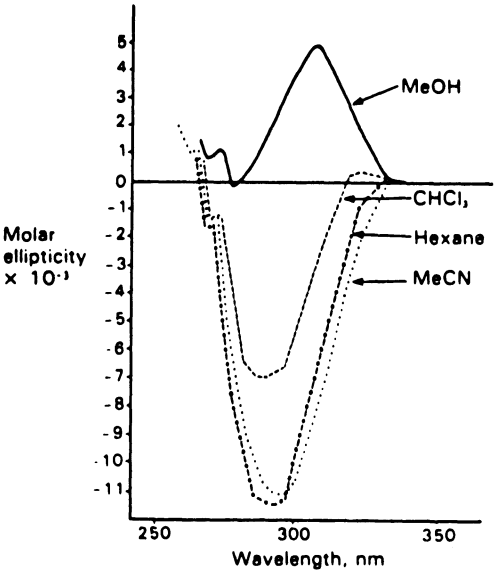


FIGURE 14.2. CD spectra of (+)-6*S*-methadone (0.1% in solvents noted) (after Henkel *et al.*).[204]

From ^{13}C-NMR (CP/MAS) spectra of methadone salts, all compounds were found to crystallize with two different conformers in the asymmetric unit.[97] There was evidence for the near-identity of solute (CD_2Cl_2, DMSO-d_6 solvents) and solid-state conformations—these were of geometries that did not allow intramolecular hydrogen bonds of the $^+NH \cdots O=C$ type.

14.4. Opioid Peptides

The isolation in 1975 of naturally occurring peptides with morphine-like properties[98–100] led to intensive investigations of peptides as potential ligands for opioid receptors, and a vast amount of information on compounds related to Met- and Leu-enkephalin (**31**) and the larger endorphins is now available.[101,102] Since most amino acid components of peptides are chiral (Gly is the chief exception), diastereosiomeric permutations, even in small peptides, are enormous, and attention in this section is confined mostly to groups of peptides that differ in configuration at a single center. Because of the vulnerability of peptides to enzymatic degradation, *in vitro* assay procedures (inhibition of contractions of GPI and MVD, and binding experiments) have dominated this field of opioid research. Work on opioid peptides also provided key evidence for the concept of subclasses of opioid receptors, the enkephalins being prototype ligands for δ-receptors,[103] although suitably modified peptides such as DAGO [Tyr-D-Ala-Gly-N(Me)Phe Gly-OH] are μ-selective.

$(H_2N)Tyr^1\text{-}Gly^2\text{-}Gly^3\text{-}Phe^4\text{-}Met/Leu^5(CO_2H)$

31

The central role played by the Tyr residue of the enkephalins is evident by the fact that D-Tyr analogues are inactive.[104] Strangely, the replacement of Gly^2 of enkephalins by a variety of chiral amino acids of unnatural (D) configuration enhances potency more effectively than the use of the L-enantiomers. GPI data for such analogues of Leu-enkephalin methyl ester (itself one-fifth as potent as Met-enkephalin in this test) reveal D-Ser to be the most effective replacement: D-Ser, 11.0; D-Met, 8.0; D-Ala, 5.6; D-Leu, 1.3; D-Phe, 1.3; potencies relative to Met-enkephalin = 1. The potency of D-Ala^2-Leu-enkephalin in GPI and MVD assays is differentially altered when L-Leu is replaced by the D-amino acid (**32**).

	IC_{50} (nM) values		
	GPI (μ)	MVD (δ)	δ/μ
D-Ala^2-Leu^5-enkephalin	28.7	1.63	17.6
D-Ala^2-D-Leu^5-enkephalin (DADL)	47.8	0.54	88.5

32

Since the GPI is regarded as a μ-rich and the MVD as a δ-tissue, presence of a D-Leu^5 residue not only enhances δ-activity three times over that of the L-Leu^5 peptide, but also enhances δ-selectivity.[106] DADL is now commonly used as a selective δ-ligand. Vas deferentia chronically treated with DADL became 8000-fold less sensitive to this peptide, but showed no cross-tolerance to μ-receptor agonists. D-Amino acids at position 2 of enkephalins increase their resistance to amino-

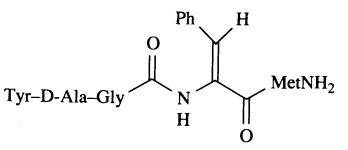
peptidase enzymes, but this factor is not considered the sole reason for their potency-enhancing influence.[107]

The D-Ala^2 unit appears in two peptides thought at one time to be of clinical promise. The D-Ala^2-MeMet^5 analogue of Met-enkephalin (with a methyl substituent on Met-nitrogen) is active after systemic administration in the mouse hot-plate test (ED_{50} μM/kg 1.5; cf. morphine 4.8 and pethidine 11.3) and exceeds morphine in potency at least 100-fold after central administration.[108] In a double-bind clinical trial on surgical patients, the analgesic activity of 70 mg of this derivative (termed met-kephamid) after im injection was significantly greater than that of a placebo and not less than that of 100 mg of pethidine.[109] The potent Sandoz peptide **33** (FX33-824, DAMME) also features D-Ala at position 2. It was 1000 times more active than morphine on a molar basis after central administration,[110] but its side-effect liability in man led to the abandonment of clinical trials.[111]

Tyr^1-D-Ala^2-Gly^3-MePhe^4-Met(O)-ol^5 (FX-33824)

33

Most variations of Gly^3 (including D- and L-Ala) depress the potency of enkephalins, as does replacement of L-Phe^4 by D-Phe^4. Imposition of local rigidity within the Phe^4 unit, as in the Δ-Phe^4 analogues **34** (5 × D-Ala^2-MetNH$_2$ in GPI assay), elevates activity,[112,113] and the significance of this finding is emphasized by comparison of the binding affinities of **34** and its *E*-isomer (prepared by photoisomerization of the *Z*-isomer).[114] The *E*-isomer was 150–260 times less effective than *Z*-**34** in displacing DADL (δ) and DAGO (μ) from rat brain membranes. Likewise, peptides with *Z*-cyclopropyl Phe residues exceeded corresponding *E*-isomers in their binding affinities[115]; remarkably, the 2*R*,3*S*-antipode of the *E*-compound (see **35**) bound to central δ-receptors of MVD but failed to activate peripheral receptors of MVD—it also blocked morphine analgesia in the rat paw test.[116]



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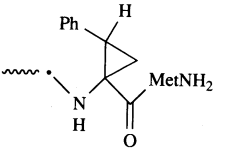


35

Stereochemical requirements at residue 5 are minimal for Leu-enkephalin but more demanding for Met-enkephalin, as is apparent from the GPI activities D-Leu^5 (0.15) and Leu-enkephalin (0.2), and D-Met^5 (0.1) and Met-enkephalin (1.0)[117] (see also DADL/D-Ala^2-leu-enkephalin).

Tyr-D-Nle-Gly-Phe-D (or L)-NleS

36

Hungarian chemists have examined a diastereoisomeric pair (**36**) in which the Gly^2 residue of the enkephalins is replaced by D-Nle (α-Bu^n analogue of Leu) and the carboxylate terminal residue 5 by the D- or L-sulfonic acid analogue of Nle

(NleS).[118,119] The D-Nle^2, L-NleS^5 peptide was 500 times more effective than the D-Nle^2, D-NleS^5 isomer in the MVD test, and had a high preference for δ-sites as judged by its MVD to GPI potency ratio of 40.5. The less active D-NleS^5 isomer showed no δ/μ discrimination.

The *dermorphin* group of opioid peptides, found in the skin of certain South American frogs, share the amino terminal Tyr residue of enkephalins, but residue 2 is D-Ala (see above) followed by Phe^3-Gly^4 in reverse of the usual order. Dermorphin (**37**) is far more potent (>50 times) than Met-enkephalin in both *in vitro* and *in vivo* assays, and replacement of D-Ala^2 by L-Ala^2 abolishes most of its activity.[120,121] Dehydro derivatives in which Phe (*Z*-configuration) is inserted at positions 3 and 5 of dermorphin depressed potency, in contrast to the potency-raising influence of Phe^4 in enkephalins discussed above.[122]

(NH_2)Tyr-D-Ala-Phe^3-Gly-Tyr^5-Pro-Ser-NH_2

37 dermorphin

14.4.1. Nonalkenic Cyclic Analogues

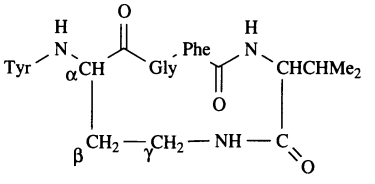
Cyclic analogues obtained by oxidative disulfide bond formation of 2,5-dicysteine analogues of enkephalinamide display large potency increased over Met-enkephalin. The D^{2}D^5 diastereoisomer **38** was nearly as active as its L^{2}D^5 analogue.[123] Corresponding disulfides made from D-penicillamine (β,β-dimethylcysteine) as residues 2 and 5 (DPDPE) are also potent agents and of high selectivity for δ-receptors.[124,125] Because of its properties, DPDPE is well suited as a probe for elucidation of structure and stereochemical features necessary for activity at δ-opioid receptors, and several conformation studies have been reported.[125–128]

Tyr-D-Cys-Gly-Phe-L (or D)-Cys-NH_2 ⟸ -S-S-bridge

38

D^{2}L^5 37.9×, D^{2}D^5 73.3× met-enkephalin in GPI test

Another approach to conformational restraint is that of side chain–carboxy terminus cyclization in which the residue-2 amino acid has an α-substituent capable of interacting with CO_2H of residue 5. An example is **39** made by substituting α,γ-diaminobutyric acid (A_2bu) for Gly^2 of Leu-enkephalin and linking the γ-NH_2 of residue 2 to CO_2H of Leu^5[129]; the D-A_2bu derivative was 17.5 and the L-A_2bu diastereoisomer 0.2 times as potent as Leu-enkephalin by GPI assay, and selective for μ-sites.[130] The open-chain congener with D-norvaline as residue 2 was somewhat less active than the cyclic derivative **39** (10.6× Met-enkephalin) and showed



39

a reduced selectivity for μ-sites.[131] Extension of the bimethylene bridge of **39** to $(CH_2)_4$ and use of D-Leu^5 gave the most active compound of the series at GPI sites [IC_{50} 2.4 nM, Leu-enkephalin 246, D-Nle^2, Leu^5-enkephalinamide (open-chain analogue) 14.6]. A variety of cyclic enkephalins of this kind have now been examined[132] and, with some exceptions, cyclization usually confers preference for μ- over δ-receptors. Highest selectivity for μ-sites in cyclic analogues of this kind is reported for the tetrapeptide **40**, which has a 13-membered ring system.[133] Data on analogues of **40** involving configurational inversion at positions 2,3, and 4, and N^{α}-methylation of Phe^3, established a relationship between **40**, and dermorphin-related peptides.[134] It is evident that conformational restriction through appropriate cyclizations permits manipulation of the receptor affinities, selectivites, and efficacies of opioid peptides, and the conformational analysis of cyclic enkephalins has attracted the attention of several groups.[132,134,135,202]

(NH_2)Tyr-D-Orn-Phe-Asp-NH_2

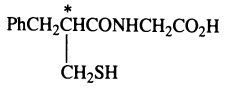
40

Conformational studies of noncyclized opioid peptides by X-ray crystallography,[136], NMR analyses, and quantum-mechanical calculations have been reviewed.[137,138].

A recent review by Schiller[201] on the development of receptor-specific peptides includes sections on cyclic analogues.

Few isomeric comparisons have been made in regard to opioid peptides composed of more amino acid units than those present in the enkephalins, such as β-endorphin (a 61 to 91 fragment of β-lipotropin) and dynorphin (both isolated from the pituitary gland), and peptides found in the adrenal medulla.[139] However, D-Tyr^1, D-Phe^4, and D-Met^5 analogues of β-endorphin are feeble or inactive analgesics, showing that stereochemical requirements of enkephalins and endorphins (residues 61 to 65 are identical with those of Met-enkephalin) are the same for the two groups.[140]

The vulnerability of opioid peptides to enzymatic attack is well known, and earlier structure–activity investigations revealed that resistance could be conferred by a D-amino acid as residue 2, conversion of terminal CO_2H to $CONH_2$, and conformational restraint. Enzyme inhibitors have also been developed that prevent the degradation of enkephalins. The first effective one was thiorphan **41**,[141] a compound which protects enkephalins against enkephalinase (cleaves Tyr^1-Gly^2 bond) under both *in vitro* and *in vivo* conditions and itself displays significant antinociceptive activity, attributed to the potentiation of endogenous enkephalins released in response to noxious stimuli.[142] The affinity of thiorphan for enkephalinase is independent of stereochemistry [inhibitory potency IC_{50} (nM): *S*, 1.90; *R*, 1.60]—in contrast, antipodal forms of *retro*-thiorphan (**42**) display a 100-fold difference in inhibitory activity [IC_{50} (nM): *S*, 210; *R*, 2.33].[143] *RS*-Kelatorphan (**43**) and its *SS*-diastereoisomer inhibit enkephalinase in equal degree, but the *RS*-isomer is also potent against aminopeptidase and dipeptidylaminopeptidase enzymes.[144]



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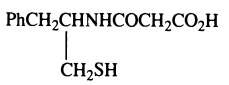

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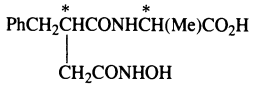


43

14.5. Miscellaneous Opioids

This section comprises groups of compounds of diverse structure with opioid ligand activity which are difficult to classify.

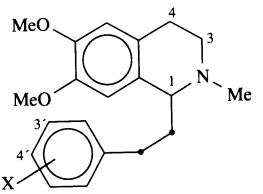
14.5.1. Tetrahydroisoquinolines

The alkaloid papaverine, a constituent of opium, is the parent isoquinoline example of this group. It lacks analgesic properties, but some of its tetrahydro derivatives with a C-1 chiral center had antinociceptive action in mice when tested by the tail-flick assay. Potency levels were low and close to those of codeine. Stereochemical specificity in the series has been established in three cases (4′-NO_2, 4′-Cl, and 3′,4′-dichloro derivatives **44**) with activity restricted to the *R*-(−)-enantiomers (Table 14.9).[145,146] ORD comparisons were used to establish the configuration.

14.5.2. Cyclohexane Derivatives

Several opioid ligands are based on cyclohexane, a ring system that serves to restrict conformational options in all derivatives that contain it. The most notable potencywise are certain 4-aryl-4-dimethylaminocyclohexanols **45**; *trans*-(*E*)-NMe_2/OH isomers have activities several thousandfold greater than that of morphine and fall in the superpotent class of opioid.[147] Treatment of precursor ketones **46** with organometallic reagents gave *E*/*Z* mixtures of tertiary alcohols which were separated by chromatography on silica gel. Configurational assignments were based on ^{1}H-NMR evidence for isomers carrying 4-CH_2R substituents with resolvable methylene proton signals and assumption of a preferred

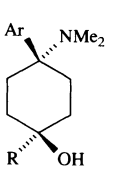
TABLE 14.9.
Antinociceptive Activities of Some Papaverine Derivatives in Mice by the Tail-Flick Assay[145, 146]



44

X	Isomer	Activity (codeine = 1)
4′-Cl	*rac*[a]	1.4
	R-(−)	3.0
	S-(+)	inactive
3′,4′-di-Cl	*rac*	1
	R-(−)	2.1

[a] Methopholine



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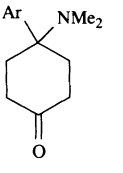
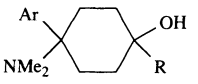


46

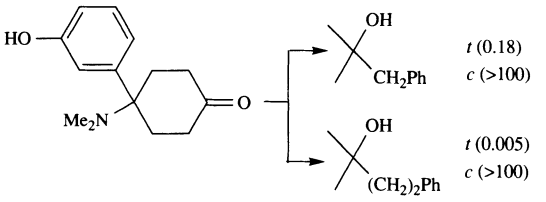
axial conformation of the 4-aryl substituent.[148] An X-ray analysis of the 4-*p*-bromophenyl -1-phenethyl derivative supported assignment of the more potent isomers.[149] Some pharmacological data are shown beneath the general structure **47**. Of special note are the pronounced *E*/*Z* potency ratios and relative activies of *E*-isomers relative to morphine (up to 1.5×10^4). Distinct preference for *E*-stereochemistry was maintained in 4-(*m*-hydroxyphenyl) analogues, but potencies were less than in nonphenolic derivatives (see **48**).[150]



47

Ar	R	Me_2N/OH configuration	ED_{50} (mg/kg sc) mouse TF[a]	*E*/Z ratio
p-ClC_6H_4	Me	*t*	1.0	2
p-ClC_6H_4	Me	*c*	2.0	
p-ClC_6H_4	CH_2Ph	*t*	0.0056	11250
p-ClC_6H_4	CH_2Ph	*c*	63	
p-ClC_6H_4	$(CH_2)_2Ph$	*t*[b]	0.0014	$>7 \times 10^4$
p-ClC_6H_4	$(CH_2)_2Ph$	*c*	>100	
p-ClC_6H_4	$(CH_2)_3Ph$	*t*	0.11	291
p-ClC_6H_4	$(CH_2)_3Ph$	*c*	32	
p-BrC_6H_4	$(CH_2)_2Ph$	*t*	0.0001	7.9×10^4
p-BrC_6H_4	$(CH_2)_2Ph$	*c*	7.9	

[a] Morphine SO_4 1.5.
[b] $CH_2CH_2C_6H_{11}$ analogue 0.014.

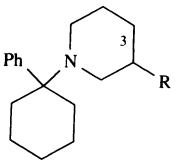


48
Mouse TF ED_{50} values (mg/kg) in parentheses

According to Upjohn workers, Dreiding models of the potent 4-*p*-bromophenyl derivative and fentanyl (**1**) may be arranged to give point-for-point coincidence of all salient structural features including the basic centers, the 4-aryl and *N*-phenethyl aryl ring of fentanyl, and the 1-phenethyl and *N*-anilido(fentanyl)

aryls.[149] Conformations of the *E*-cyclohexanol required to achieve this superposition are not the preferred ones, but energy increases on this account could be offset by that released on formation of the ligand–receptor complex. These ideas are attractive but need to be supported by comparative structure–activity analysis (phenolic analogues of fenanyl have low potencies while those of *E*-**47** retain activity). The 4-bromophenyl derivative was also included in a matching exercise of superpotent analgesics using computational methods[151]; the compound fitted one of the proposed models, but only when in its unfavored equatorial 4-aryl chair conformation. It is a pity that relatively little attention has been given to this group in SAR analyses, particularly to seeking reasons for the profound potency difference between geometrically isomeric pairs.

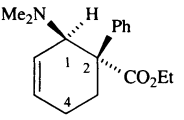
Aromatic and *t*-basic features are also linked to a quaternary center in *phencyclidine* (**49**, R=H PCP), a well-known drug of abuse.[152,153] Insertion of 3-methyl (**49**, R=Me) depressed affinity for the PCP receptor, more so for the *R*-antipode [relative affinities in binding assay vs. [^{3}H] PCP: PCP 100, *S*-(−)-3-Me 23, *R*-(+)-3-Me 4.1].[154]



49

The chiral derivatives (**49**, R=Me) were synthesized from antipodal 3-methylpiperidines of known configurations.[155] Analogues of PCP which incorporate elements of the reversed ester of pethidine have already been mentioned (Chap. 13, page 494).

Tilidine (**50**) and *tramadol* (**51**) are two chiral analgesics of potencies in the pethidine to morphine range developed and studied clinically in Germany. The configuration of tilidine is *trans*-NMe_2/CO_2Et (*E*). The corresponding *cis*-isomer, although still effective, is the less potent isomer. Most of the activity of *rac*-**50**



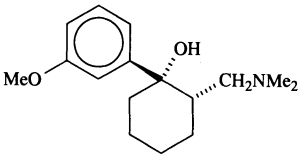
	ED_{50} mg/kg MWR
(+)	4.95
(−)	116
rac	8.8
pethidine	6.3

50

resides in the dextro isomer (see ED_{50} data).[156] Tilidine is the minor component of cycloaddition of *trans*-1-dimethylamino-1,3-butadiene to ethyl atropate, a reaction with favored *cis*-stereochemistry[157]; a modified procedure yields the *trans*-derivative exclusive.[158] Configurational assignments were made by X-ray crystallography, spectroscopy, and chemical transformations. Some "*p*-tilidine" analogues (1-NR_2, 4-CO_2Et) were active in the writhing test, and in these cases *cis*-isomers proved superior to *trans*-isomers.[159]

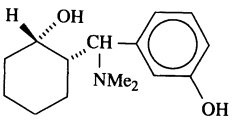
Tramadol **51** (Tramol), another cyclohexane with opioid properties, has *trans*-1-*m*-methoxyphenyl/2-dimethylamineo substituents and is in clinical use in West

Germany as the racemic mixture.[160] The *cis*-analogue of tramadol (itself of codeine-like potency) is a feeble analgesic. Stereoselectivity typical of opioids is displayed by tramadol, as seen by ED_{50} values (mg/kg) measured in mice by an electroshock test: *trans*-**51**-*rac* 10.3, (−) 67, (+) 6.2; *cis*-**51** 7.8; morphine 1.8 (absolute geometry is unreported).



51

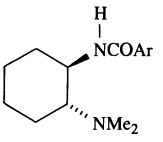
Remarkably, transposition of aryl (*O*-demethylated) and one of the α-hydrogens of the aminomethyl side chain of tramadol also yields an active compound. The formally derived analgesic *ciramadol* (actually made by reducing the unstable adduct formed from a benzilidine cyclohexanone and dimethylamine) is the *levo* antipode of the *cis*-diastereoisomer **52**. It is about twice as potent as morphine in the rat tail-flick assay and classified as a dualist, since it antagonizes morphine in rats with a potency close to that of nalorphine. The corresponding (+)-antipode and *trans*-racemic mixture lack agonist activity in rats.[161]



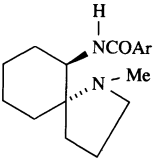
52

14.5.3. Kappa (κ) Agonists: U-50488 and Its Relatives[162]

The benzamide **53**, of *E*-configuration, behaves as a potent μ-agonist in mice (7.5 × morphine by tail-flick assay),[163] while the related azaspirodecane **54** is also a μ-agonist.[164]



53

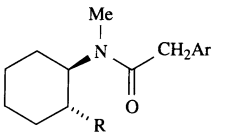


54

see **55** for Ar

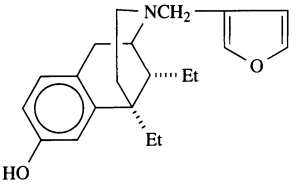
However, derivatives related to **53** which are N-methylated, and have a methylene group interposed between amido carbonyl and the aryl substituent, are classified as κ- rather than μ-agonists and have achieved considerable notoriety in studies of κ-opioid receptors. The prototype molecule, *rac*-**55**b (U-50488), developed by Upjohn, is an analgesic that lacks morphine-like behavioral effects in mice, and is up to half as potent as morpine in mice and rats in a variety of

antinociceptive tests with its actions reversed more effectively by Mr2266 (**56**) (a κ-antagonist, page 462) than by naloxone.[165] In binding assays U-50488 bound primarily to a subpopulation of [^{3}H]ethylketazocine sites (EKC, another κ-ligand) that were not blocked by the μ-ligand dihydromorphine. Compound **55**b had no effect on respiration in rats at a dose of 32 mg/kg and did not reverse or inhibit the morphine withdrawal syndrome in dependent monkeys.[166]

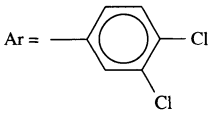


(a) R = NMe_2; (b) R = $N(CH_2)_4$

55

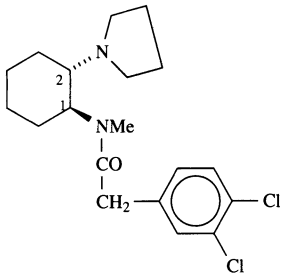


56 (–)-Mr226

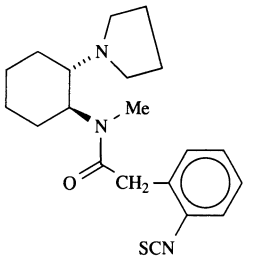


Several analogues of U-50488 have now been investigated, one of the most potent and specific being the *levo* antipode of *rac*-**60**a with a 4-benzo[*b*]furanyl aromatic substituent (κ-binding affinity 780 × μ, equipotent with morphine in the rat-paw pressure test); the corresponding *dextro* isomer had low κ- and μ-affinities.[167] U-50488 itself has now been resolved, and initial binding experiments show the *levo* antipode (K_d 124 nM) to have a greater affinity for κ-sites in guinea-pig brain than the *dextro* isomer (K_d 90, 300 nM).[168] In this work, the diamine intermediate (**55**b, $COCH_2Ar$ replaced by H) was readily resolved to give antipodal forms of high optical purity as judged by the HPLC of ureas formed by reaction with (+)-α-methylbenzylisocyanate. X-ray crystallography of the (+)-base *R*-(−)-mandelate established its 1*S*,2*S* configuration—this base gave 1*S*,2*S*-(−)-**57** (U-50488) when acylated with 3,4-dichlorophenylacetylchloride.

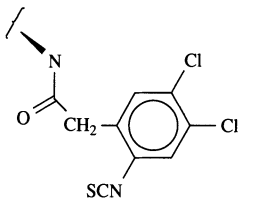
Tritiated (−)-U-50488 has been described[169] and also the irreversible acylating agent **58** (1*S*,2*S* shown).[170]



57 (–) 1*S*,2*S* (eutomer)



58

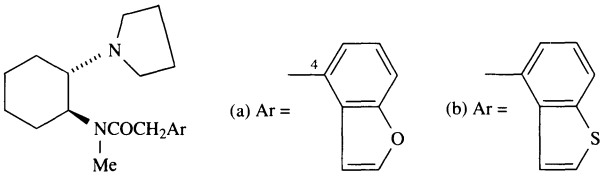


59

While the (+)-1*R*.2*R* antipode had little influence on uptake of [^{3}H]U-69593 (a nonhalogen containing analogue of U-50488, see later) by κ-receptors of guinea-pig brain (100→95.9%), (−)-**58** brought about a reduction amounting to 11.2% of

the control value. Neither antipode influenced the binding of [^{3}H]-bremazocine (when μ, δ sites blocked)—evidence of κ-sites heterogeneity (see page 536). Since 1*S*,2*S*-**58** was ineffective in an *in vivo* experiment (icv injection), antipodes of the analogue **59** were prepared, a compound directly related to U-50488. In binding vs. [^{3}H]U-69593, IC_{50} values (nM) were: *rac* 115; 1*S*,2*S* (UPHIT) 25.9; 1*R*,2*R* 827. After icv injection into guinea-pig brain followed by analysis of remaining κ-binding sites 24 hours later, *rac*-**59** was found to deplete 98% of κ-receptors that bind to [^{3}H]U-69593 and 40% of those that bind [^{3}H]bremazocine.

Levo isomers of the 4-benzo[*b*]furanyl **60**a and 4-benzo[*b*]thiophenyl **60**b derivatives were also the eutomeric forms in κ-binding and the rat-paw pressure test[167]—see data below the formulas. The optical purity of the resolved diamine precursor of **60**a and b was established as >98% by the influence of the chiral solvating agent *R*-(−)-2,2,2-trifluoro-1-(9-anthranyl)ethanol[171] on antipodal NMR spectra, and absolute configuration assigned by X-ray analysis of the levo precursor base. Eutomeric correlations for **55**b (U-50488), **60**a, and **60**b (benzoheterocycles) now extend to other derivatives, as noted below.



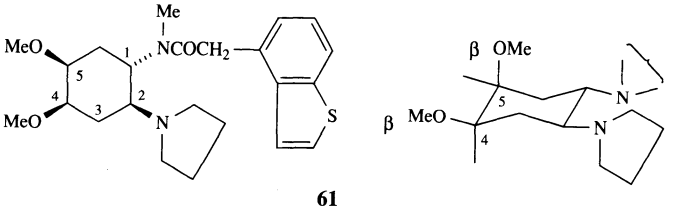
60

		K_1 (κ) nM	μ/κ[a]	MPE_{50} (mg/kg po)[b]
60b	(−) *S*,*S*	9.6	100	8.3
	(+) *R*,*R*	1500		>100
60a	(−) *S*,*S*	4.2	780	12
	(+) *R*,*R*	1500		

[a] κ-Binding measured by displacement of [^{3}H]etorphine from guinea-pig brain pretreated with DADLE and DAGO to block δ and μ sites, respectively. μ-Binding measured using [^{3}H]DAGO.

[b] Dose to produce 50% of the maximum possible analgesic effect in rat-paw pressure test.

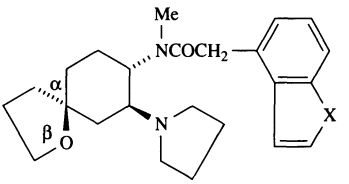
Following observation of the high *in vitro* κ-opioid receptor affinity of the *cis*-4,5-dimethoxyl analogue **61** (K_1 16 nM) (patent reference to synthesis),[172] a variety of 4- and 5-monomethoxy analogues have been examined. Kappa-affinity rankings wer 4β > 4α > 5β as were those for κ-selectivity, except for reversal of 4α and 5α.[173] The same paper reported some spiro ethers **62** related to spiradoline (U-62066, see below). Again levo antipodes were the eutomeric forms of *S*,*S* configuration. Levo **62a** had high affinity for κ-sites (K_1 0.83 nM, μ/κ ratio 1520) and was 25 times more potent than morphine in the rat-paw pressure test. Evidence for



61

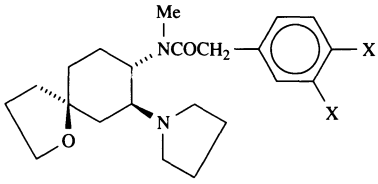
the relative stereochemistry of 4-Meo, 5-MeO, and spiro derivatives was submitted as supplementary data to the 1990 paper. It is to be noted that the β-O configuration of the spiro ether **62** correlates with the optimal 4 β-OMe position of **61**.

Spiradoline itself (**63**a) has also been characterized as an potent κ-agonist[174]; its activity was found to reside in the *levo* antipode (>30 × dextro isomer in antinociceptive tests) while (+)-**63**a behaved as a μ-agonist of low potency, as shown by its induction of physical dependence and cross-tolerance in morphine-tolerant mice.



(a) X = O (PD 129290); (b) X = S

62



(a) X = Cl (spiradoline, U-62006)

(b) X = H (levo isomer, U-69593)

63

A Parke Davis group confirmed these results.[175] Racemic and levo forms of spiradoline and the benzo[*b*]thiophenyl derivative **60**b had κ-binding K_1 (nM) values in the range 0.31–0.5 (420–1360 nM for dextro isomers). In the guinea-pig ileum assay (μ/κ-preparation), levo isomers far exceeded the potency of dextro forms; evidence that their inhibitory actions were mediated chiefly at κ-sites was provided by the fact that the κ-selective antagonist nor-BNI blocked the actions of these agonist far more effectively than did μ-selective naloxone (Table 14.10). All compounds were ineffective at 10nM–3μM concentrations in the rat vas deferens (δ-preparations) while racemic mixtures and levo antipodes fully inhibited the twitch response in the rabbit vas deferens (κ-preparation). Dextro spiradoline had the profile of a weak μ-agonist.

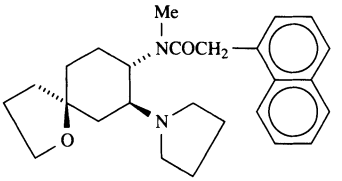
The chlorine-free analogue (**63**b, U-69593 levo antipode) of spiradoline is often used as a standard κ-agonist in pharmacological tests. Its stereochemistry (1*S*,2*S*,4*S*) has been confirmed by an X-ray analysis.[176]

TABLE 14.10.
Antagonism of U-50488 Analogues by Naloxone and nor-BNI in the Guinea-Pig Ileum Assay[175]

Compound	Form	IC_{50} (nM)	pK_B	
			naloxone	nor-BNI
Spiradoline	*rac*	0.88[a]	7.7	10.2
(**63**a)	(−)	1.13	7.7	9.6
	(+)	576	8.2	7.1
Benzo[*b*]	*rac*	1.04[a]	7.8	10.3
Thiophenyl	(−)	1.99	7.4	10.2
Analogue (**66**)	(+)	621	7.4	9.7

[a] The fact that *rac* exceeds (−) suggests that (+) potentiates (−).

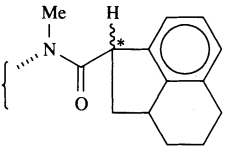
In a later paper the PD group,[177] after finding that **64** (the 1-naphthyl analogue of **62a**) retained κ-affinity, discovered that conformational restraint about the CH_2-Ar linkage as in the acenaphthene derivatives **65** enhanced the affinities of both diastereoisomers for κ-sites (K_1 nM dextro isomer 0.37; levo 1.2, see legend to **64**, with μ/κ ratios of 659 and 227, respectively).



64

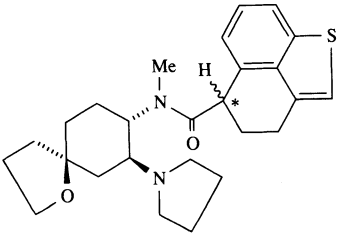
K_1 (nM) 3.35; μ/κ 258

vs. [^{3}H]etorphine in the presence of μ and δ peptide ligands

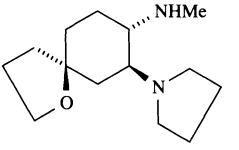


65 (diastereoisomers)

Related restrained analogues of the benzothiophene **60b** were also examined; the (−)-diastereoisomer **66** exceeded the affinity of the (+)-form for κ-sites (K_1 nM 4.65 and 11.1, respectively) and was over twice as effective as the U-50488 standard in this regard. The (−)-intermediate **67** was employed in the synthetic work. Configurations about the restrained centers were not reported.

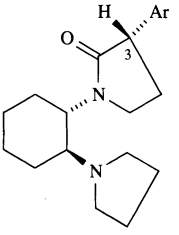


66



67

Constraint of the amido substituent of U-50488 was also achieved by synthesis of the lactams.[200] Of the two diastereoisomers epimeric about C-3, that of relative configuration shown (**67a**) had the higher affinity for μ- and κ-sites (μ/κ ratio 9.4);

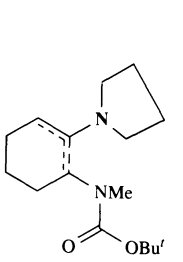


67a

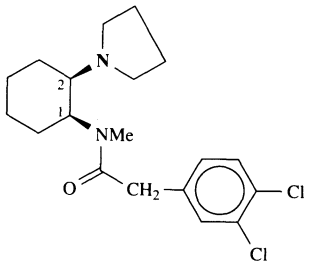
Ar = 3,4-di-ClC_6H_3

K_1 (nM) values (epimer value in parentheses) were: κ 10 (92), μ 225 (864); cf. 15 (κ) and 825 (μ) for U-50488. The compounds were examined in racemic mixture form, and relative configurations are tentative.

In a study of the conformational preferences of U-50488 by MM2-87 computations and NMR spectroscopy, reasonable geometrical agreement between the relatively rigid κ-agonist (−)-ketazocine and a *gauche* conformer of U-50488 was observed.[208] *cis*-Analogues of U-50488 have also been investigated.[178] An NIH group obtained these by catalytic hydrogenation of the enamine intermediate **68**.



68



69

1*S*, 2*R* shown

The diamine precursor of *cis*-**69** was resolved and its absolute configuration established by X-ray crystallography. Binding data for antipodes of U-50488 and its *cis*-analogue are given in Table 14.11. The higher affinity of 1*S*,2*S* (−)-U-50488 over its antipode was confirmed; its greater affinity for sites labeled by [^{3}H]-(−)-U-69593 than those by [^{3}H] bremazocine is evidence for the existence of different populations of κ-receptors[179]—*cis*-antipodes had low affinties for κ-sites of either kind. Among other sites investigated, σ-receptors (labeled by [^{3}H]-(+)-3PPP, see page 461) showed significant interactions with both the 1*S*,2*R* and 1*R*,2*S* *cis*-antipodes while the σ-affinities of the *trans*-isomers were much lower.

In further tests K_1 (nM) values of 1.3 and 6.0 for displacement of [^{3}H]-(+)-3-PPP from guinea-pig brain membranes were reported for (−)-1*S*,2*R* and (+)-1*R*,2*S* **69**, respectively.[180] 2-Naphthyl analogues (**69**, $C_6H_3Cl_2$ replaced by

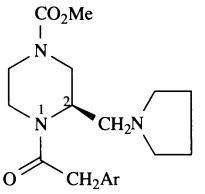
TABLE 14.11.
Affinities of Antipodes of U-50488 and its *cis*-analogue (69) for κ- and σ-Sites of Guinea-Pig Brain[178]

Compound	Affinity K_i values (nM)		
	vs [^{3}H]-BREM[a] (κ)	[^{3}H]-U-69593[b] (κ)	[^{3}H]-(+)-3-PPP (σ)
U-50488 (*trans*)			
rac	109	not done	874
1*S*,2*S*-(−)	44	0.98	594
1*R*,2*R*-(+)	1298	299	1270
cis-**69**			
1*R*,2*S*-(+)	no inhibition	2715	221
1*S*,2*R*-(−)	no inhibition	167	81

[a] Bremazocine in the presence of μ- and δ-blockers.
[b] See **63**b.

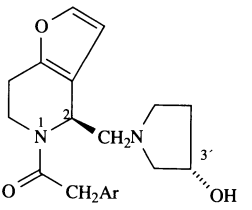
2-$C_{10}H_7$) were less effective competitors but showed a greater eudismic ratio (K_1: 1*S*,2*R* 8.66, 1*R*,2*S* 1375; ratio 160). All isomers had feeble affinities for DA_2, κ-opioid (vs. [^{3}H]bremazocine and U-69593), and PCP sites, and their use is advocated for study of σ-receptors. (See Ref. 206 for further studies of antipodes.)

A Glaxo research group have reported some U-50488 variants which retain the $NCCN(CH_2)_4$ unit of the parent compound but possess a different cyclic system otherwise. The compounds **70** and **71** both had high potencies in the rabbit vas deferens (κ-preparation) and mouse writhing tests: eutomers were the 2*R* (**70**) and 2*R*, 3′*S* (**71**) isomers (there is X-ray evidence of configuration in the last case).[181]



70

2*R* shown

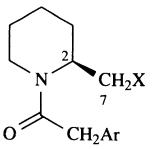


71

2*R*,3′*S* shown

Detailed pharmacological results are now available[182]; rabbit VD IC_{50} values (nM) were 0.09 for *rac*-**70**, 0.02 for 2*R*-**70**, and 300–540 for U-69593. The compounds were ineffective in vas deferentia from rat (μ-preparation) and hamster (δ-preparation), and induced diuresis in rats at sc dose levels much lower than those required with the U-65953 standard.

Vecchietti *et al.*[183] based their analogues of U-50488 on piperidine and produced several derivatives which exceeded the effectiveness of the parent compound in both antinociceptive (MTF) and binding affinity tests (Table 14.12). High κ:μ affinity ratios were observed, such as 6500:1 for 2*S* **72**a. The absolute geometry of the eutomeric forms (2*S* **72**) corresponds to that of the piperazine (**70**).



(a) X = 

Ar = 3,4-di-ClC_6H_3

(b) X = NMe_2, Ar = 3,4-di-ClC_6H_3

(c) X = 

Ar = 4-$CF_3C_6H_4$

72

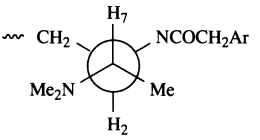
The authors argue that an $N_1C_2C_7N_8$ torsion angle of 60° (which obtains in the equivalent structural unit of U-50488 and its congeners) is a requirement for κ-activity. Evidence in support followed from results on the pair of *rac*-diastereoisomers obtained by methylating C-7 of **72**b (Table 14.12). In the inactive form, preferred geometry about this fragment of the molecule was shown to be **73**

TABLE 14.12.
Antinociceptive Activities and Binding Affinities of Some Piperidine Derivatives Related to U-50488[183]

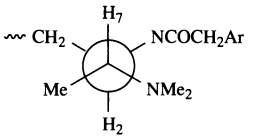
Compound	Mouse tail-flick ED_{50} (mg/kg sc)	Binding affinity K_i (nM)[a]	
		κ	μ
rac-**72a**[b]	0.09	0.53	1860
S	0.05	0.24	1560
R	17.53	15.4	7110
rac-**72b**	0.92	1.36	3050
S	0.87	0.70	2160
rac-**72c**	0.2	0.78	3520
S	0.11	0.57	2340
7-Me (**72b**)			
erythro	0%[c]	1000	> 10000
threo	0.38	0.6	762
7-Me (**72a**)			
erythro	40%[c]	11.7	864
threo	0.40	0.33	464
U-50488	1.9	0.97	616
morphine	2.8	151	3.3

[a] *vs* [^{3}H]U-69593 (κ), [^{3}H]DAGO (μ), guinea-pig brain membranes.
[b] Also reported by a Glaxo group[182] who found it 30–50 times more effective than U-69593 in the rabbit vas deferens preparation.
[c] % Protection at 10 mg/kg sc.

on the basis of the large ^3J coupling (9.0 Hz) between H_2 and H_7, and enhancement of aromatic signals when the 7-Me resonance was irradiated; in this arrangement $\tau(N_1C_2C_7N_8)$ is close to 180°. In contrast, the conformation **74** of the active form ($^3J_{H\text{-}2,H\text{-}7}$ 11.5 Hz) permits a dihedral angle of 60° in this respect. Antipodal forms of **72** of known configuration were obtained by a synthetic route employing *S*-(−)- and *R*-(+)-pipecolic acids.[184] (See Ref. 207 for recent Glaxo work in this area.)



erythro-**73**
inactive diastereoisomer

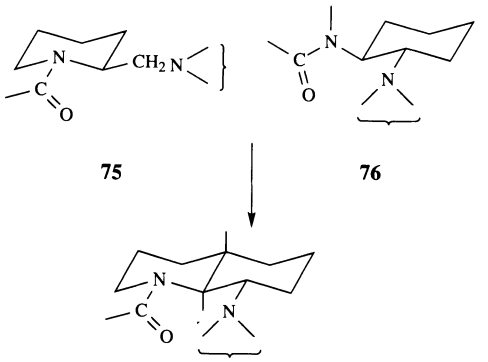


threo-**74**
active diastereoisomer

Eudismic forms of U-50488 and related cyclohexanes (**76**) and also the piperidines (**75**) may be superimposed to form a decahydroquinoline ring system **77** with correspondence of both the amido and *t*-amine nitrogen centers. The same correspondence may not be achieved between 2*R*-**75** (distomer) and 1*S*,2*S*-**76**.

Further pharmacological details of the 2*S*-eutomer **72c** (BRL52656A) were reported at the British Opioid Colloquium held in Cambrdige, April 1991.

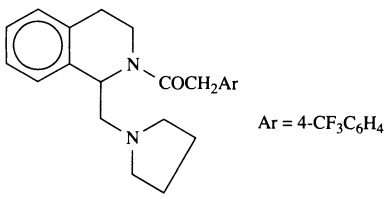
Benzo analogues of the piperidine **72**, represented by the tetrahydroisoquinoline **77a**, proved from 3 to 7 times more potent as antinociceptive agents than the original compounds.[203] As in the piperidines **72**, eutomers had the *S*-con-



75 76

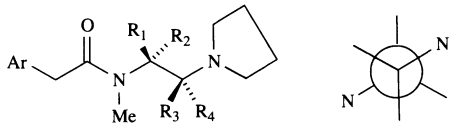
77

figuration (established by X-ray analysis of the dextro antipode **77a** HBr·2H_2O). Data for one of the more potent agents **77a** (typical of the series) were: mouse tail-flick ED_{50} (μM/kg sc) *S*-(−) 0.25, *R*-(+) > 25; kappa-binding affinity K_i (nM) *S*-(−) 0.24, *R*-(+) > 1000 (see also Refs. 209 and 210).



77a

The theme of the governing role of the NCCN torsion angle in U-50488 and its relatives has also been pursued by an ICI group.[151] The work involved acyclic analogues of type **78**. The unsubstituted derivative **78** ($R_1 = R_2 = R_3 = R_4 = H$) of preferred *trans*-N/N conformation **79** had only a feeble affinity for



78 79

[^{3}H]bremazocine labeled sites of GP brain membranes (Table 14.13). Insertion of methyl α to amido nitrogen raised affinity by a large factor to a value almost twice that of *rac*-U-50488, provided the chiral center so created had the *S*-configuration (**80**) and hence related to the eutomer of the cyclic parent (the *R*-analogue failed to bind to κ-sites) In the α-methyl derivative the *gauche*-N/N conformation is favored. α-*S*, β-*S*-Dimethyl substitution also led to a product of high κ-affinity—the α*S*,β*R*-diastereoisomer bound far less well; only the α*S*,β*S*-derivative **81** provides a favored *gauche*-N/N conformation of the required configuration.

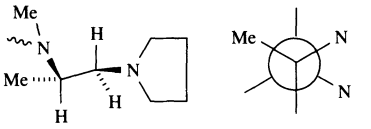
α-Isopropyl (*S*), phenyl (*S*), and *t*-butyl (*RS*) analogues had even higher

TABLE 14.13.
Biological Data for Some Acyclic Analogues of U-50488[151]

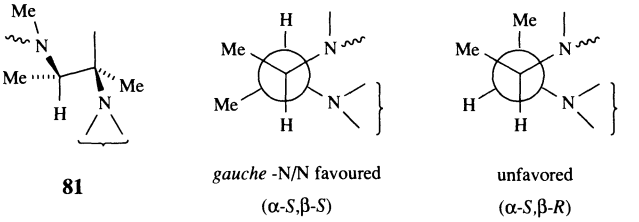
78 substituents	κ-Binding IC_{50} (nM)	MVD potency[a] (EKC = 1)	Mouse writhing (HAc)[b] ED_{50} (mg/kg)
None	6120	not active	—
S-α-Me	53.2	0.14	0.67
R-α-Me	~10000	not active	—
S-β-Me	1540	0.002	>10
α,α-di-Me	2060	0.004	>10
S-α-Me, *S*β-Me	84.6	0.013	4.4
S-α-Me, *R*β-Me	3410	not active	—
S-α-Pr^i	6.3	4.3	0.05
RS-α-Bu^t	24.3	2.5	0.03
S-α-Ph	6.9	16.1	0.004
U-50488	96	0.11	1.1
Morphine	2390	0.13	0.4

[a] Action blocked by naloxone: K_e values of naloxone were ~15 nM, indicative of κ-affinity.[185]
[b] Action reversed by 3 mg/kg naloxone.

binding affinities than the methylated derivative **80**. α,α-Dimethyl and *S*-β-methyl congeners were both of low κ-affinity—in neither case were conformers of the N/N *gauche*-type preferred.



80



81

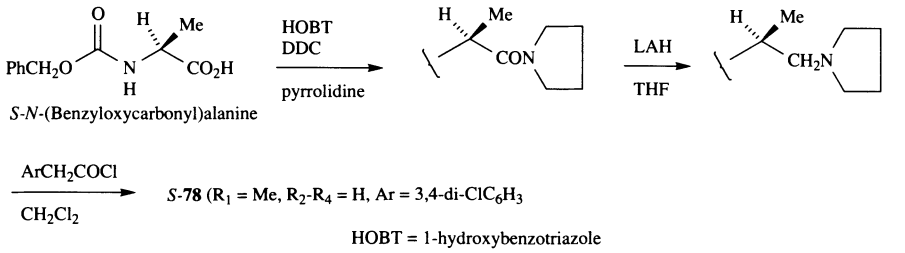
gauche -N/N favoured
(α-*S*,β-*S*)

unfavored
(α-*S*,β-*R*)

All compounds of high κ-affinity proved effective agonists in the mouse vas deferens and mouse writhing test assays (Table 14.13).

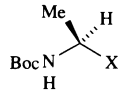
Chemistry. Derivatives of known chirality were prepared from *R*- and *S*-antipodes of suitably protected amino acids. One example is shown in Scheme 14.11.

Synthesis of the α,β-dimethyl diastereoisomers started from (*S*)-Boc-alanine amide **82**a via the nitrile **82**b (formed after reaction with methanesulfonyl chloride) and the Boc-protected diamines **83** [treat **82**b with MeMgBr, THF followed by LAH, and finally $(CH_2CHO)_2$ $NaBH_3CN$, H_2O pH 5]. The diastereoisomers were



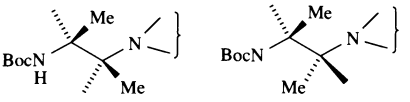
Scheme 14.11.

separated by chromatography and their relative configurations assigned by NMR methods reported in supplementary material. Conformational preferences of the acyclic derivatives were estblished by computational methods.



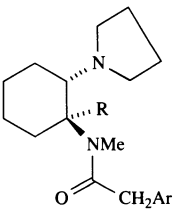
(a) X = $CONH_2$;
(b) X = CN

82



83

The homochiral derivatives **84**a and b, with an absolute geometry at C-1 identical with that of 1*S*,2*S* (−)-U-50488, both bound with high affinity to κ-receptors of guinea-pig brain and were potent agents in the field-stimulated mouse vas

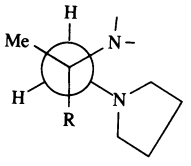


(a) R = $CHMe_2$; (b) R = Ph

84

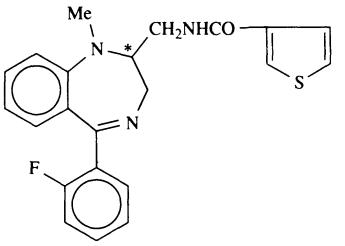
deferens and guinea-pig ileum preparations and in an *in vivo* antinociceptive test (mouse writhing, acetic acid).[185] These results are puzzling, however, since the derivatives tested represent the antipodal (*R*) forms of the potent acylic analogues (**80**, Me replaced by Pr^i or Ph) of *S*-configuration.

Perhaps derivatives of type **85** will prove active as κ-ligands.



85

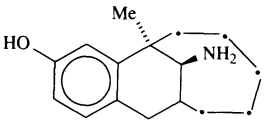
The benzodiazepine **85a**, termed tifluadom, acts selectively at κ-receptors.[186] Differences in the diuretic effects of (+)- (biphasic response) and (−)-tifluadom (modest antidiuresis) have been reported.[187]



85a

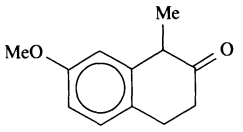
14.5.4. Aminotetralins

There has been some interest in analgesics based on 2-aminotetralin.[188] The bridged derivative **86** was the most effective racemic mixture in the rat tail-flick

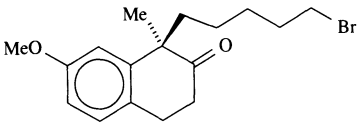


86

assay, most of its action being traced to the levo antipode (WY-16225, dezocine) [ED_{50} (mg/kg) *rac* 1.11, (−) 0.53, (+) > 100].[189] Its amino function (β-orientation) is primary in contrast to the usual *t*-basic character of opioid ligands. The animal pharmacology of dezocine is unusual in that it acts as an agonist in opioid-naive animals (17 × morphine in mice, 8 × in rats ip tail flick) and an antagonist in morphine-dependent rhesus monkeys.[190] It is devoid of activity in the guinea-pig ileum test. A stereospecific synthesis of (−)-dezocine has been reported which involved a stereoselective alkylation of the tetralone **87** to give *R*-**88**.[191]

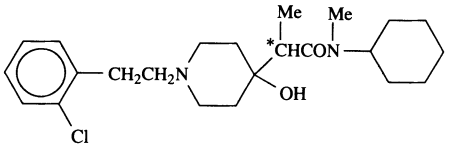


87

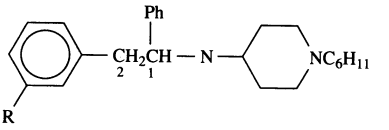


88

Chiral influences among opioids based on 4-piperidol[192] and diphenylpiperazines have been reported.[193] In the case of the 4-piperidinol (**89**) the levo form did not show the expected doubling of potency in comparison with the racemic mixture. Most of the activity of the piperazine **90a** (*rac* 0.8 × morphine, rat tail-flick test) was due to the *S*-(+)-isomer. When a *m*-OH was present in the C-2 aromatic group, a compound over 20 times more potent than morphine resulted. The *S*-(+)-form of **90b** was again the eutomer, but its antipode retained significant potency



89



(a) R = H; (b) R = OH

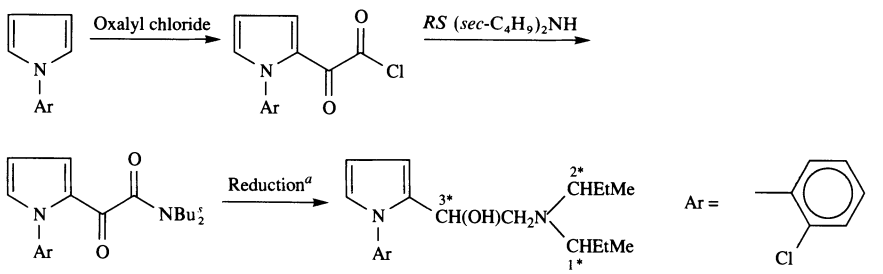
90

(ED_{50} mg/kg tail-pressure test: *S* 0.027; *R* 0.35; morphine 1.17.[194] The analogue of **90b** with a terminal *N*-3,3-dimethylallyl substituent provided another *S*-(+)-antipode of high potency (ED_{50} 0.031 mg/kg) while its *R*-(−)-form (ED_{50} 2.99 mg/kg) along with that of **90b** were capable of reversing the analgesic effects of morphine. Stereochemical assignments were made from ORD data (no details provided).

14.5.5. Viminol

This analgesic is a mixture of diastereoisomers obtained by the reaction sequence shown preceding formula **91**.[195,196] The molecule has three chiral centers and viminol is a mixture of the five isomers detailed; individual isomers are accessible by using *R*- or *S*-*sec*-butylamine in the synthesis and resolving the secondary alcohol formed at the end of the sequence. The configuration of the C-3 center was established by X-ray crystallography.[197]

Isomer	Configuration 1	2	3
R_1	*R*	*R*	*R*
R_2	*R*	*R*	*S*
S_1	*S*	*S*	*R*
S_2	*S*	*S*	*S*
meso	*RS*	*RS*	*RS*



91

(asymmetric centers denoted by asterisks).

[a] Using sodium *bis*-(methoxyethoxy)aluminium hydride.

In the rat tail-flick test, isomer R_2 was the most potent (ED_{50} 0.9 mg/kg ip, morphine 5.0 mg/kg). Other isomers had ED_{50} values greater than 20 mg/kg, while

the mixture itself was about one-third as effective as morphine. Only the R_2 isomer produced physical dependence in mice (the dependence capacity of viminol was very low). Remarkably, isomer S_2 antagonized R_2 (and morphine) as judged by the jumping response in tolerant mice. Viminol thus contains both an agonist and an antagonist component, a combination that leads to a mixture having moderate analgesic potency coupled with a low order of physical dependence capacity.[198] The R_2 but not the S_2 isomer was found to bind to synaptosomal sites.[199]

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Appendix and Postscript

Appendix

Given unlimited time and space, chapters devoted to many other biological areas subject to stereochemical control could be written along the same lines as the material already presented. To aid readers seeking information on topics outside the scope of this monograph, a brief guide to the literature (especially review publications) of a variety of pharmacodynamic and other areas influenced by the stereochemistry of their molecular participants is provided in this concluding appendix. The material was mostly gathered during literature searches carried out during the preparation of the current book or encountered by the author over the recent past.

To continue in the realm of *neurotransmitter receptor systems*, the stereochemical and conformational requirements of *GABA (4-aminobutyric acid)* synaptic mechanisms have been reviewed by Krogsgaard-Larsen.[1–3] Also implicated in this domain are *benzodiazepines* (which bind to the GABA-A receptor subtype),[4–10] antagonists such as bicuculline,[11] and ligands such as baclofen (a GABA-B agonist)[12–14] and β-phenyl-GABA.[12,15] Steric considerations of receptors for *excitatory amino acids*[16,17] include discussion of antipodal forms of MK-801[18]—the dextro isomer is a potent antagonist of the *N*-methyl-D-aspartate (NMDA) subclass of glutamate receptors.[19] The antihypertensive agent ifenprodil is also an NMDA antagonist—the (+)-*threo* isomer is the eutomeric form.[19a]

Studies of *ion channels* relevant to this appendix relate to calcium (see below) and chloride ions. The stereospecificity of chloride ion channels has been established by use of chiral analogues of *clofibric acid.*[20,21] Apart from agents which activate or block adrenoceptors (Chapters 4 and 5), several other groups of cardiovascular drugs fall under the influence of molecular geometry. These include calcium channel agonists and antagonists,[22] antiarrhythmic agents,[23,24] and angiotensin-converting enzyme (ACE) inhibitors,[25] all reviewed as noted. Some specific references are as follows: niguldipine,[26] verapamil,[27] felodipine,[28] and disopyramide.[29]

The identification of D-*myo*-inositol 1,4,5-tris(phosphate) as the intracellular second messenger linked to a variety of pharmacological receptors has led to the synthesis and testing of many stereochemical variants,[30] and the topic has been extensively reviewed.[31,32]

The field of *CNS stimulants*, especially of amphetamine and its relatives, has been well documented in regard to stereochemical influences,[33,34] together with the related areas of antidepressants,[35,36] hallucinogens,[37] and psychotics.[38] Studies of the role played by monoamine oxidases in these classes of drug provide much information on the stereochemical demands of such enzymes.[39] References to chiral *anticoagulants*,[40] *nonsteroidal antiinflammatory* drugs (NSAIDs), and the *barbiturate* class of antidepressants have already been given in Chapters 2 and 3 of this book. Chiral *hypocholesterolemic*[41] and *hypoglycemic* agents[42] have also been reported.

The chiral features of ligands of the enigmatic sigma (σ) receptor form part of both dopamine (Chapter 6) and opioid (Chapter 13) sections of this book. Stereochemical and other information are available in a recent review.[43]

Turning to *intracellular receptors*, i.e., those existing within a cell rather than being anchored across a cell membrane, it is important to note those interacting with *thyroid* hormones[44] and *steroids*.[45] The range of action of steroid-derived products is great, but since all endogenous and most semisynthetic agents originate from natural products, all carry the built-in stereochemistry that results from natural biosynthetic pathways. Hence the majority of active agents are available only in a single isomeric form, although there are exceptions where total synthesis and resolution give access to antipodal pairs or racemic mixtures, such as levonorgestrel and *rac*-norgestrel. Diastereoisomeric sets produced by the epimerization of one or more chiral center are, however, reasonably common, e.g., testosterone and its 17-α-OH epimer.[46] Simonyi[4] includes a section on steroids in his general review of chiral drug action.

The same limitations to stereochemical study obtained in the field of *antibiotics* and *antimicrobials* reviewed by Mitscher *et al.*[47] Thus most penicillins are derived from 6-aminopenicillanic acid (6-APA) of specific configuration at its three chiral centers, an intermediate obtained from species of the penicillium mould. Access to enantiomeric forms (even to epimers) is difficult; one of the rare epimeric examples is 5-epibenzylpenicillin.[48] In contrast, the natural product chloramphenicol (the D-*threo*-form) and its three stereoisomers are relatively easy to synthesize and separate, and antibacterial potency data on this particular quartet of molecules are available.[49,50]

The chiral features of *chemotherapeutic agents* directed against the malaria parasite have been reviewed[51] as have those of *antineoplastic* agents,[52] e.g., studies of geometrical *Z*/*E* isomeric pairs related to tamoxifen.[53] Tetramisole is a well-known example of stereospecificity among *anthelmintic* agents.[54] Finally, moving outside systems of direct importance to human biology, the reader's attention is drawn to a comprehensive account of the stereoselectivities of *pesticides*[55] (already mentioned in regard to the inhibition of cholinesterases, in Chapter 8) and to *plant growth* hormones.[56]

Postscript

This book gives some idea of the vast amount of data now available on the variation of biological activity of diverse kind among sets of stereoisomers. It also

illustrates the large amounts of effort, money, and time expended by chemists and life scientists in accumulating this information.

In view of this outlay of human and material resources, it is pertinent to raise the question of the value of such activity to society, apart from the intellectual satisfaction of the scientists involved. In principle, work of this kind (and indeed structure–activity analyses in general) should advance knowledge of bioactive components of the body at the molecular level, as outlined in the introductory chapter of this book. It should also promote understanding of the workings of the human system in health, and lead to the rational design of medicinal agents required to counteract disease and bodily malfunction. While there are examples of stereochemical analyses that have been put to direct clinical advantage, e.g., evidence that has led to the replacement of *rac*-drugs by single antipodes, it must be admitted that, by and large, the wealth of present-day knowledge of molecular geometry in pharmacology has yet to be exploited. The work has already led to significant advances in the characterization of receptors, to the differentiation of receptor subtypes, and to the identification of molecular assemblies that constitute the active sites of bioactive macromolecules.

Perhaps our inability so far to derive much direct benefit in this regard is inevitable as a result of the labyrinthine complexity of biological macromolecules as compared with the relative simplicity of most of their ligands. However, since technological progress in macromolecular and receptor science is rapidly rectifying this imbalance of knowledge, one may be condifent that macromolecule–ligand interactions in the field of the life sciences will be far better understood in the immediate future. A point in time must soon be reached, therefore, where our fund of stereochemical–structure–activity knowledge will realize its full potential and justify the work of all who have been involved in its collection.

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