Molecular Biology Biochemistry and Biophysics

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Trevor Robinson

The Biochemistry of Alkaloids

Second Edition

With 35 Illustrations

Springer-Verlag
Berlin Heidelberg New York 1981

Trevor Robinson, Ph. D.
Department of Biochemistry
University of Massachusetts
Amherst, Massachusetts 01003, U.S.A.

Library of Congress Cataloging in Publication Data
Robinson, Trevor.
The biochemistry of alkaloids.

(Molecular biology, biochemistry, and biophysics
; v. 3)
1. Alkaloids. I. Title. II. Series.
[DNLM: 1. Alkaloids—Metabolism. W1 MO195T no. 3/
QV 628 R665b]
QK898.A4R6 1981 581.19'242 81-8931
AACR2

9 8 7 6 5 4 3 2 1

ISBN-13: 978-3-642-61832-1 e-ISBN-13: 978-3-642-61830-7
DOI: 10.1007/978-3-642-61830-7

Preface

The alkaloids were of great importance to mankind for centuries, long before they were recognized as a chemical class. The influence they have had on literature is hinted at by some of the quotations I have used as chapter headings. Their influence on folklore and on medicine has been even greater. The scientific study of alkaloids may be said to have begun with the isolation of morphine by Sertürner in 1804. Since that time they have remained of great interest to chemists, and now in any month there appear dozens of publications dealing with the isolation of new alkaloids or the determination of the structures of previously known ones. The area of alkaloid biochemistry, in comparison, has received little attention and today is much less developed.

There is a certain amount of personal arbitrariness in defining "biochemistry", as there is in defining "alkaloid", and this arbitrariness is doubtless compounded by the combination. Nevertheless, it seems to me that in any consideration of the biochemistry of a group of compounds three aspects are always worthy of attention—pathways of biosynthesis, function or activity, and pathways of degradation. For the alkaloids, treatment of these three aspects is necessarily lopsided. Much has been learned about routes of biosynthesis, but information on the other aspects is very scanty. It would be possible to enter into some speculation regarding the biosynthesis of all the more than 4,000 known alkaloids. I have for the most part limited consideration to those alkaloids for which there is experimental information. In a few cases, however, rigid adherence to this principle would have resulted in the exclusion of important

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Chapter 1

Introduction

Glory to God for dappled things—
All things counter, original, spare, strange;
Whatever is fickle, freckled (who knows how?)
With swift, slow; sweet, sour; adazzle, dim;
He fathers forth whose beauty is past change:
Praise Him.

Pied Beauty, GERARD MANLEY HOPKINS[1]

The alkaloids are a group of naturally occurring organic compounds containing nitrogen. Their name, meaning "alkali-like," was given them because most of them are basic in nature and form salts with acids. Basicity is a common characteristic of organic nitrogen compounds, which can be regarded as derived from ammonia by the substitution of organic radicals for hydrogen. The simple amines are considered a class apart from the alkaloids, although with increasing complexity of structure the dividing line between amine and alkaloid tends to become indistinct. According to one definition, alkaloids contain nitrogen in heterocyclic rings while the nitrogen of amines is aliphatic. Compounds such as colchicine and mescaline would be excluded from the alkaloids by such a definition, but the exclusion seems inadvisable on the basis of historical usage. Introduction of the term "protoalkaloid" for a group of borderline compounds leaves very few compounds unclassified. A few otherwise impeccable alkaloids, such as rutaecarpine and ricinine, that have electron-withdrawing functional groups either adjacent to or conjugated with their nitrogen atoms, do not show the characteristic of basicity.

The chemical classification of alkaloids is based on their carbon-nitrogen skeletons. Some of the commonest skeletons are shown in Fig. 1-1. The chemistry of alkaloids is outside the scope of this book. Many textbooks of organic chemistry and heterocyclic chemistry offer an adequate introduction to the general chemical properties of alkaloids. Except for some increase in complexity, alkaloids show no striking peculiarities to set them

[1]Reproduced by kind permission of Oxford University Press from the "Poems of Gerard Manley Hopkins."

apart from simpler compounds with the same functional groups. Readers interested in the proof of structure and synthesis of alkaloids are referred to the comprehensive treatise of Manske et al. [28] or the books of Pelletier [47] and Dalton [8]. The last book, in particular, is organized according to metabolic pathways of biosynthesis. The Chemical Society (London) publishes annual Specialist Reports reviewing the literature on alkaloids; and two encyclopedic works are valuable for finding information on both structures and occurrence of alkaloids [12,48].

Physically, most alkaloids are colorless, crystalline solids slightly soluble in neutral or alkaline aqueous solution but readily soluble in acid or in organic solvents such as ether, chloroform, or ethanol. Although most alkaloids are basic, a few N-oxides and some that have carbonyl groups adjacent or conjugated with nitrogen are neutral and can be missed in the customary isolation procedures. A few alkaloids (e.g., nicotine and coniine) are liquid at room temperature, and some (e.g., berberine and sanguinarine) are colored.

Many alkaloids are optically active, and the fact that they rarely occur as racemic mixtures is taken as evidence that they are synthesized at least partially by enzymatic catalysis. In some cases both enantiomorphs are known to be naturally occurring, but each from a different source. Because of their polar, basic nature, most alkaloids occur dissolved in plant saps as cations which, on evaporation of the sap, form salts with the organic acids that are also present. Just as some plants are noted for containing certain alkaloids, they may be noted for containing a preponderance of certain

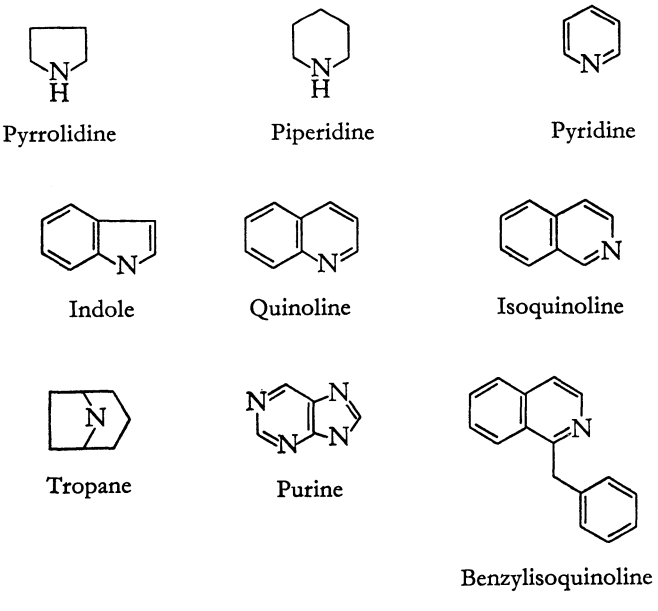

Fig. 1-1. Some common alkaloid ring skeletons

acids; therefore, a particular alkaloid may occur most often as the salt of a particular acid. However, there appears to be no necessary correlation between the biosynthesis of the alkaloid and of its accompanying acid.

The distribution of alkaloids in nature, while not completely random, cannot be described in any simple and unambiguous way. Higher plants are the chief source of alkaloids, yet alkaloids are also known from club mosses (*Lycopodium* spp.), horsetails (*Equisetum* spp.), and fungi [53]. Surveys of ferns and bryophytes have revealed that alkaloids are lacking or extremely rare [46,59]. Results suggestive of the presence of alkaloids are given by bacteria, but none have actually been identified [29]. Certain nitrogenous compounds occurring in animals are sometimes referred to as animal alkaloids, but many of them are relatively simple bases such as histamine, noradrenaline, and spermine. However, a few compounds with more typical alkaloid structures have been found in animals. Some of these may simply be derived with slight structural modification from plants eaten by the animal, but the possibility that some are synthesized in the animal body from simple precursors cannot be ruled out. The best examples of animal alkaloids are the salamander compounds (see Chap. 13) and the quinazolones excreted as a repellent by certain millipedes. Arthropod alkaloids are reviewed in [56,63]. Marine worms and bryozoans have also produced some peculiar alkaloids [5,19]. The extremely powerful toxins of puffer fish and certain toads could also be classed as alkaloids [2,72]. Among the seed plants a greater variety of alkaloids has been found in dicotyledons than in monocotyledons or gymnosperms. It has been estimated that about 20%–30% of all plants contain alkaloids, but the accuracy of any such estimate is limited by the sensitivity of the analytical methods used. Among the dicotyledons there are also considerable differences between taxa. Not a single alkaloid has been found among the thousands of plants in the orders Pandanales, Salicales, and Fagales [21]. In the family Solanaceae about two-thirds of the species have alkaloids [57]. A famous alkaloidal family, the Apocynaceae, has produced nearly 900 different alkaloids [21]. A convenient listing of plants and alkaloids isolated from them is found in [70,71].

Three times as many alkaloid-producing plant families are found in the tropics as in temperate zones, but this only reflects the greater number of tropical plants generally, since the ratio of tropical alkaloid families to total tropical families is the same as the ratio for temperate families [34]. Although some alkaloids are taxonomically quite restricted in occurrence, others are found widely in unrelated plants. A statistical analysis of 3600 alkaloid plants showed caffeine occurring in the largest number of families, lycorine in the largest number of genera, and berberine in the largest number of species [69]. Some conflicting ideas on the geographical distribution of alkaloid-bearing plants can be found in [23,24] and [32].

When the same alkaloid has been found in several different plant species, it is tempting to assume an evolutionary relationship between the plants and therefore to describe the occurrences as "homologous." However, decisions about homology must depend more on knowledge of biosynthetic pathways

and mechanisms than on knowledge of end products. Far too little is known to make any generalization that the same alkaloid is always produced in the same way. If different pathways or mechanisms are found in different plants to lead to the same alkaloid, the occurrences must be described as "analogous," and they then can offer no information regarding phylogenetic relationships. A striking example of what must be an analogous occurrence is the presence of the same hallucinogenic indole alkaloids in ergot and morning glory (cf. Chap. 10). Mothes has stressed the important point that the presence of an alkaloid in a plant shows not only that the biosynthetic pathway is present but also that the plant can tolerate the alkaloid. The absence of an alkaloid does not mean necessarily that the biosynthetic pathway is absent; it may mean that an active degradative pathway is also present [36]. The foregoing considerations show that taxonomy can be based only approximately on accumulated products of metabolism. For the most part, classical taxonomy has taken little account of alkaloid distribution. If the same type of alkaloid is observed in two plants already thought to be related, its presence is used as evidence to support the relationship; its occurrence in two plants thought to be unrelated is cited as an example of independent evolution. The appearance of two apparently quite different structural types of alkaloid in supposedly closely related species cannot be taken as grounds for questioning the closeness of relationship, since it is not difficult to visualize a single-gene mutation setting off an entirely different biosynthetic pathway—for instance, using many of the type reactions of the original pathway but in a different order or with a different starting material (see Chap. 2). Still, alkaloids of similar structure are often found within plants of a given taxonomic grouping, and the lower the grouping in the taxonomic hierarchy, the greater the similarities in structure. Practically, this generalization is useful in locating plant material for the study of alkaloid biochemistry. Theoretically, it probably does argue for similar biosynthetic pathways in plants that are otherwise similar.

The occurrence of "chemical races" has been frequently observed among alkaloid plants. A chemical race is an intraspecific variety distinguished from other plants of the same species only by its composition. The intensive breeding of medicinal plants for high content of some active constituent has produced some of these races, but others appear to have arisen naturally and to have persisted in geographical isolation. Some examples of chemical races are tabulated as follows:

Species	Distinguishing features
Duboisia myoporoides	Hyoscyamine or hyoscine or norhyoscyamine
Sedum acre	Sedamine and nicotine, or sedridine
Hordeum vulgare	Hordenine or N-methyltryamine
Papaver somniferum	Narcotine or absence of narcotine
Claviceps purpurea	Ergotamine or ergocristine

Many examples can be found in [60]. Extensive breeding of the oil poppy [44] and forage lupines [14] for low alkaloid content has not succeeded in producing plants that are completely alkaloid-free. In a few cases thorough genetic analysis of alkaloidal plants has been carried out, and some such studies have been useful in delineating biosynthetic pathways [67]. Differences in ploidy have sometimes resulted in differences in composition. It has been consistently found among *Datura* and *Atropa* spp. that tetraploid plants have a higher alkaloid content than diploid plants. [54].

The formation of alkaloids varies notably from tissue to tissue within the same plant and also changes during the course of ontogeny. This kind of variability as well as the occurrence of chemical races can account for disagreements over the presence or absence of a particular alkaloid in a given plant [38]. In general, alkaloids tend to accumulate in:

1. Very active tissues
2. Epidermal and hypodermal tissue
3. Vascular sheaths
4. Latex vessels

Many examples of alkaloid localization are cited by James [17] and Mothes [35]. It must be emphasized that the sites of accumulation are not necessarily the sites of synthesis. Nicotine, for example, is synthesized in the roots but is translocated and accumulates in the leaves. In other cases the complete synthesis may require cooperation of different plant tissues. Thus, those species of tobacco that have nornicotine in their leaves make nicotine in the root, translocate it to the leaves, and then demethylate it (see Chap. 4). Tropane alkaloids first made in the roots of *Datura* spp. are extensively modified in the leaves [35]. Although prominent in very actively growing tissues, alkaloids are found not in the youngest cells of these tissues but in somewhat older cells which are becoming vacuolated. The presence of alkaloids in vacuoles rather than the surrounding cytoplasm was amply shown in early studies and has more recently been demonstrated using modern techniques such as electron microscopy [7,30,41]. Vacuoles isolated from *Chelidonium maius* take up sanguinarine from the surrounding medium, and it has been suggested that vacuoles act as traps for alkaloids because phenols in the vacuolar sap form complexes with alkaloids [30]. Besides in vacuoles, alkaloids have been observed attached to cell walls and to thickenings in xylem vessels [66,41]. In some plants, alkaloids are confined to special cell types [20,40,74]. In the opium poppy there are alkaloidal vesicles in lacticifer cells, and these vesicles appear to form by a dilation in the endoplasmic reticulum [40]. An overall theory to account for the way alkaloids are distributed in a plant has been proposed by McKey [33], based on the assumption that alkaloids are protective substances and that specialized locations are adaptive. As a result of translocation and of different steps taking place in different tissues, it may be quite difficult to determine the real site of alkaloid synthesis. Grafting experiments and culture of isolated plant parts have been the two techniques giving the

greatest amount of useful information, but difficulties exist with both approaches. The chief problem is that negative findings may not necessarily mean that the cultured or grafted organ has no synthetic ability, but only that alkaloid synthesis requires contributions from other parts of the plant. Interspecific grafts have been known to result in the appearance of alkaloids that are absent from either graft partner growing singly [26,67]. The same phenomenon also occurs in tissue cultures [1,15]. Experiments where labeled alkaloids were fed to plants have shown that alkaloids native to the particular plant migrate and become localized where they are normally found, but foreign alkaloids may become immobilized at the site of application [43,65]. There are certainly exceptions to this observation, though, because on the one hand alkaloids do cross interspecific graft unions, and on the other hand, alkaloids applied externally can be toxic to plants that normally contain them internally [37].

Ontogenetic changes in alkaloid content of plants have been known for many years and are the basis for various empirical rules about the proper time for harvesting plants whose alkaloids have commercial value. The most general principle of ontogenetic change is that alkaloid content increases rapidly at the time of cell enlargement and vacuolization, the increase being followed by a slow decline in concentration during senescence. This pattern has been observed in several tissues of different plants, although leaves have been most often studied. The time of maximum alkaloid content will differ according to whether concentration or total amount is stated, with the concentration maximum coming earlier. Several other circumstances may have striking effects on the overall ontogenetic pattern. The initiation of flowering may stop or inhibit alkaloid formation [35] or stimulate it [39]. A young leaf on an old plant may reflect in its alkaloid content the plant's stage of development rather than its own [17], but in some perennial, woody plants leaves follow a pattern of declining concentration through the growing season [50,75]—even to containing no detectable alkaloid in the fall [50]. There is no consistent pattern for the ontogeny of alkaloids in developing seeds and germinating seedlings, although unfertilized ovules of alkaloid plants normally have alkaloids. In some species the alkaloid concentration may decrease after fertilization, giving mature seeds with little or no free alkaloid (e.g., *Nicotiana*, *Papaver*, *Hordeum*, *Datura*, and *Erythroxylum* spp.). The word "free" is included in the previous sentence because some seeds that have been traditionally regarded as not containing alkaloids do, in fact, contain bound forms of them [13,68]. Seeds of other species may contain high concentrations of alkaloid (e.g., *Lupinus* and *Physostigma* spp.). During germination, alkaloid synthesis may begin within a few days (*Hordeum*) or only after several weeks (*Datura*). The alkaloid content of alkaloid-rich seeds may actually decline in the early stages of germination—both in concentration and, more significantly, in total amount per plant. A striking example of ontogenetic change is offered by *Catharanthus roseus*, which contains virtually no alkaloid in its seed, then develops alkaloids until at 3 weeks after germination they are present throughout the

plant. They then disappear almost completely and reappear at 8 weeks [38]. Besides changes over longer periods, there have also been observed cyclic diurnal variations in alkaloid content, with certain alkaloids more than doubling or halving their concentration within a few hours [51].

Several external factors have been found to influence alkaloid content, but direct biochemical explanations for these influences are mostly lacking. Light is, of course, essential for growth of higher plants, so that its beneficial influence on total alkaloid content is expected. However, chlorophyll formation and alkaloid formation are not always influenced in the same way by light [16]. For the most part the influence of light is probably indirect; however, more specific light effects have also been observed. Etiolation increases both concentration and total amount of ricinine in *Ricinus communis*. In the light *Catharanthus roseus* has vindoline as its predominant alkaloid, but in the dark this alkaloid is absent [38]. The effect of photoperiod on alkaloid content are obviously related to the effects of photoperiod on initiation of flowering. *Lycopersicon glandulosum*, a short-day plant, if grown under long days contains five times as much tomatine as under short days. Species less dependent on day length for flowering show a lesser response of tomatine content to photoperiod [55]. Such findings raise the possibility that tomatine serves specifically as an inhibitor of flowering and acts as a chemical mediator of photoperiodic flower induction. Another interpretation would be that decomposition of tomatine gives rise to other steroids related to pregnenolone which serve as flowering hormones (see Chap. 13). With tobacco, long days favor alkaloid production; this is largely a phytochrome response rather than due to increased photosynthesis [62].

Effects of nutrition and plant growth substances on alkaloid content have received some attention, with the rather commonplace conclusion that factors favoring growth generally favor alkaloid formation [51,67]. It does appear that nitrogen supplied to plants in the form of ammonium salts is somewhat better than nitrates for increasing alkaloid formation [17]. This result suggests a rather direct use of ammonia in alkaloid synthesis, while nitrate acts more indirectly by increasing overall growth. A potassium-calcium antagonism has also been observed. A high K/Ca ratio favors protein synthesis, but a low ratio favors alkaloid synthesis [17]. Effects of more unusual ions such as lithium [10] and manganese [11] have also been observed. Such growth factors as kinetin, gibberellins, and auxins have been investigated. Several of these growth regulators increase the alkaloid content of lupine embryos in sterile culture [27]. Auxins depress the nicotine content of tobacco [73]. Gibberellins have diverse effects, depending on the plant and the experimental conditions [51].

Tissue cultures and cell-suspension cultures derived from alkaloid plants are being used more and more as a convenient tool for studying alkaloid biosynthesis, but also with the hope that they can be used in production of some commercially valuable alkaloids. So far it must be concluded that alkaloids are especially difficult to produce in this way [3,6,58]. Some cultures grow well but produce little or no alkaloid. Sometimes supple-

mentation of the medium with necessary substrates or growth factors has been able to increase alkaloid production. Sometimes alkaloids are accumulated only if dedifferentiation occurs because specialized cells are required for alkaloid synthesis or storage [42]. At the theoretical level many fascinating findings have resulted, but industrial exploitation still seems far off.

Any discussion of the function of alkaloids in plants runs the danger of becoming teleological, but the matter has been of too much interest to too many people to ignore it altogether. Most often alkaloids have been called nitrogenous "waste products" analogous to urea and uric acid in animals. Their occurrence in vacuoles rather than the living parts of protoplasm supports such a view. However, nitrogen is often scarce for plants, and its reutilization is more often the rule in plant metabolism. The metabolic activity of some alkaloids also puts them in a class apart from ordinary waste products. Fluctuations in concentration and frequently rapid conversion to other products have been observed [51]. Knowledge of alkaloid catabolism is still scanty, so that generalizations are not possible. However, there is evidence that some of the pyridine alkaloids can serve as precursors of nicotinic acid and thus can be reservoirs of this vitamin (see Chap. 4). A possible role of alkaloids in protecting plants against herbivores has been frequently proposed, and a few examples can be given in support of such an idea [17,25,52], but there are many more examples of plants whose high content of alkaloids confers no protection against their major enemies. Defense against microbial pathogens is another proposed function; the generalization has been put forward that most resistance to herbivores is constitutive but most resistance to microorganisms and nematodes is induced [22]. Although alkaloids are constitutive, some do have activity against fungi [22], bacteria [61,64], nematodes [4], and even viruses [61,69]. The role of alkaloids as detoxication products appears plausible in some instances, as in the case of alkaloids that may remove such active molecules as indole-3-acetic acid or nicotinic acid from sites where they could unbalance metabolism. Because of the great diversity among alkaloids, it seems likely that theories which give plausible functions to several of them cannot be universally applicable. Furthermore, it is impossible to state what kind of evidence is acceptable for proving the function of an alkaloid. Complete removal of an alkaloid from a plant, where experimental manipulation has made this possible, usually has no effect on the plant; of course, 70%–80% of all plants never contain alkaloids. However, alkaloids do have effects on plant growth—usually inhibiting such processes as elongation or seed germination [9,31,49]. Some of these inhibitions could be significant in the competition between plants [45].

It is evident that biosynthesis of alkaloid molecules often must require energy and in some instances the presence of highly specific enzymes. The perpetuation of such a low entropy system through the course of evolution seems to call for an explanation in terms of useful function, but no generally adequate explanation has been forthcoming.

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Chapter 2

General Theories of Alkaloid Biosynthesis

O my soul! if I realize you I have satisfaction,
Animals and vegetables! if I realize you I have satisfaction,
Laws of the earth and air! if I realize you I have satisfaction.

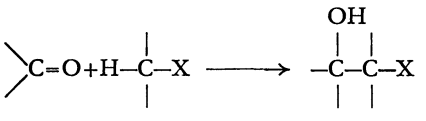
Leaves of Grass, WALT WHITMAN

In spite of the large numbers and great diversity of alkaloid structures, it seems possible now to discern a few general principles that apply to the biosynthesis of many different alkaloids. Some broad theories of alkaloid formation have borne the test of many experimental investigations, some have received no support, some have been refuted, and some newcomers have not yet been tested adequately.

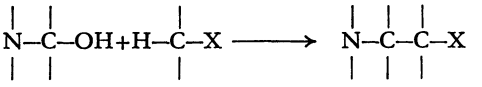
In the present chapter, brief mention will be made of experimental findings that have general significance. More information on biosynthetic pathways can be found by consulting the appropriate specific chapters or by referring to the Index for names of compounds.

Various earlier proposals for the derivation of alkaloid structures from common amino acids were considered and incorporated into the far-reaching proposals presented in 1917 by Sir Robert Robinson [23] and later expanded [24]. These comprehensive proposals were based on analogy with reactions of organic chemistry and on comparisons of structure rather than on biochemical evidence. Nevertheless, biochemical experiments have confirmed the predicted pathways to a great extent. The assumptions of Robinson's scheme of biosynthesis may be summarized as follows:

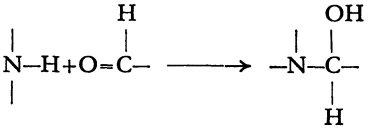
1. The fundamental skeletons of alkaloids are derived from common amino acids and other small, biological molecules.
2. A few simple types of reaction suffice to form complex structures from these starting materials. For example, the aldol condensation:



the carbinolamine condensation:

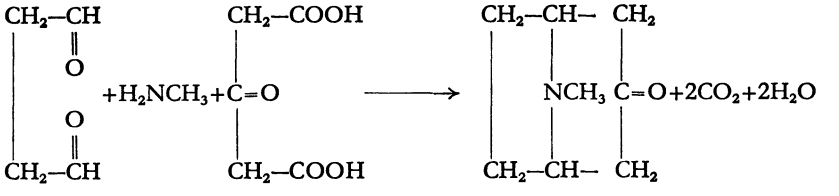


the aldehyde-amine condensation:



as well as simple dehydrations, oxidations, and decarboxylations. (X represents an "activating" group, such as carbonyl.)

It is important to understand that the Robinson proposals were never intended to apply to specific compounds but only to general groups of structurally related compounds. Thus a possible formation of the tropane skeleton from succindialdehyde, methylamine, and acetonedicarboxylic acid could be represented as:



However, the actual reactants in vivo might resemble more closely ornithine, glycine, and citric acid, which by relatively simple reactions could be transformed into the three represented precursors. Summarizing this categorical approach, Fig. 2-1 shows some common structural elements of alkaloids and the types of precursors from which they might be derived. A great number of experiments have been done to show that with low concentrations and under very mild conditions of temperature and pH it is possible to bring about reactions of the hypothetical precursors to form complex structures resembling alkaloids. Biochemical experiments first carried out about 1955 have continued to lend support to the overall ideas of Sir Robert Robinson, although certain discrepancies and variations have been found. The biochemical experiments will be considered in the separate chapters on the different groups of alkaloids.

Another useful generalization in considering pathways of alkaloid biosynthesis is the probable importance of amine oxidases in the production of alkaloid structures. Mann and Smithies [19] and Hasse and Maisack [8] showed that cyclic compounds were formed as the result of the action of plant diamine oxidase on 1,4-diaminobutane (putrescine) or 1,5-diaminopentane (cadaverine). Initial formation of an imine and ring closure with loss of ammonia or formation of an aldehyde and ring closure with loss of water were two possible mechanisms suggested.

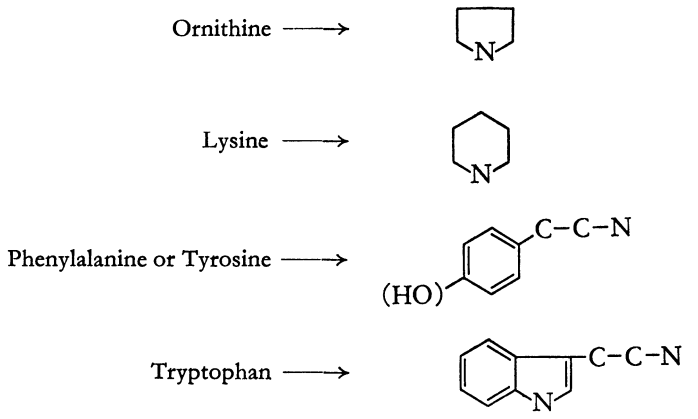
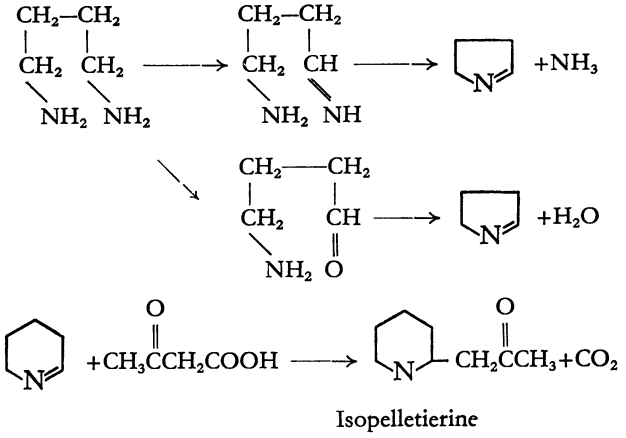


Fig. 2-1. Some common structural elements of alkaloids and their presumed precursors

The Δ^1-pyrroline formed in the above reaction or the Δ^1-piperideine formed from cadaverine could then by suitable condensations (since they are very reactive molecules) give rise to various alkaloids containing pyrrolidine or piperidine rings. It was in fact found that reaction of Δ^1-piperideine with acetoacetic acid resulted not only in condensation but also in spontaneous decarboxylation with formation of the known alkaloid isopelletierine [4].



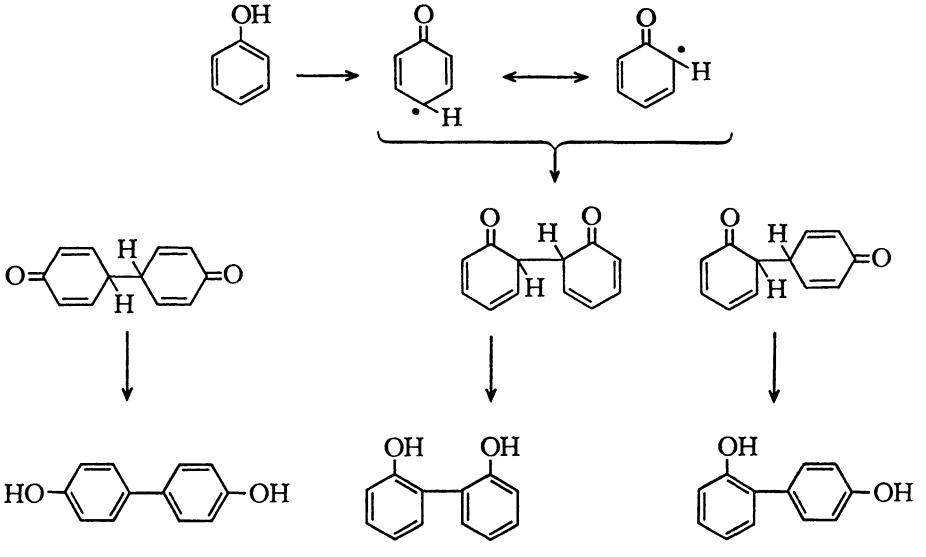
Isopelletierine

More examples of alkaloids believed to be formed through the action of diamine oxidase will be found in Chaps. 4 and 6. Kaczkowski has reviewed the probable role of diamine oxidase in alkaloid biosynthesis [12]. Some conversions which have been attributed to amine oxidation could just as well have been brought about by transamination, since the action of

transaminase also results in formation of a carbonyl group from an amino group. Cases where alkaloid synthesis does not correlate well with the presence of amine oxidase should therefore be investigated to determine the activity of transaminase. Jindra et al. [11] have shown the presence of enzymes catalyzing transamination in a number of alkaloid-containing plants, but so far the direct participation of these enzymes in alkaloid biosynthesis has not been established.

It has become clear that the O-methyl, methylene dioxy, and N-methyl groups found in the great majority of alkaloids are derived by direct transfer from S-adenosylmethionine. Feeding experiments with [^{14}C]-labeled methionine have shown this for many different alkaloids. In a few cases this transmethylation has been brought about in vitro by cell-free extracts. Tracer experiments have shown in a few alkaloids the possible origin of methyl groups from formate. Presumably this is first converted to the methyl group of methionine. The state at which methylation occurs may well vary from one alkaloid to another. With some (e.g., nicotine) it appears that the methyl group may be introduced at a very early stage, perhaps to the amino acid precursor. Other methyl groups may be added very late—indeed, methylation may preclude any further reaction, as in the case of laudanosine.

A feature of many alkaloid structures is the attachment of two aromatic rings to each other. A general explanation for all such structures was put forward by Barton and Cohen [1] and since then has been applied to many specific alkaloids and thoroughly accepted [2]. Oxidation of a phenol by a one-electron transfer process gives a transient free radical with the odd electron more or less localized at the positions *ortho* and *para* to the phenolic hydroxyl group. Coupling of the free radicals with each other then occurs:



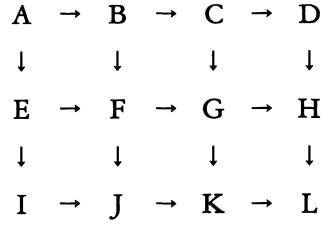
These free radical oxidations have been brought about with inorganic oxidants such as ferricyanide or ferric chloride and are useful in chemical syntheses [13,28]. In vivo it is presumed that they are catalyzed by such plant enzymes as laccase or peroxidase [26]. The requirement for a free hydroxyl group *ortho* or *para* to the coupling position means that O-methylation of precursors can prevent coupling; or when several hydroxyl groups are present in the precursors, methylation of certain ones can control the direction of coupling. A further elaboration of this kind of mechanism is the suggestion of Franck et al. [5] that oxidative coupling reactions most likely occur with alkaloids having quaternary nitrogen. With nonquaternary nitrogen, alternative reactions are more likely. Although this is true in the chemical oxidations, there are certainly cases where it is not true in vivo. Since oxidative coupling mechanisms have been applied most extensively to the morphine alkaloids, further consideration is given to these mechanisms in Chap. 8.

Many alkaloids are now known to incorporate into their structures units derived from mevalonic acid, the well-known precursor of terpenoids and steroids. With some alkaloids, indeed, the entire carbon skeleton comes from mevalonic acid by typical terpenoid pathways. These terpenoid and steroid alkaloids are considered in Chap. 13. In other alkaloids terpenoid units are more incidental, as for instance the C_5 unit of lysergic acid (Chap. 10), or the C_9 or C_{10} unit which in several variations is quite widespread (see p. 117).

Several alkaloids having an α-pyridone structure are known to coexist with corresponding pyridinium salts (e.g., oxysanguinarine and sanguinarine in *Sanguinarea canadensis*, oxydemethoxyalstonidine and demethoxyalstonidine in *Ourouparia gambir*). It has been proposed as a general principle that enzymatic oxidation of pyridinium salts to pyridones accounts for the biosynthesis of pyridone alkaloids. Such a reaction has been catalyzed by an enzyme that is present in many plants of the Euphorbiaceae [6,7]. Another way that this structure could arise is by substitution of nitrogen from ammonia or an amine for oxygen in a pyrone or lactone precursor [27].

The teleological question of why alkaloids exist in plants has been touched on in Chap. 1. A somewhat more approachable question is, "How did the biochemical pathways of alkaloid formation evolve?" In a few cases we are close to knowing most of the intermediates, but knowledge of the nature of the mechanisms, enzymatic or otherwise, for bringing about the series of transformations is scarcely approached. Thus speculation regarding evolution of a pathway necessarily remains vague and generalized. James [9,10] proposed that many of the steps in an alkaloid synthetic pathway are probably not specific to that pathway. Some of them may be catalyzed by enzymes of low specificity which perform other functions in plant metabolism. Some steps may occur spontaneously, without mediation of any enzyme. Thus plants that do not synthesize any alkaloids may nevertheless possess considerable segments of potential biosynthetic path-

ways, and mutation of a single gene might be enough to start off the entire sequence. Such a single gene mutation might result in appearance of a new compound or it might result in a structural modification within a cell, with the result that cellular constituents kept segregated in the parent cell are able to interact in the mutant. Several observations support this overall viewpoint. Pea plants do not contain alkaloids, but homogenates of pea plants provided with cadaverine synthesize anabasine [20]. Beans, which do not contain alkaloids, if fed certain lupine alkaloids will transform them into other lupine alkaloids [21]. One can argue that peas, beans, and lupines, all members of the Leguminosae, are descended from an alkaloid-containing ancestor and that certain parts of the alkaloid biosynthetic mechanism were lost in the evolution of peas and beans but retained by the lupines. It can as well be argued that the ancestor was alkaloid-free but, like peas and beans, had the potential for synthesizing alkaloids once a mutation had occurred and that such a mutation did occur somewhere during the phylogeny of the lupines. Although a choice cannot be made between these alternatives at the moment, the question is not meaningless. What we need to answer it is detailed knowledge of the pathway and of which steps are missing in which species. An elaboration of the "one special reaction" point of view has been applied by Bu'Lock and Powell [3] to account for the formation of secondary products of microorganisms. It might, however, be just as validly applied to higher plants. Using a schematic treatment, these authors have shown that given a single special product outside the general metabolism of a cell and a few enzymes that are not absolutely specific (i.e., which can act on several compounds of related structure), a great number of products could be formed. Inclusion of some nonenzymatic reactions would, of course, greatly enlarge the number of possibilities. In this kind of "metabolic grid" several sequences may all occur simultaneously as in the following diagram:



With A as precursor and L as product there are nine possible sequences of intermediates [22,29].

All of the previously described approaches to the elucidation of alkaloid biosynthetic pathways have been rather hypothetical. They have centered on considerations of structures and possible reaction mechanisms for producing them or they have taken note of the co-occurrence of compounds and postulated their interconversions. The more direct approach of feeding

isotopically labeled materials to plants, then isolating alkaloids, and determining label incorporation, has provided most of the information we have about synthetic pathways in vivo. Early radiotracer experiments, often with randomly labeled substrates, merely showed that some atoms of the substrate reached the alkaloid. The pathway may have been indirect. Specific labeling of the precursor and degradation of the isolated alkaloids so that the label incorporated into individual atoms could be measured is now a routine approach and gives at least a qualitative idea of how directly the precursor goes to the alkaloid. Finally, double and even triple labeling has been applied in a few cases to establish almost beyond a doubt that a precursor has gone directly to an alkaloid. If a single molecule labeled with tritium, [^{14}C], and [^{15}N] is converted to an alkaloid with the same isotopic ratio (after correction for any loss of atoms), there can be little doubt that it has served as a direct precursor without degradation and reassembly.

Still, the tracer technique, however carefully applied, does not usually avoid the problem arising from the likelihood that many nonspecific enzymes and reaction pathways exist in plants. A labeled compound could travel through these pathways and arrive at an alkaloid that might normally be made along quite different pathways from a different precursor. The compound fed must be shown to be a normal constituent of the system, and the rate of the proposed steps must be consistent with the normal, overall rate of product formation. Leete [15] has made a useful distinction between two types of aberrant synthesis that can occur. In type I an unnatural precursor gives rise to a natural product. An example of this is the conversion of N-methyl-Δ^1-piperideinium chloride to anabasine in *Nicotiana* spp. [17]. In type II an unnatural precursor gives rise to an unnatural analogue of the normal alkaloid. Examples of this are seen when halogenated or C-methylated precursors are incorporated into alkaloids that retain the abnormal substituent groups [14,16,25]. The only carbon compound that is a normal substrate for higher plants is carbon dioxide. Studies with labeled carbon dioxide are not frequent but they have given some very useful information regarding the biosynthesis of nicotine (Chap. 4) and the opium alkaloids (Chap. 8).

Some generalizations about the use of tissue or cell-suspension cultures for the study of alkaloid biosynthesis are given in Chap. 1. Particular results from this approach are scattered through the following chapters and may be found by consulting the Index under "Tissue culture" or "Cell culture."

In vitro studies of alkaloid biosynthesis using more or less purified plant enzymes are becoming more and more frequent. A review of some of these experiments is [18]. Mostly simple reactions involving oxidations and methylations have been investigated; but, especially in the field of indole alkaloids, much more complex transformations have been examined at the enzymological level (cf. Chap. 10). Some details of regulatory mechanisms have been revealed by this approach, and the future appears exciting.

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Chapter 3

Simple Amino Acid Derivatives and Protoalkaloids

With shepherd's purse, and clown's all-heal,
The blood I staunch and wound I seal.
Only for him no cure is found,
Whom Juliana's eyes do wound;

Damon the Mower, ANDREW MARVELL

A number of naturally occurring nitrogen compounds have basic properties in common with the alkaloids but are usually not classified as alkaloids because their structures are relatively simple. Their nitrogen atoms are not incorporated into heterocyclic skeletons; and they are seen to be derivable from amino acids by a few, simple reactions. Mere decarboxylation of amino acids produces simple amines which are never classed as alkaloids. Further modification of such simple amines by the introduction of methyl groups or hydroxyl groups or both gives rise to a group of ambiguously classified compounds that are sometimes called alkaloids. However, it seems useful to distinguish them by the name "protoalkaloids," suggesting both their simple structures and their possible role as precursors of more typical alkaloids (cf. Chap. 1).

Simple amines are widely distributed in higher plants, though usually in low concentration. A survey of 220 species revealed amines in many of them. Since some plants contained only trace amounts, the exact percentage of amine-containing species cannot be stated with precision. Isopentylamine was most widespread, occurring in 75 species [41]. Other reviews on the occurrence and biochemistry of plant amines are [89,91]. The volatile amines given off by some flowers seem to serve to attract insect pollinators [85]. Table 3-1 lists some of the simple amines found in plants with the amino acids from which they are derived by decarboxylation. Radioactive leucine and valine fed to influorescences of *Sorbus aucuparia* and *Crataegus monogyna* gave rise, respectively, to labeled isoamylamine and isobutylamine. Enzyme preparations were also found to carry out these decarboxylation reactions [79]. Tracer experiments have shown alanine to be a precursor of ethylamine [103]. On the other hand, free ethanolamine, although often

Table 3-1. Some simple amines occurring in plants, with their parent amino acids

Amine	Amino acid precursor
CH_3NH_2 Methylamine	COOH | CH_2NH_2 Glycine
$HOCH_2CH_2NH_2$ Ethanolamine	NH_2 $HOCH_2CHCOOH$ Serine
$(CH_3)_2CHCH_2NH_2$ Isobutylamine	NH_2 $(CH_3)_2CHCHCOOH$ Valine
$(CH_3)_2CHCH_2CH_2NH_2$ Isopentylamine	NH_2 $(CH_3)_2CHCH_2CHCOOH$ Leucine
$H_2N(CH_2)_4NH_2$ Putrescine	NH_2 $H_2N(CH_2)_3CHCOOH$ Ornithine
$H_2N(CH_2)_5NH_2$ Cadaverine	NH_2 $H_2N(CH_2)_4CHCOOH$ Lysine

detected in leaves, was not found to acquire label when radioactive serine was fed to sugar beet leaves [7]. Possibly the actual decarboxylation occurs in some other tissue. Serinol of sugar cane is not made by reduction of serine but by transamination of dihydroxyacetone phosphate followed by hydrolysis of the phosphate group [5]. It is physiologically interesting because it stimulates toxin production by the pathogenic fungus *Helminthosporium sacchari* [5]. Other aliphatic amines might be made by transamination of aldehydes rather than by decarboxylation of amino acids [108]. Putrescine (1,4-diaminobutane), although obviously derivable by a decarboxylation of ornithine, may be made in a more indirect way from arginine via agmatine (1). Arginine, as a protein amino acid, is much more abundant than ornithine, which is only a transient intermediate of arginine biosynthesis. Thus under conditions of protein turnover the formation of putrescine from

arginine seems more likely than its formation from ornithine. Agmatine fed to excised barley leaves was found to be converted to putrescine with N-carbamylputrescine as an intermediate [52,86,88]. Studies on this sequence have shown that it proceeds at a greater rate in plants under potassium deficiency, and such plants are therefore unusually high in putrescine [87]. Both enzymes of the sequence are less active in potassium-deficient plants, but neither is inhibited by potassium ion in vitro [87, 93,100]. The main soluble nitrogen compound of *Galega officinalis*, galegine (3), is also an amidine like agmatine; and its amidine group has been shown by tracer experiments to come from arginine, but the dimethylallyl group cannot be derived simply from any natural amino acid [78]. More likely, it comes from the terpenoid precursor dimethylallyl pyrophosphate (Fig. 3-1).

The next higher homologue of putrescine is cadaverine (1,5-diaminopentane), and it also is a normal plant constituent [2]. As expected, its precursor is lysine [76,110].

The polyamines spermidine and spermine can be recognized as containing a putrescine unit to which has been added, respectively, one or two propylamine groups:

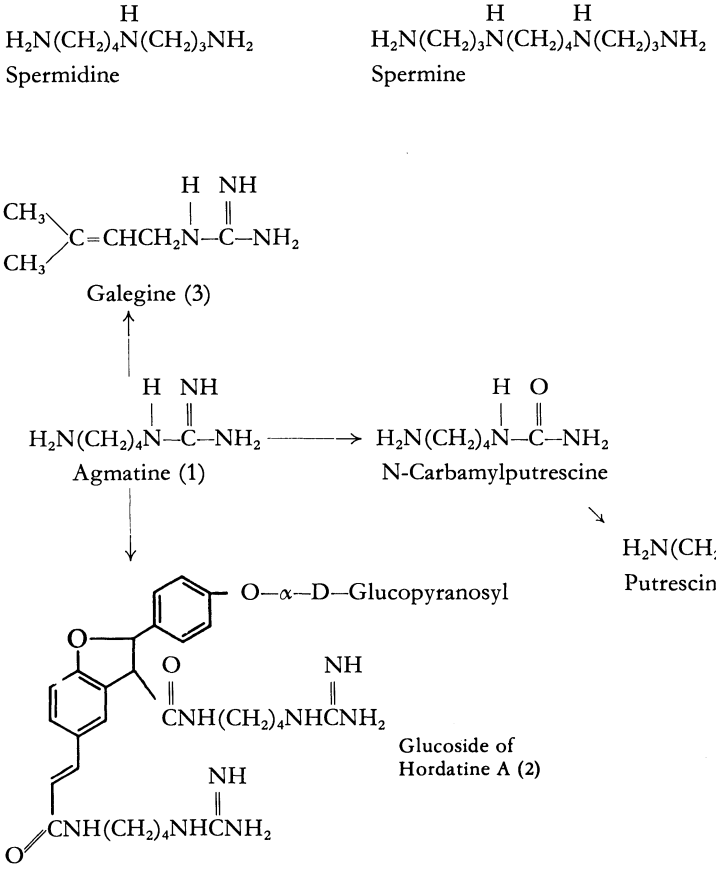


Fig. 3-1. Amidine compounds

Polyamines are reviewed in [18]. They are present especially in ovules, pollen, and embryos of higher plants [6,54]. Many studies point to them as having some regulatory role in growth and development. They do stimulate ribosomal protein synthesis in vitro [17,72], but this may not be their only mode of action [3,16,66]. Amides of the di- and polyamines with organic acids are also turning out to be present and physiologically interesting in plants. Their production is associated with infection, wounding, and floral induction [14,15,61,94,119]. Barley contains not only coumaroylagmatine but also dimers of it known as hordatines (2) that have antifungal activity [94,99]. A cyclic derivative of spermidine is present in *Cannabis sativa* [106].

The possible role of diamines as alkaloid precursors is discussed generally in Chap. 2. Their transformation into heterocyclic rings is presumably mediated by diamine oxidase, which oxidizes one amino group to an aldehyde that then condenses with the other amino group. A survey of the plant kingdom has shown diamine oxidase to be present in many different dicotyledonous families but absent from monocotyledons and gymnosperms [111]. Some purification and characterization of diamine oxidase from pea and soybean plants has also been reported [63,102]. Monoamine and polyamine oxidases are also present in plants [75]. The former converts primary amines to aldehydes—e.g., methylamine to formaldehyde [101]. The latter acts on spermine and spermidine to produce a variety of products [37,90]. In young pea seedlings, the activity of amine oxidases reaches a maximum at the same time as the content of amines [95].

Choline (4), acetylcholine, and related compounds are widely distributed in plants both as components of phospholipids and also as simpler derivatives [4]. Phosphorylcholine functions as an important phosphate carrier in plant sap, and the appearance of free choline or other choline derivatives in leaves may signal removal of phosphorus from phosphorylcholine translocated from the roots [60]. During germination of *Cicer arietinum*, choline content increased to a maximum in 96 h and then decreased. Availability of methyl groups apparently limited choline formation, since supplying methyl donors increased choline content while supplying other acceptors decreased it [1]. In this case it seems likely that de novo choline synthesis was occurring; in other cases choline appearance may only indicate breakdown of phospholipids. The biosynthesis of choline has been investigated using leaf discs and homogenates of *Beta vulgaris* [20]; the probable pathways are outlined in Fig. 3-2. As expected, the methyl groups are derived from methionine [62]. It is interesting that betaine seems not to be formed by direct methylation of glycine but, rather, by oxidation of choline [19, 97]. Acetylcholine, best known as a neurotransmitter in animals, is also present in plants where it shows some properties as a growth regulator [40,109]. It reaches an extraordinarily high concentration in the hairs of stinging nettle (*Urtica dioica*) and is synthesized in the leaves of this plant from choline plus acetyl-CoA [7]. A compound similar in properties to choline is muscarine (5), which is at least partially responsible for the toxicity of *Amanita* and *Inocybe* spp. of fungi [116]. It is biosynthesized from pyruvate plus the middle three carbon atoms of glutamate [71].

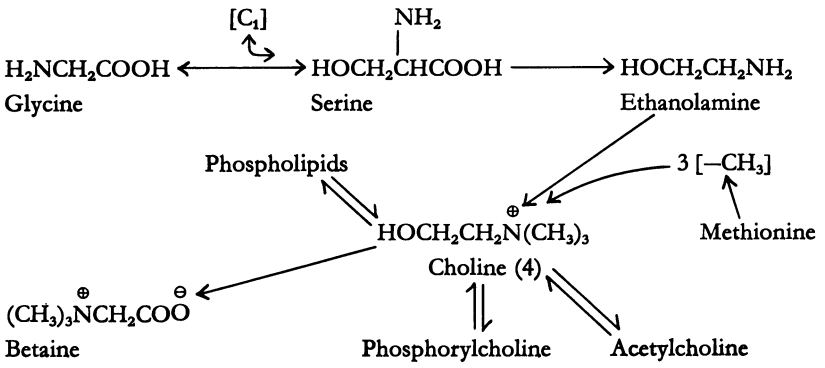
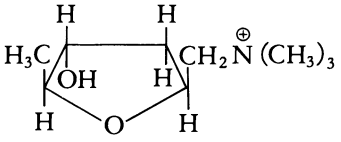
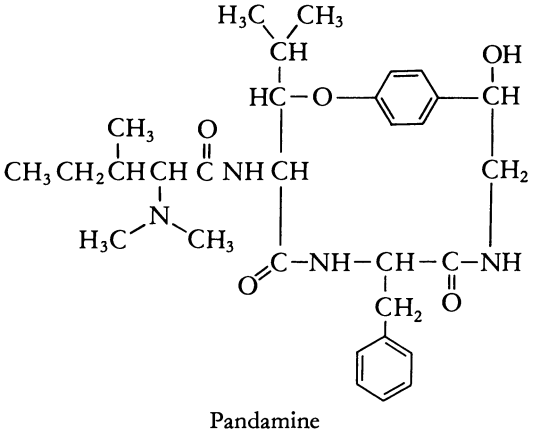


Fig. 3-2. Pathways of choline metabolism



Muscarine (5)

Certain basic substances isolated from plants have been first classed as alkaloids and later found to be more correctly classed as peptides or slightly modified peptides. They are reviewed in [73,105]. Most are based on the phenylcyclopeptine ring. The structure of one of these, pandamine from *Panda oleosa*, is given below:



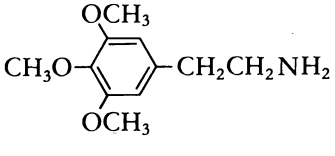
Pandamine

Other compounds of this group are julocrotine from *Julocroton montevidensis* [70], zizyphin from *Zizyphus oenoplia* [120], and ceanothine B from *Ceanothus* spp. [47]. Peptides are also found as an essential part of the lysergic acid

group of alkaloids (see Chap. 10) as well as being toxic constituents of *Amanita* spp. [114] and of *Viscum album* [65,82].

Protoalkaloids related to the aromatic amino acids tyrosine and dihydroxyphenylalanine are rather common in their own right and are also important as postulated precursors of other groups of alkaloids. Phenethylamine derivatives of plants are reviewed in [92]. While phenylethylamine (6) formed from phenylalanine is of widespread occurrence in plants, tyramine (7) and derivatives of it are more common. A survey of 188 plant species showed tyramine to be present in 15% of them [113]. Although dihydroxyphenylalanine is not a common plant amino acid, its decarboxylation product dopamine (8) is well-established as a plant constituent and occurs at high concentration in banana peel where it is the chief substrate for the browning reaction [31]. Separate decarboxylases for tyrosine and dihydroxyphenylalanine have been characterized from plants. The former has some activity with L-DOPA but none with D-tyrosine [38]. The latter acts on both D- and L-DOPA [104]. Successive methylations of the nitrogen in tyramine give rise in sequence to N-methyltyramine (9), hordenine (10), and candicine (11). All four of these compounds have been found to occur together in barley roots [38], and they are found singly in a variety of other plants. Tracer experiments with barley have demonstrated the origin of tyramine and hordenine from phenylalanine [104] or tyrosine [13,64,104]. Such experiments have also shown the origin of the methyl groups of hordenine from methionine [62]. This methylation is evidently reversible [38]. Some studies have been done on the enzymatic system responsible for methylation of tyramine in *Panicum miliaceum* [13] and *Hordeum vulgare* [98]. The enzyme tyramine methylpherase appears in germinating barley roots at the same time as tyramine and hordenine, and its formation in isolated embryos can be stimulated by the addition of kinetin [98]. It appears that the activity of the methylase is the controlling factor on the rate of hordenine accumulation in barley [98]. The conversion of hordenine-α-[^{14}C] to a radioactive, nitrogen-containing lignin fraction in barley has been reported and is of interest as indicating an active metabolic role for hordenine. Demethylation to N-methyltyramine also occurs [28]. Cell-suspension cultures of barley degrade hordenine to 3,4-dihydroxyphenylacetic acid and thence to ring-fission products [8].

Mescaline, a hallucinogenic drug from the peyote cactus [*Anhalonium lewinii (=Lophophora williamsii)*], has the structure:



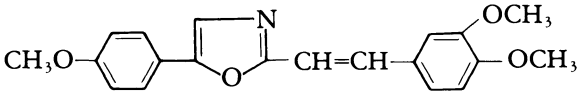
Dopamine is a precursor of mescaline, and, as shown in Fig. 3-3, it can be derived either by decarboxylation of DOPA or by hydroxylation of tyramine [80]. Relative percentage recoveries of radioactivity from α-[^{14}C] com-

pounds were phenylalanine 0.025, tyrosine 0.32, tyramine 1.53, DOPA 1.58, and dopamine 1.90 [80]. The sequence of intermediates between dopamine and mescaline appears to be [9,55,74]:

3-Methoxy-4-hydroxyphenethylamine
↓
5-Methoxy-3,4-dihydroxyphenethylamine
↓
3,5-Dimethoxy-4-hydroxyphenethylamine
↓
Mescaline (3,4,5-trimethoxyphenethylamine)

Methionine is the source of methyl groups [10,56], and the second reaction shown above commits the sequence to mescaline. If at this step *para* methylation occurs, the product (3,4-dimethoxy-5-hydroxyphenethylamine) does not go on to mescaline but is, rather, a precursor of the isoquinoline alkaloids that co-occur with mescaline (cf. Chap. 7). Mescaline does not serve as a precursor to the isoquinolines [56], but to some extent it does appear to be converted into several amides [42]. Some tracer experiments using tyrosine as a precursor of peyote alkaloids have been interpreted as showing that the utilization of tyrosine for protein synthesis is segregated from the alkaloid pathway and that the former is subject to feedback control, whereas the latter is not [81]. L-tyrosine, rather than D-tyrosine, is used for alkaloid biosynthesis, as it is for protein synthesis [81].

Several physiologically interesting compounds are obviously related to the phenethylamines but distinguished by having a hydroxyl group at the β-position of the side chain. Best known of such compounds are the animal neurotransmitters noradrenaline (norepinephrine) (13) and octopamine (*p*-hydroxyphenethanolamine). Both of these substances are also found in plants [25,27,36,45] as are various N- and O-methyl derivatives of them [43,44,112]. There does not appear to be an obligatory, straight-line sequence of methylation and hydroxylation reactions leading to these compounds [43,112]. The enzyme responsible for oxidation of dopamine to noradrenaline has been partially purified from bananas, and molecular oxygen has been shown to be the oxident [96]. In *Lolium multiflorum* (rye grass) octopamine co-occurs with annuloline and may be a precursor of it [36].



Annuloline

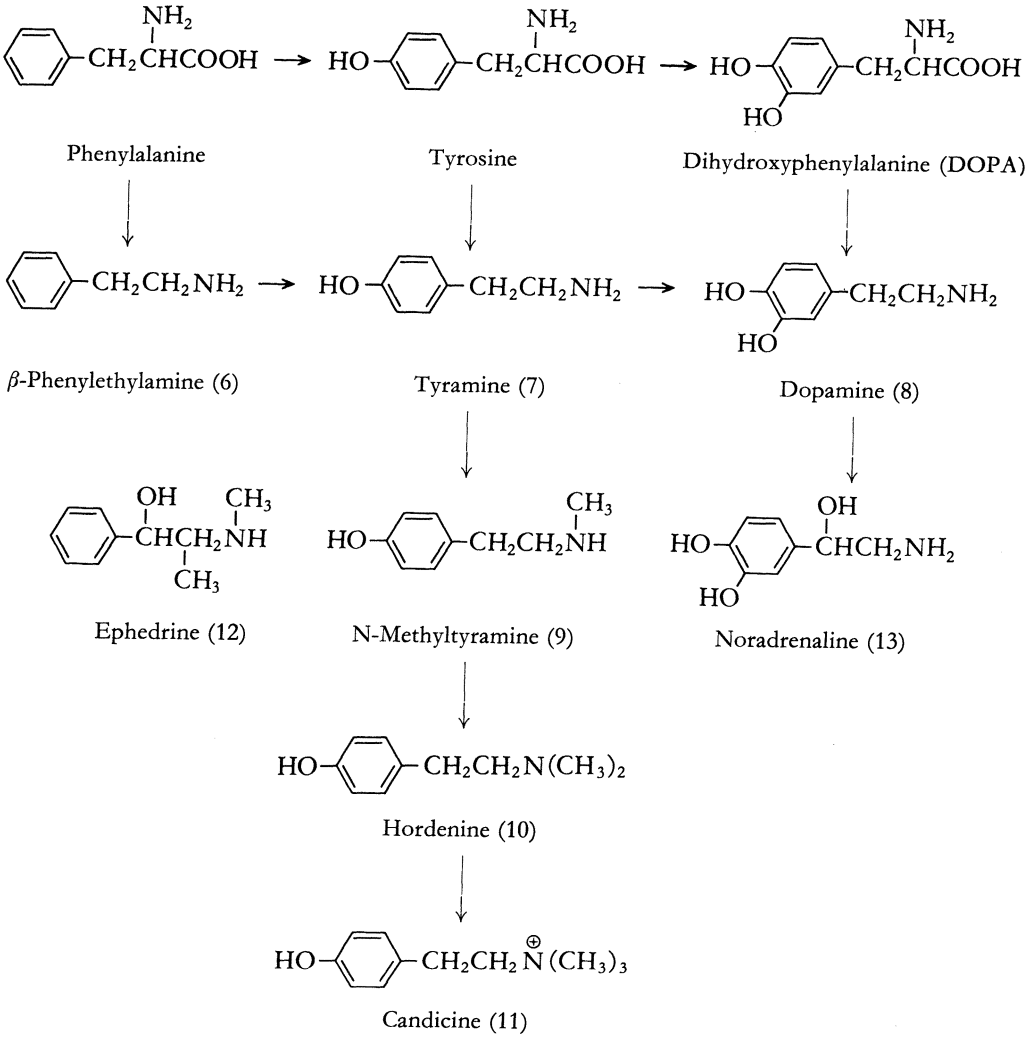
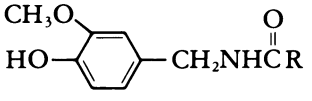


Fig. 3-3. Amines and protoalkaloids related to phenylalanine and tyrosine

Ephedrine (12) is also a product of this β-hydroxylation pathway, but coming from phenylalanine rather than tyrosine. Surprisingly, phenethylamine does not appear to be an intermediate, because tracer experiments have shown that the α-carbon of phenylalanine is lost in its conversion to ephedrine. Probably a C_6–C_1 fragment is made and then condensed with a C_2–N fragment [117,118]. Formate is a good precursor of both methyl groups but methionine only of the N-methyl group [118]. Factors influencing ephedrine production by callus tissue cultures of *Ephedra gerardiana*

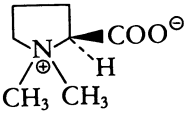
have been investigated, and it appears that active protein synthesis inhibits ephedrine synthesis [77].

The capsaicinoids of red pepper are a group of similar amides formed between vanillylamine and various short-chain fatty acids. Tracer experiments have shown that the vanillylamine unit is derived from phenylalanine and hydroxycinnamic acids [11,39].

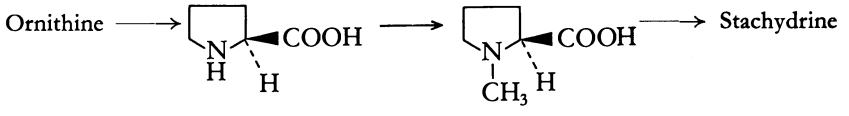


A Capsaicinoid

(−)-Stachydrine, the betaine of L-proline,



is known to occur in a large number of unrelated plants. Racemic stachydrine also occurs widely. The two isomeric betaines of hydroxyproline, betonicine and turicine, are known but less common. Homostachydrine, the homologous betaine of pipecolic acid, is known from alfalfa (*Medicago sativa*). Biosynthesis of stachydrine and homostachydrine has been investigated by tracer feeding experiments using such likely looking precursors as ornithine, proline, and lysine [24]. After many negative results it was concluded that young alfalfa plants (3 weeks old) do not synthesize stachydrine although they can make proline from ornithine; or if hygric acid is provided, they can methylate it to stachydrine. The block between proline and hygric acid is removed as the plant matures, and mature plants convert proline to stachydrine so rapidly that free proline cannot be detected in them [24]. Evidently, then, two different methylating enzymes function in the following scheme:



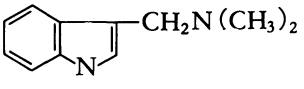
L-Proline Hygric Acid

The amino acid tryptophan is a precursor of the important plant hormones related to indole-3-acetic acid as well as of the indole alkaloids

(Chap. 10). A small group of indole derivatives derived from tryptophan by decarboxylation probably includes precursors of the indole alkaloids as well as compounds related in a presently obscure way to the indole hormones [67]. Tryptamine has been reported to occur in many plants of the family Leguminosae. N-methyl and N-dimethyl tryptamine have also been found in several different plants [29]. Serotonin (5-hydroxytryptamine) (14) is also widely distributed, although in small amounts [46]. These compounds are derived from tryptophan [83]. Various N- and O-methylated derivatives of serotonin are best known as constituents of toad secretions but also occur in certain fungi and higher plants [26,83]. Canary grass (*Phalaris* spp.) contains several such compounds that have low toxicity to grazing animals [59]. The N-methyltransferases responsible for their biosynthesis have been purified and characterized [58]. Methylated derivatives of 4-hydroxytryptamine are of interest as the hallucinogenic components of certain mushrooms [12,53]. Structures of some of the naturally occurring tryptamine derivatives are given in Table 3-2.

Tryptamine can act as a plant growth substance since it apparently is converted to indole-3-acetic acid, but the pathway involving tryptamine is not thought to be the only route for the formation of indole hormones from tryptophan [67].

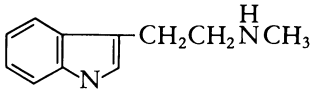
The widely occurring protoalkaloid gramine (3-dimethylaminomethylindole)



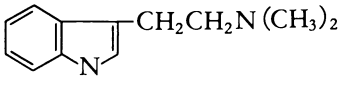
Gramine

is peculiar in that although it is derived from tryptophan [48,83,115], it has a shorter side chain than tryptamine. The β-carbon of tryptophan is retained in gramine [33,50], but it is not known whether the nitrogen of tryptophan is retained in the alkaloid. Nitrogen from glycine can be incorporated into the side chain of gramine, but this may be indirect [34]. A cell-free preparation from barley catalyzes the formation of gramine if supplied with tryptophan, ATP, and methionine [68], and other work also shows that methionine is the source of methyl groups [49]. Although gramine is not present in ungerminated barley seeds, it appears (along with 3-aminomethylindole and 3-methylaminomethylindole) within 4 days after planting and then persists for at least 50 days [69,107]. During this time, although the total content remains relatively constant, both synthesis and degradation are occurring [32]. Tracer experiments have shown that degradation of gramine by barley plants apparently leads to an indole derivative that can be reconverted to tryptophan [21,22]. In flowers of *Arundo donax*

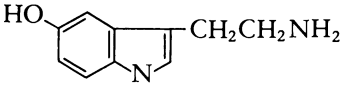
Table 3-2. Some derivatives of tryptamine found in higher plants and fungi



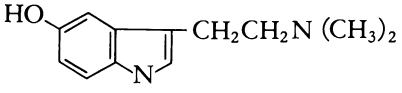
Dipterin



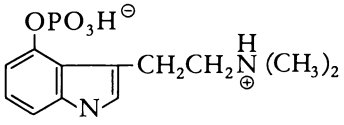
N, N-Dimethyltryptamine



Serotonin (14)



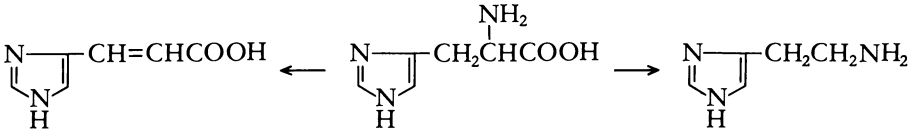
Bufotenine



Psilocybin

gramine appears early but is gradually replaced by quaternary derivatives of it [30].

Histamine, the decarboxylation product of the amino acid histidine, is, like serotonin, more familiar as an animal hormone than as a plant product. However, it and derivatives of it are firmly established as plant constituents. In the case of nettles (*Urtica* spp.), the presence of histamine in the stinging hairs may account at least partially for their irritant effects [23]. One of the best plant sources of histamine and its derivatives is spinach (*Spinacea oleracea*) [4], and the formation of histamine from labeled histidine has been studied in germinating spinach seeds. While the decarboxylation occurred rapidly in spinach, it was negligible in 10 other plant species tested [35]. *Phaseolus* spp. convert histidine to urocanic acid (imidazoleacrylic acid) [57,84]. Histidine is also presumably a precursor of several imidazole alkaloids (Chap. 12).



Urocanic Acid Histidine Histamine

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Chapter 4

Pyrrolidine, Piperidine, and Pyridine Alkaloids

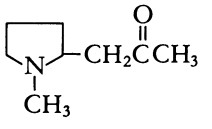
My heart aches, and a drowsy numbness pains
My sense, as though of hemlock I had drunk,
Or emptied some dull opiate to the drains
One minute past, and Lethe-wards had sunk:

Ode to a Nightingale, JOHN KEATS

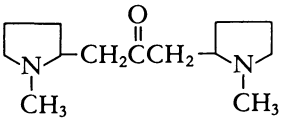
Pyrrolidine Alkaloids

There are only a few simple pyrrolidine alkaloids found in nature, although pyrrolidine (or pyrrole) rings combined into larger structures are relatively common (e.g., in the indole alkaloids). Methylated derivatives of proline or hydroxyproline could be called alkaloids but are placed here, with proto-alkaloids in Chap. 2; β-methylpyrroline is considered with the terpenoid alkaloids in Chap. 13.

The two best-known pyrrolidine alkaloids are hygrine and cuscohygrine, both found in *Erythroxylum coca* and various other plants.

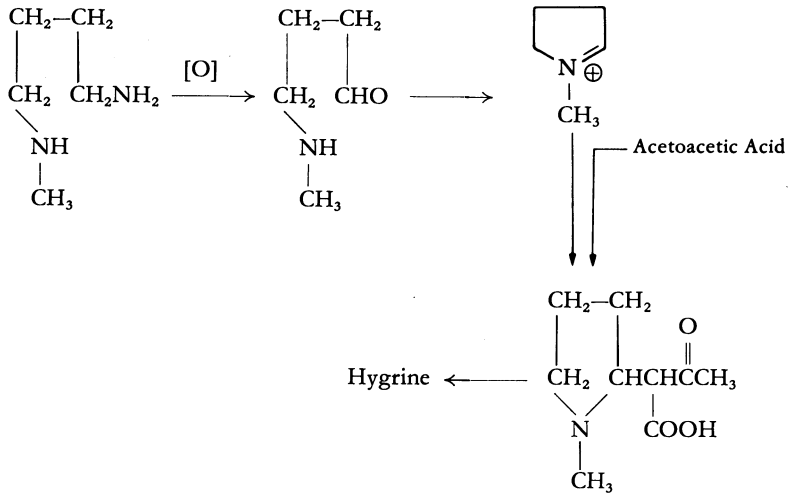


(±)-Hygrine



Cuscohygrine

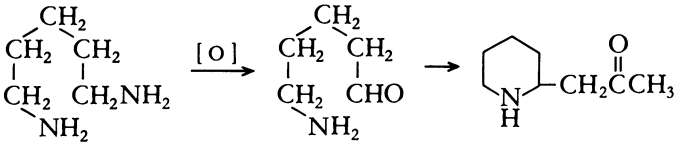
Both are formed nonenzymatically in mixtures of acetonedicarboxylic acid and N-methyl-2-hydroxypyrrolidine [28]. They are also produced by exposing a mixture of N-methylputrescine and acetoacetic acid or acetonedicarboxylic acid to the action of diamine oxidase [193]. Presumably in the latter case an aminoaldehyde is first formed and condenses to a pyrrolinium salt that reacts with the acid [85]:



Norhygrine is made in a similar system substituting putrescine for its N-methyl derivative [19]. Such in vitro reactions are believed to be analogous to the in vivo biosynthesis. Tracer experiments have shown that ornithine, putrescine, and N-methylputrescine are efficient precursors of the pyrrolidine ring of hygrine and cuscohygrine [108,146]. Hygrine is apparently a precursor of cuscohygrine by condensation with a second molecule of pyrrolinium ion [108,146]. Acetate is a precursor of the C_3 chains of both these alkaloids [7,108,146], and acetoacetic acid may be the more immediate precursor. Hygrine is also a precursor of some tropane alkaloids, so that experiments performed to elucidate the biosynthesis of the tropane ring also provide information on precursors of hygrine (cf. Chap. 5). The alkaloids of *Tylophora* spp. that have a complex phenanthroindolizidine structure are apparently derived from Δ^1-pyrroline plus two different phenethyl units [59,130].

Piperidine Alkaloids

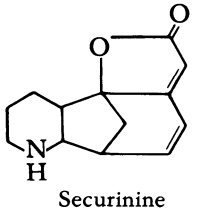
Some piperidine alkaloids, such as isopelletierine and coniine, are usually considered to be made in the same way as hygrine but starting with cadaverine (or lysine) rather than putrescine [48]:



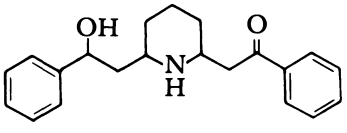
Isopelletierine

The same in vitro systems that are found to produce hygrine are found with suitable modification to produce isopelletierine or N-methylisopelletierine [19,28,193]. Tracer experiments, also, have shown that L-lysine (or cadaverine) is a precursor of the piperidine ring and acetate of the C_3 chain in N-methylisopelletierine of *Punica granatum* [107,145] and *Sedum sarmentosum* [50,106] as well as several related alkaloids in these and other plants [145,107]. Although cadaverine is an efficient precursor, the results with lysine indicate an unsymmetrical intermediate [50], which is not pipecolic acid [107]. One possibility is that cadaverine formed from lysine remains bound to the decarboxylase in an unsymmetrical way until it is acted on by diamine oxidase [106]. Cyclization of the 5-aminopentanal to $\Delta^{1,2}$-piperideine is then followed by condensation with acetoacetic acid and decarboxylation to isopelletierine [86]. Halosaline of *Haloxylon salicornicum* is also derived from lysine but has a C_5 side chain rather than C_3 [142]. The Lycopodium alkaloids at first appeared to be dimers of isopelletierine; but tracer experiments have shown, rather, that only one isopelletierine unit is incorporated and combined with a different precursor derived from malonic acid [13,14,120].

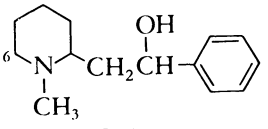
Several, more complex piperidine alkaloids appear to be derived by condensation of Δ^1-piperideine with an aromatic acid rather than acetoacetate. In this group are securinine of *Securinega suffruticosa*, santiaguine of *Adenocarpus foliosus*, sedamine of *Sedum* spp., and several alkaloids of *Lobelia* spp.



Securinine



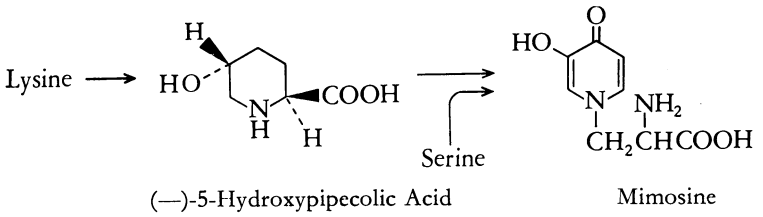
Lobeline



Sedamine

For securinine, tyrosine is a precursor of the C_6–C_2 unit attached to the piperidine ring, but closer precursors are unknown [41,150,162]. For the others, phenylalanine is a precursor of the aromatic portion, and cinnamic acid is probably a closer precursor [53,143,144,147]. The incorporation of lysine into sedamine proceeds via an unsymmetrical intermediate [50–52,77] which is not yet identified [50–52,77]. A group of alkaloids from the family Lythraceae incorporates two aromatic units from phenylalanine condensed in complex ways with a piperidine ring from lysine [49,63].

Mimosine, found in *Mimosa pudica* and *Leucaena glauca* has its pyridone ring derived from lysine and the alanine side chain from serine [65,140,184,185]. Since *L. glauca* also contains 5-hydroxypipecolic acid, this is a possible intermediate between lysine and mimosine, but hydroxylysine was found not to be a good precursor [184].



Extracts from *L. glauca* catalyze the degradation of mimosine to 3,4-dihydroxypyridine, pyruvate, and ammonia [177]. In the presence of a thiol compound, the alanyl side chain, rather than being decomposed to pyruvate and ammonia, is added to the thiol to make a substituted cysteine [132]. Mimosine also occurs to a small extent as a glucoside, mimoside; and extracts of *L. glauca* catalyze the formation of the glucoside from mimosine plus UDP-glucose [133]. It appears that mimosine is toxic to bean plants because dihydroxypyridine is formed and chelates iron [177].

Besides the piperidine alkaloids that are derived from lysine, there is another distinct group whose precursor is acetate. The best-studied of this group is coniine of *Conium maculatum*, which evidently is made by the condensation of four acetate units to a C_8 intermediate, then addition of nitrogen and ring closure [83] (Fig. 4-1). A reasonable intermediate in this scheme is 5-ketooctanal. An aminotransferase present in *C. maculatum* catalyzes the transfer of an amino group from L-alanine to it, and the 5-ketooctylamine that is produced cyclizes spontaneously to γ-coniceine [156,157]. The role of γ-coniceine as a precursor of coniine is generally accepted. When γ-coniceine-1′-[^{14}C] was fed to *C. maculatum* plants, 9.9% incorporation into coniine was observed [91]. An enzyme that catalyzes the reversible transfer of hydrogen between coniine and γ-coniceine has also been found in leaves and fruits of the plant. It uses NADP as its coenzyme

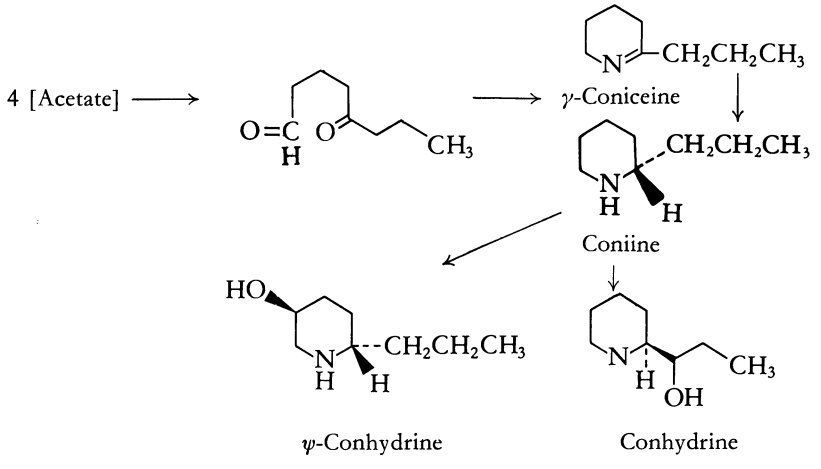
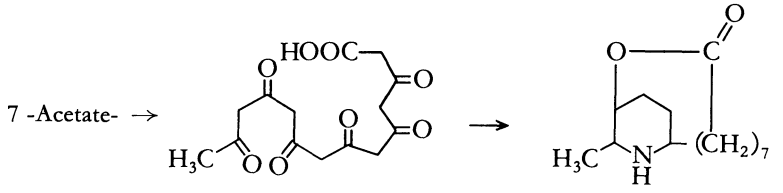


Fig. 4-1. Pathways to coniine and related alkaloids

[155]. Further conversion of coniine to conhydrine or pseudoconhydrine also occurs [91], and N-methylconiine can be produced by a methyl transfer from S-adenosylmethionine [154]. Other piperidine alkaloids that seem to come from the polyacetate pathway are carpaine of *Carica papaya* [10],



Carpaine

pinidine of *Pinus jeffreyii* [93,98], pyrindicin of *Streptomyces griseoflavus* [69], and several alkaloids of *Cassia spectabilis* [129].

The foregoing discussion makes it clear that there are at least two known pathways to the piperidine alkaloids: one from lysine and one from acetate. It should also be mentioned that some alkaloids with a piperidine ring are probably derived from terpenoids (see Chap. 13).

Pyridine Alkaloids

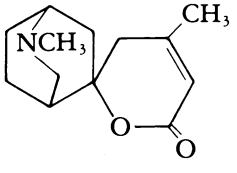
Although the pyridine alkaloids bear a superficial resemblance to the piperidine alkaloids, there is a distinct pathway leading to the pyridine ring which is different from any of the piperidine pathways. Cases of formation of a pyridine alkaloid by dehydrogenation of a piperidine or vice versa are almost nonexistent (but see anabasine, later in this chapter).

The vitamin, nicotinic acid, is the key compound involved in the biosynthesis of the pyridine alkaloids. Unfortunately, details of the synthesis of nicotinic acid itself in higher plants are still unknown. Indeed, more has been learned about the biosynthesis of nicotinic acid in higher plants from the study of alkaloids known to be derived from it than by any direct investigations. Therefore the pathway from nicotinic acid to several alkaloids will be discussed first, and finally the biosynthesis of nicotinic acid itself. There is a review on naturally occurring pyridine derivatives [45].

Trigonelline and Other Simple Derivatives of Nicotinic Acid

The simplest pyridine alkaloid is N-methylnicotinic acid, or trigonelline. Trigonelline occurs widely distributed in the plant kingdom. An enzyme catalyzing its formation from nicotinic acid and S-adenosylmethionine has been found in the pea plant [66]. Trigonelline can evidently serve as a storage form of nicotinic acid and can also contribute its methyl group to the

C_1 pool [11,47,58,66,73]. Since free nicotinic acid is rapidly destroyed in pea plants, its storage in a more inert form may be advantageous [73]. There is some indication that trigonelline may have additional physiological significance since it has been found to act similarly to kinetin in inhibiting yellowing of isolated wheat leaves [74]. It also inhibits growth in peas [30]. Whereas legumes appear to use trigonelline as a storage form of nicotinic acid, other plants store the N-arabinoside of nicotinic acid instead [105]. A survey of 40 cell-suspension cultures showed that the arabinoside was more restricted in occurrence than was trigonelline [137]. A transferase that catalyzes formation of the arabinoside from UDP-arabinose and nicotinic acid has been purified from parsley cells [104]. Other, rarer derivatives of nicotinic acid are sesquiterpenoid esters ("cathedulins") of *Catha edulis* [9], substituted amides of rutaceous plants [16], and 6-nicotinoylglucose present in *Cryptolepsis buchanani* [26]. When nicotinic acid is fed to tobacco plants, a major product formed is its N-glucoside [124]. Nicotianine of tobacco appears to be derived from nicotinic acid plus homocysteine [139]. Dioscorine of *Dioscorea hispida* is made from nicotinic acid plus a C_8 unit from acetate [90].



Dioscorine

Ricinine

The most intensively studied of the pyridine alkaloids with a single heterocyclic ring is ricinine (1) of the castor bean plant (*Ricinus communis*). The advantages of ricinine as an object of study are that the plant is easily grown and that it contains high concentrations of only one alkaloid. Ricinine synthesis is clearly associated with rapid growth both in whole plants [197] and in isolated root cultures [54]. In young seedlings, the quantity per plant may increase up to 50-fold within a week after planting [197]. Derivation of the pyridine ring of ricinine from nicotinic acid or nicotinamide was first shown by Leete and Leitz [99] and has since been amply demonstrated by others [195]. Castor bean seedlings also convert a large percentage of fed nicotinic acid to trigonelline and do it at a rate faster than they make ricinine [121]. Taken together with the finding that quinolinic acid is a somewhat more efficient precursor of ricinine than is nicotinic acid [208], this result suggests that free nicotinic acid is not on the normal "main line" to ricinine but that a nucleotide of it may be. This nucleotide could be derived from

nicotinic acid but more directly from quinolinic acid [61]. It is clear that pyridine nucleotide metabolism is closely related to ricinine biosynthesis because inhibitors of the pyridine nucleotide cycle cause parallel inhibition of ricinine biosynthesis [72]. Also, the enzyme responsible for synthesis of nicotinic acid mononucleotide from quinolinic acid shows a six-fold increase in activity just preceding the rapid increase in ricinine formation [118]. It thus seems likely that the pathway to ricinine branches off from the pyridine nucleotide cycle; but these branches may occur at more than one point, giving a metabolic grid [72,198]. Arguing against the essentiality for nucleotides in the ricinine pathway is the observation that when nicotinamide labeled with [^{15}N] in the amide group and [^{3}H] at positions 2, 4, 5, and 6 of the ring was fed to castor bean plants, the amide nitrogen and the ring were incorporated as an intact unit [195]. All other evidence has shown that nicotinamide must be deaminated in order to enter the pyridine nucleotide cycle [15,62]. Again, this conflict can be reconciled by suggesting a metabolic grid with separate pathways to ricinine [198] (Fig. 4-2).

The two methyl groups of ricinine are, expectedly, derived from methionine [25] or formate [195]; but the point in the sequence when they are added is unknown, as is the point at which the pyridine ring becomes oxygenated, and the point at which the amide group is dehydrated to a cyano group. Castor bean seedlings (as well as other plants of the Euphorbiacae) contain an enzyme that oxidizes 1-alkylnicotinonitriles to the corresponding 4- and 6-pyridones [34–36]. Since the enzyme is not specific for the 1-alkyl group, oxidation could occur at a nucleotide stage or after substitution of a methyl group at this position has occurred. This enzyme could ac-

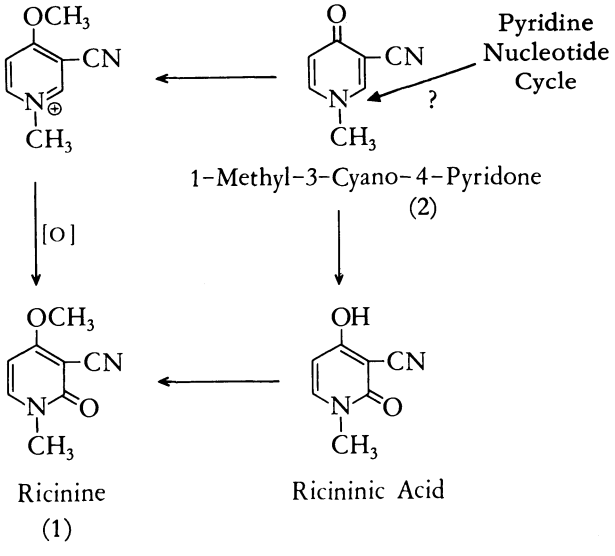


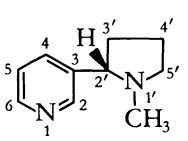
Fig. 4-2. Possible pathways of ricinine biosynthesis

count for the 4-oxidation, but no enzyme has been found for the 2-oxidation. 1-Methyl-3-cyano-4-pyridone, a reasonable intermediate in the ricinine pathway, has been identified in another plant of the same family, *Mallotus repandus* [60]. 1-Methyl-3-cyano-6-pyridone, 1-methyl-3-cyano-2-pyridone, and 1-methyl-3-carboxamido-6-pyridone are all present in *Trewia nudiflora*, another Euphorb which also has the oxidizing enzyme [34,38,128, 163]. 3-Cyano-4-methoxypyridine occurs in the non-Euphorb, *Hernandia nymphaefolia* [206]. Thus the processes leading to ricinine are not as unique to the castor bean plant as they once appeared to be.

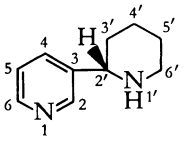
Some information is available regarding later transformations of ricinine in the castor bean plant. Labeled ricinine injected into castor bean plants was degraded up to 95% over a period of several weeks even though the total ricinine content of the plants increased greatly. No degradation occurred in maturing seeds, where the concentration increased as a result of both synthesis in situ and translocation from other parts [200]. Ricinine is almost completely absent from yellowed, senescent leaves. It is not only translocated from old leaves as such, but is also N- and O-demethylated and the demethylated products also translocated to younger parts of the plant [80,175,196]. Remethylation to ricinine occurs in actively growing regions, but some ricinine is also lost by degradation to CO_2 [196,200]. To some extent ricinine is also converted back to nicotinic acid and therefore, like trigonelline, it may be able to serve as a storage form of nicotinic acid [197].

Tobacco Alkaloids

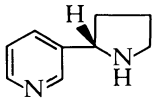
The alkaloids of tobacco (*Nicotiana* spp.), especially nicotine (3), have probably been investigated from biochemical standpoint as thoroughly as any other group of alkaloids. Considerable information is available regarding their route of biosynthesis and degradation. The typical tobacco alkaloids contain a pyridine ring attached at a β-position to the α-position of a pyrrolidine or piperidine ring as illustrated in Fig. 4-3. Tracer experiments have amply demonstrated that the pyridine ring is derived from nicotinic acid and the other ring from ornithine or lysine as in the case of simple pyrrolidine or piperidine alkaloids. Dawson and co-workers [21,23,178] fed nicotinic acid variously labeled with tritium or [^{14}C] to tobacco root cultures and showed that while the pyridine ring went intact to nicotine or anabasine (4), the hydrogen atom from C-6 and the carboxyl group were lost in the process. More recently, it has been established by tracer feeding experiments with whole plants that the pyrrolidine ring of nicotine becomes attached to the same carbon atom that held the carboxyl group in nicotinic acid. The pyridine ring therefore does not become symmetrical in the course of nicotine biosynthesis [100,173]. Nicotinic acid was a better precursor than quinolinic acid for nicotine, giving 10% incorporation of label vs 5% for quinolinic acid [173], or 6.9% vs 5.4% [33]. This difference argues against participation of nucleotide intermediates in nicotine biosynthesis. Al-



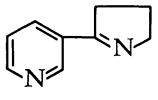
(—)-Nicotine (3)



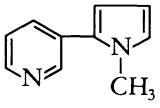
(—)-Anabasine (4)



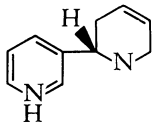
(—)-Nornicotine (5)



Myosmine



Nicotyrine



(—)-Anatabine

Fig. 4-3. Structures of some tobacco alkaloids

though NAD was incorporated to 5.8% [33], its prior degradation to nicotinic acid could not be ruled out. Circumstantial evidence for some role of pyridine nucleotides in nicotine biosynthesis is the finding of a correlation between high activity of quinolinic acid phosphoribosyl transferase and high rate of nicotine synthesis. The correlation is both temporal within a plant [118] and also genetic when high- and low-nicotine varieties of tobacco are compared [203].

Considerable detail has been learned regarding the biosynthesis of the pyrrolidine and piperidine rings of nicotine and anabasine. Glutamic acid-2-[^{14}C] or ornithine-2-[^{14}C] fed to *Nicotiana rustica* label equally the two α-carbons of the pyrrolidine ring of nicotine [103]. This result suggests the expected conversion of glutamic acid to ornithine and then to a symmetrical intermediate such as putrescine where label becomes randomized between C-1 and C-5. Other precursors that can transfer label to the pyrrolidine ring (e.g., acetate, aspartate, glycerol, propionate) can be understood as precursors of glutamic acid through well-established metabolic pathways [204,205]. Some results with other labeled compounds which do not follow obviously from known pathways have been interpreted in terms of different pool sizes and recycling of intermediates [88]. For instance, the pool size of acetate in tobacco leaves is extremely small, so that addition of labeled acetate or compounds rapidly converted to acetate could disturb the steady state and result in extensive recycling before appreciable label was supplied to alkaloid synthesis. Conversely, the pool size of succinate being much larger, any labeled compounds that come rapidly into equilibrium with succinate could contribute label to metabolites of succinate with less opportunity for recycling. These expectations were borne out. Label from acetate-2-[^{14}C] was extensively randomized before incorporation into nicotine, and label from succinate-1- or 2-[^{14}C] went to nicotine with negligible randomization. Malonate is presumably converted to acetate before being used in nicotine synthesis, but the labeling pattern of nicotine derived from

fed malonate differed from the pattern obtained from fed acetate. It is proposed that malonate is converted slowly to acetate so that the steady-state concentration is undisturbed, and that malonate feeding therefore can give a truer picture of the metabolism of endogenous acetate than can acetate feeding [18].

Putrescine is indeed a good precursor for the pyrrolidine ring of nicotine [82,210], and is probably the normal intermediate. If they are provided, both δ-N-methylornithine and N-methylputrescine are incorporated into nicotine unsymmetrically, but the former compound cannot be a natural intermediate because it would not allow for the symmetrical incorporation from ornithine [39]. N-methylputrescine is probably a normal intermediate, and C-1 from it goes specifically to C-5′ of nicotine [170,171]. The presence of putrescine N-methyl transferase in tobacco roots is further support for the pathway going through putrescine and N-methylputrescine [120,125,165]. Methionine is the source of the methyl group [42]. Earlier proposals that Δ^1-pyrroline or pyrroline-5-carboxylic acid were intermediates have been ruled out by later work [78,85]. The finding that ornithine labeled with [^{15}N] in the α-amino group contributed no [^{15}N] to the pyrrolidine ring while the δ-amino group did, seems at first sight to invalidate putrescine as an intermediate [96]. However, it can be explained away by suggesting that α-transamination of ornithine is a much faster process than decarboxylation. In this way replacement of essentially all the α-[^{15}N] with [^{14}N] could occur before significant decarboxylation to putrescine occurred. The action of a diamine oxidase on N-methylputrescine produces γ-methylaminobutyraldehyde, which is in equilibrium with N-methylpyrrolinium ion. The latter ion is probably the actual reactant in an electrophilic attack on nicotinic acid [89,123].

These reactions are summarized in Fig. 4-4. The loss of hydrogen from C-6 of the pyridine ring can be explained by supposing that the hydrogen that is added at the time of condensation is not the same one that is removed to reestablish the aromatic system [100].

Studies on the relationship between nicotine and nornicotine have made it clear that while some nornicotine is made by demethylation of nicotine, little or no nicotine is made by methylation of nornicotine [5,79,122]. Aside from tracer experiments that showed this relationship, it also follows from the fact that naturally occurring nornicotine is partially racemic, whereas nicotine is always (−) [75]. Also, although ornithine-2-[^{14}C] labels equally both α positions of the pyrrolidine ring of nicotine, in nornicotine isolated from the same plant C-2′ has twice as much label as C-5′ [122]. It appears that there are probably two routes to nornicotine—one from nicotine, giving the symmetrically labeled pyrrolidine ring, and another directly from ornithine, in which C-2 of ornithine goes exclusively to C-2′ [122]. Those species of tobacco that are noted for a high content of nornicotine probably owe this characteristic to high activity of nicotine demethylation in their leaves. Their roots synthesize nicotine just as high-nicotine plants do [20,75, 89,168]. The demethylation is largely oxidative, with the methyl group

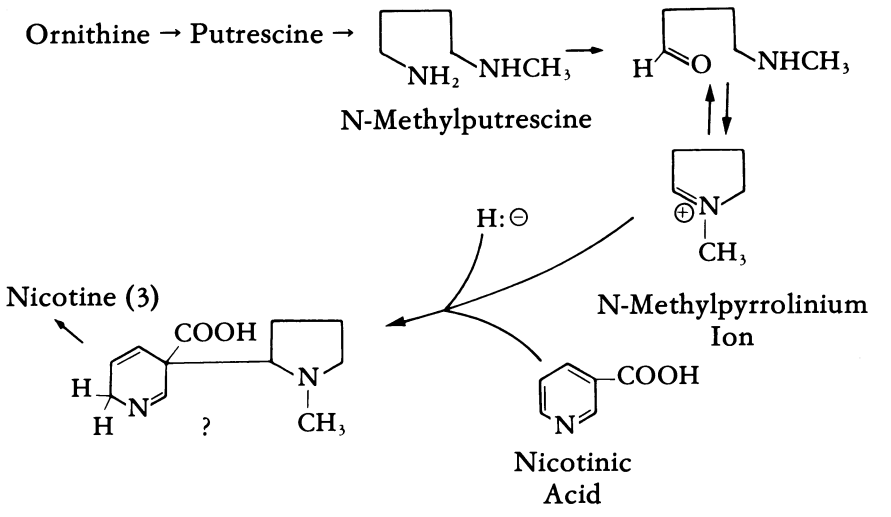


Fig. 4-4. Pathway to nicotine

appearing as CO_2 [68,75]. The mechanism of demethylation is still mysterious. Nicotine-1'-N-oxide does not seem to be an intermediate [3] and hydrogen is lost from C-2' [94].

Studies of tobacco alkaloid biosynthesis with isotopically labeled carbon dioxide or nitrate as the precursors are of special interest since these simple substances are the natural substrates for all plant biosynthesis. This approach, begun and continued by Tso and co-workers, has shown that movement of [^{14}C] from CO_2 into alkaloids occurs much later than its incorporation into such active metabolites as sugars and amino acids [191]. The various tobacco alkaloids were shown to arise rather independently of each other and not to be interconverted [188–190].

Further work with [$^{14}CO_2$] has been done by Rapoport and co-workers [2,4,34,109,160], who in addition to following the total incorporation into various alkaloids have degraded them for isolation of individual carbon atoms. Although these workers agree with previous observations that the primary site of nicotine biosynthesis is the root, they have shown that some nicotine of the shoot must arise in situ. So 2 or 4 h after exposure to [$^{14}CO_2$], nicotine in the root had 90% and 84%, respectively, of its radioactivity in the pyridine ring, but nicotine from the shoot had only 74% after 4 h. Such a result cannot be explained simply by translocation of nicotine from the root, but requires assumption of different pathways or precursor pool sizes in the shoot. Short-time experiments (i.e., less than 4 h) allowed estimation of the extent of synthesis in root vs shoot before translocation could obscure the results. It was concluded that about 16% of the total nicotine of *Nicotiana glutinosa* is synthesized in the shoot. Different species of *Nicotiana* may show quite different patterns of labeling from [$^{14}CO_2$]. Experiments were done primarily with *N. glutinosa*. While the reports of others that nornicotine

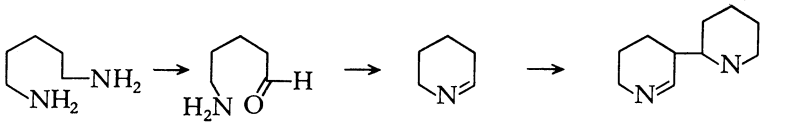
arises largely from demethylation of nicotine were confirmed, it was also found that in short-time experiments C-2′ of nicotine was labeled in preference to C-5′ [109,160]. This result throws into doubt the concept of a symmetrical intermediate under natural conditions. Interpretation of other unexpected labeling patterns led these workers to propose a complex scheme involving CO_2 fixation into glycolic acid and ultimate conversion of this to an unsymmetrically labeled glutamic acid. While some other work has confirmed the unsymmetrical incorporation of CO_2 into the pyrrolidine ring [64,134], this result is still controversial [88].

Nicotine is not an inert end product in tobacco plants. It has a half-life of less than 24 h, and more than 10% of the carbon dioxide fixed by a mature tobacco plant passes through nicotine on its way to other products [158]. The nature, and especially the relative amounts, of these other products is still obscure. Some of them are minor alkaloids of related structure, such as nornicotine, nicotine-1′-N-oxide, and myosmine [76,94,155,190]. Some methyl groups from nicotine appear to enter the C_1 pool and can be transferred to make choline or betaine [27,92]. Conversion of nicotine back to nicotinic acid has been reported [43], and myosmine may be an intermediate [94]. However, other evidence suggests that there is no simple, direct pathway from nicotine to nicotinic acid [111]. The reutilization of nicotine in the synthesis of amino acids and proteins has been shown by feeding [^{14}C]-labeled nicotine to *N. tabacum* and then isolating labeled amino acid and protein fractions [67,152].

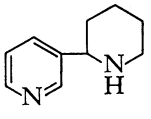
Studies on the biosynthesis of anabasine (4) have been less extensive than studies on nicotine biosynthesis. Most have utilized *N. glauca*, whose chief alkaloid is anabasine. As with nicotine, the pyridine ring comes from nicotinic acid [87,178]. There has never been any suggestion of a symmetrical intermediate in the biosynthesis of the piperidine ring of anabasine. Lysine-2-[^{14}C] labels only C-2′ of anabasine [81,114], in analogy with the transfer of label to nornicotine from ornithine, but unlike that to nicotine from ornithine. Feeding of lysine labeled with [^{15}N] in the α or ε amino group has shown that it is the α nitrogen that is lost in forming the piperidine ring [97]. In *N. glutinosa*, labeled anatabine and anabasine were formed from labeled CO_2, but anatabine always had a higher specific activity than anabasine and the two are evidently not interconvertible [75,101,141]. Anatabine derives both of its rings from nicotinic acid [102], but its isomer 1,2-dehydroanabasine is made from anabasine in the same way that nornicotine is converted to myosmine [15].

It has been found that anabasine can be made in rather simple model systems. A crude enzyme preparation from peas (*Pisum sativum*) can catalyze the formation of anabasine, along with related products such as tetrahydroanabasine and Δ^1-piperideine [56,57,119,127] from cadaverine. Lysine under similar conditions gives rise to Δ^1-piperideine-6-carboxylic acid and its dimer tetrahydroanabasinedicarboxylic acid [55]. Diamine oxidase is believed to be responsible for the initial reaction, as in the simpler cases of hygrine and isopelletierine, and the secondary condensations are

probably nonenzymatic. Formation of anabasine presumably occurs by dehydrogenation of tetrahydroanabasine, but the hydrogen acceptor and the enzyme involved (if any) are unknown. Lupine seedling extracts can also dehydrogenate tetrahydroanabasine to anabasine [172]. Neither compound is normally present in pea plants, and only traces of anabasine in lupine; so such results may point out that the enzymes needed for alkaloid biosynthesis are widespread and that only differences in cellular organization or degradative ability prevent all plants from accumulating alkaloids. Some possible intermediates in these in vitro systems are indicated below:

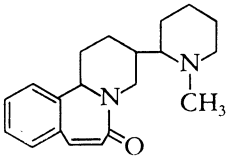


Cadaverine Δ^1-Piperideine Tetrahydroanabasine

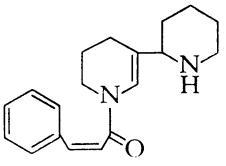


Anabasine (4)

Alkaloids related in structure to anabasine are astrocasine of *Astrocaria phyllantoides* and adenocarpine of *Adenocarpus viscosus*, and santiaguine of *Adenocarpus foliosus*. Tracer experiments have indicated synthesis of the latter two from cadaverine or tetrahydroanabasine [169]. A cinnamic acid unit appears to make up the rest of these molecules [143].



Astrocasine



Adenocarpine

Anatomical and ontogenetic studies on nicotine biosynthesis have revealed that seeds contain no free alkaloids but do have bound forms that are released by boiling hydrochloric acid [201]. Free alkaloids appear after 2 days, but the total amount declines up to about 3 days and then increases rapidly [12,112,201]. The major site of synthesis is in mature root cells [115]. Accumulation in leaves appear to be by a process of passive permeation dependent on a negative cell membrane potential [131,136]. Within a leaf cell there is accumulation in cell walls and vacuoles [131,164]. In some species, flowering appears to activate alkaloid synthesis [113]. Various effects of photoperiod, light intensity, and light quality have been observed

[192,201]. Among the plant growth regulators, both auxins and gibberellins inhibit alkaloid biosynthesis [151,179,209]. It has been possible to obtain normal-appearing haploid plants of *Nicotiana sylvestris* and to show that they had a very wide range of nicotine content and relative amounts of the different alkaloids [116].

Tissue and cell cultures similarly show a very wide range of alkaloid content, some close to the level of the parent plants although most are much lower; and some lack alkaloids altogether [148,180]. As with whole plants, 2,4-D inhibits alkaloid synthesis in tissue cultures [37,182]. Two mechanisms have been suggested—lowering the concentration of required precursors [181], and increasing the rate of degradation [8]. The availability of precursors may be more important in some strains and some growth conditions than in others [138,149]. In callus cultures the inhibition of protein synthesis by cycloheximide increased alkaloid synthesis, but in suspension cultures there was no effect of cycloheximide on alkaloid production up to concentrations that inhibited growth [138].

Nicotinic Acid

The biosynthesis of nicotinic acid in plants is reviewed in [22].

Once it became established that the pyridine rings of ricinine, nicotine, and anabasine all had the same pyridine ring compound, quinolinic acid, as a precursor and that nicotinic acid fell into this same group of compounds, it became possible to apply evidence regarding precursors of the pyridine ring of one of these compounds to all of them. Thus, it is now clear from tracer results that the middle two carbon atoms of various four-carbon dicarboxylic acids such as succinic, malic, and aspartic go rather directly to C-2 and C-3 of the pyridine ring [166,194]. A carboxyl group from these same acids becomes the carboxyl group of nicotinic acid or the nitrile carbon of ricinine [174,208,211], but both carboxyl carbons are lost when these acids go to nicotine or anabasine. Since through the glyoxylate cycle the middle two carbons of four-carbon dicarboxylic acids are derivable from C-2 of acetate and the carboxyl groups from C-1, tracer results with acetate have shown that C-2 and C-3 of ricinine, nicotine, and anabasine are derived from C-2 of acetate, while the carboxyl group of acetate goes to the nitrile carbon of ricinine.

Except for randomization, however, the carboxyl group does not enter the pyridine portion of the other two alkaloids [166,174,194,208,211]. Carbon atoms 4, 5, and 6 of the pyridine ring evidently enter as a C_3 unit closely related to glycerol, since glycerol-2-[^{14}C] puts label primarily into C-5, while glycerol-1-[^{14}C] labels equally C-4 and C-6. Entry of carbon from glycerol into C-2 and C-3 can be explained by conversion of some of the glycerol to acetate [31,95, 207,208]. Several other compounds have been tested as possible pyridine ring precursors. These compounds include malonic acid [18], propionic acid [44,271], formate [167], lysine [167],

alanine [18], and β-alanine [18,183]. Results with them are most conveniently explained by considering them to be precursors of glycerol or the four-carbon acids, which are regarded as more direct precursors. One rather surprising precursor is inorganic cyanide, which is a specific precursor of the nitrile group of ricinine [166]. While this is probably an in vitro artifact, it can be explained by reaction of HCN with serine to form β-cyanoalanine and hydrolysis of the β-cyanoalanine to aspartic acid, although a more direct pathway is not ruled out [186,187]. The source of the pyridine ring nitrogen is still controversial. In bacteria, carbons 2, 3, and 4 of aspartic acid along with the α-amino group are evidently incorporated as a unit into the pyridine ring of nicotinic acid [46,135,202]. A similar finding has been reported for the biosynthesis of ricinine in castor beans [199,208]. Other results showing that malate or aspartate labeled at C-3 puts nearly twice as much label at C-3 of the pyridine ring in nicotine as at C-2 also support the idea of an unsymmetrical C_4 precursor closer to aspartate than to succinate [70]. However, conflicting results that indicate a more indirect route for incorporation of aspartic acid have also been reported [71,183]. Some randomization of label in as active a metabolite as aspartic acid is of course inevitable. The chief argument against incorporation of aspartic acid as a unit into the pyridine ring of ricinine is the finding that when aspartic acid-[^{15}N] was fed, the same [^{15}N] abundance was found present in both nitrogen atoms of ricinine. This result was taken to indicate splitting off of nitrogen and equilibration of the label before incorporation [183]. The result could also be explained, however, by postulating a pathway wherein both nitrogens come immediately from aspartic acid (for instance, one by direct incorporation and the other by transamination). The latter explanation, while unlikely, cannot be ruled out. Leete proposed a condensation of glyceraldehyde-3-phosphate with aspartic acid to give a six-membered ring that goes on to quinolinic acid [84]. Glyceraldehyde-3-[^{14}C], when fed to *Nicotiana glauca*, labeled nicotine mostly at C-4 and was a more efficient precursor than glycerol [32]. From this result, if the scheme shown in Fig. 4-5 is followed, the aldehyde group of glyceraldehyde-3-phosphate condenses with the amino group of aspartic acid and C-3 with the β-carbon of aspartic acid to form an intermediate such as the one shown in brackets. Results from bacteria have shown that the actual reactants in the biosynthesis of quinolinic acid are a dehydrogenated form of aspartic acid (perhaps iminoaspartate) and dihydroxyacetone phosphate [135,202]. In higher plants it seems likely that a dihydroxyacetone derivative is involved because glycerol-2-[^{3}H] loses all tritium when it is converted to ricinine [159]. Dihydroxyacetone phosphate could, however, simply be an intermediate between glycerol and glyceraldehyde-3-phosphate. One clear difference between the bacterial and higher plant systems is that glycerol-1(3)-[^{3}H] does not contribute tritium to the pyridine ring in the former but does in the latter [17,159].

Whatever the precise C_3 and C_4 units are that condense, there is no doubt that the first pyridine derivative produced is quinolinic acid. It is not

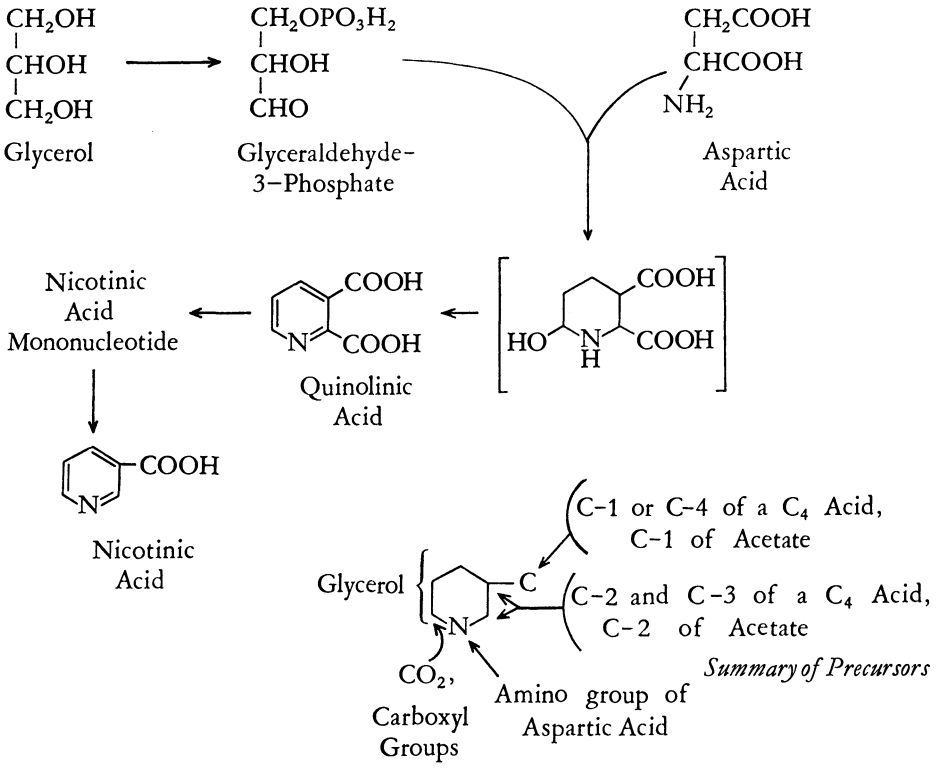


Fig. 4-5. Possible precursors of nicotinic acid

decarboxylated to free nicotinic acid, but in one-step reaction is both decarboxylated and converted to the nucleotide, nicotinic acid mononucleotide [117]. This nucleotide can be hydrolyzed to free nicotinic acid or can enter a cycle of pyridine nucleotides that leads both to synthesis of the ubiquitous dehydrogenase coenzymes or the pyridine alkaloids discussed in this chapter [40,161]. Whereas free nicotinic acid can be converted to its nucleotide, free nicotinamide is evidently converted to a nucleotide only after loss of its amino group [15]. It would seem, then, that any alkaloids derived from nicotinamide with retention of the amino group must be made without involvement of nucleotides.

Although the pathway to nicotinic acid that is described above is most probably the pathway used by higher plants, bacteria, and anaerobic yeast, many other organisms including aerobic yeast [1] and higher fungi [24,29] utilize a pathway from tryptophan. Both pathways converge on quinolinic acid as shown in Fig. 4-6. As a general principle, it has been proposed [110] that those organisms that make lysine from α-aminoadipic acid use the tryptophan pathway to nicotinic acid, and that those organisms that make lysine from 1,5-diaminopimelic acid use the other route.

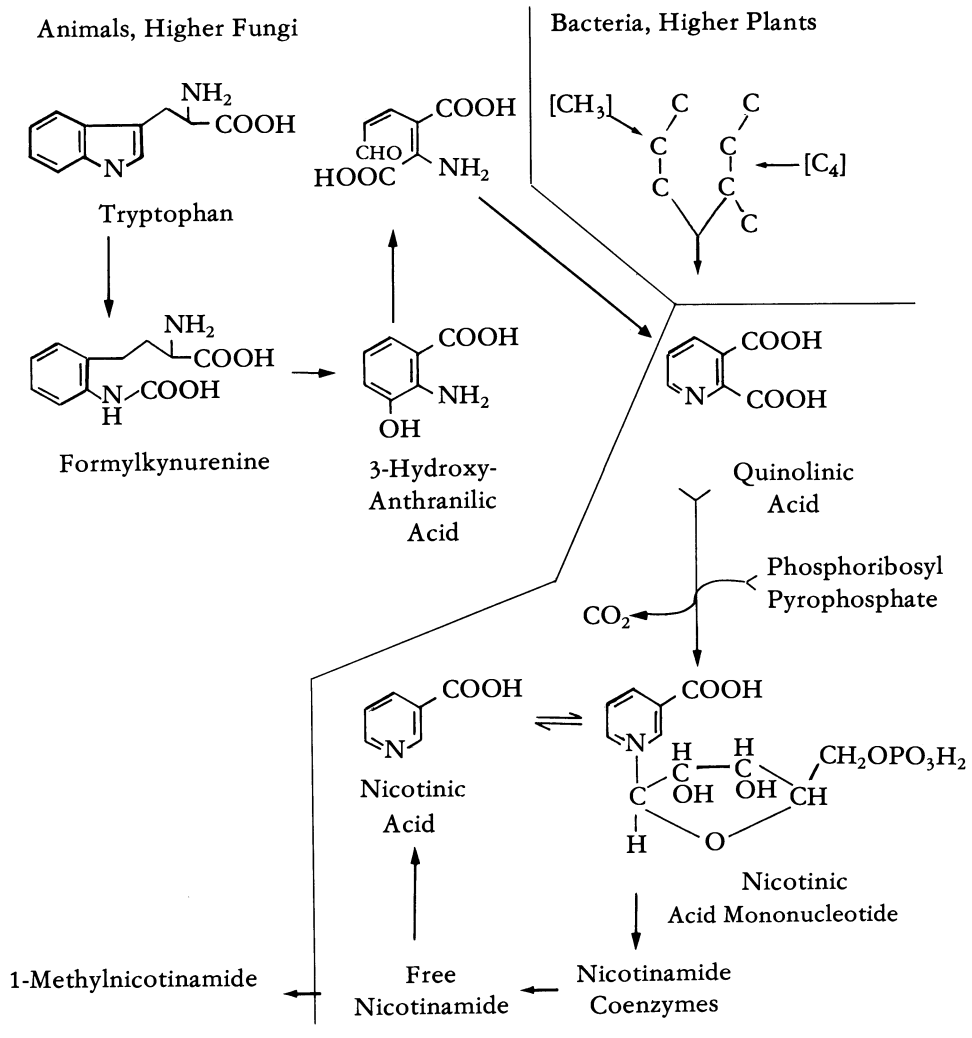


Fig. 4-6. Pathways of nicotinic acid biosynthesis

Some lingering doubt remains as to whether the tryptophan pathway to nicotinic acid is completely absent from higher plants. Tobacco plants do degrade tryptophan to a variety of products [176], and it is possible that some nicotinic acid gets made this way [6].

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Chapter 5

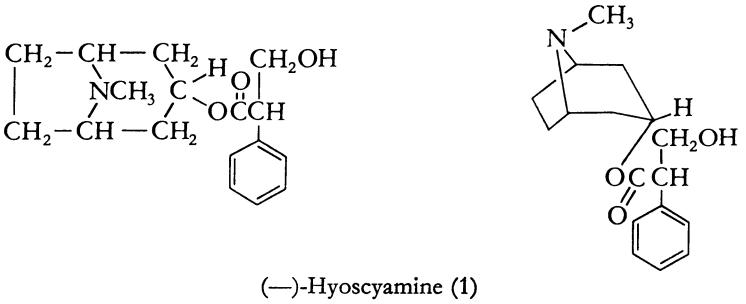
Tropane Alkaloids

Belladonna, n. In Italian a beautiful lady;
in English a deadly poison. A striking example
of the essential identity of the two tongues.

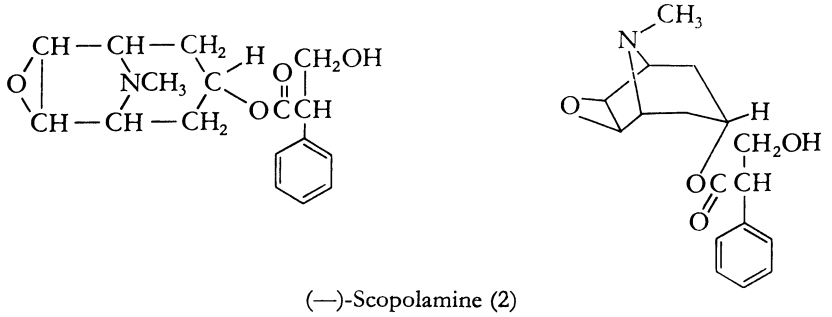
The Devil's Dictionary, AMBROSE BIERCE

The tropane alkaloids are divided into two major groups, those found in certain genera of the Solanaceae and those found in *Erythroxylum* spp. The solanaceous group includes species of *Atropa* (esp. *A. belladonna*), *Datura*, *Duboisia*, *Hyoscyamus*, *Mandragora*, and *Scopolia*. The best-known alkaloids of this group are (−)-hyoscyamine (1) and (−)-scopolamine (2) (hyoscine). (−)-Hyoscyamine and (−)-scopolamine are esters of tropic acid with the bases tropine and scopoline, respectively. Optical activity is contributed solely by the tropic acid residue since the bases have a plane of symmetry passing through C-3, the nitrogen atom, and the methyl group. In the accompanying conformational structures the C-3 ester group is shown as *trans* to the nitrogen atom, since it has this configuration in the best-known solanaceous tropane alkaloids. However, compounds with the opposite configuration are known both as synthetic derivatives and as naturally occurring alkaloids. They are designated by the prefix pseudo-. During pharmaceutical preparation of the esters of tropic acid, racemization readily occurs since the asymmetric carbon atom is adjacent to a carbonyl group and can enolize. DL-hyoscyamine resulting from this racemization is known as atropine.

Tropane Skeleton

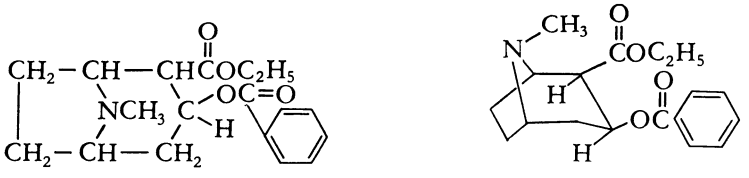


(—)-Hyoscyamine (1)



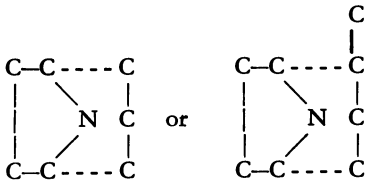
(—)-Scopolamine (2)

Species of *Erythroxylum* (especially *E. coca*) are the only known producers of cocaine (3) and alkaloids of related structure. This group of tropane alkaloids differs from the solanaceous group in having one more carbon atom as a carboxyl group attached at C-2. In (−)-cocaine both ester groups are arranged *cis* to the nitrogen atom as shown in the conformational structure below.



(—)-Cocaine (3)

The tropane alkaloids were among the first to have their route of biosynthesis investigated by the radiotracer technique, and the results obtained provided one of the best confirmations for Sir Robert Robinson's hypothetical ideas advanced nearly 40 years previously [40,64]. Leete [34] has reviewed recent work on the biosynthetic pathway. The tropane ring system is best regarded as made up of two parts, a pyrrolidine ring and a C_3 or C_4 chain attached across the α carbons of the pyrrolidine ring:



The pyrrolidine portion (as in proline and the simpler alkaloids discussed in Chap. 4) is shown by tracer evidence to be derived from ornithine or glutamic acid, but details are lacking as to the nature of the first cyclic intermediate. The frequent co-occurrence of hygrine with tropane alkaloids is suggestive of a close metabolic relationship [45,66]. Tracer experiments have shown that with ornithine-2-[^{14}C] as a precursor of hyoscyamine in *Datura stramonium* only one of the two bridgehead carbons (C-1 and C-5) became labeled, ruling out participation of a symmetrical intermediate like putrescine or succindialdehyde [32,33]. However, if putrescine labeled in its α-carbon atoms was fed, it was also an efficient precursor and labeled both bridgehead carbons [37,46,47]. Root cultures of *Datura metel* fed N-methyl-putrescine-[$^{14}CH_3$], [^{15}N] incorporated this compound into hyoscyamine and scopolamine with retention of the methyl group [43]. Therefore, more than one pathway is possible (Fig. 5-1). N-methylation of ornithine before its decarboxylation could give rise to the unsymmetrical N-methylputrescine and thus lead to labeling of only one bridgehead carbon. Decarboxylation to putrescine followed by methylation would give rise to labeling of both bridgehead carbons. The best indications are that δ-N-methylornithine is the true natural intermediate [1] and that it goes on to N-methyl-putrescine, which is acted on by an amine oxidase to give N-methyl-Δ^1-pyrrolinium ion just as in the pathway to hygrine (Chap. 4). Indeed, hygrine has been found by tracer experiments to be a precursor of tropane alkaloids [44,56]. Some plants can use both D- and L-hygrine equally as precursors of tropanes, but *Datura innoxia* uses preferentially the D(+) isomer [50,51].

Since hygrine is a precursor, it is not surprising that the C_3 or C_4 portion of the tropane alkaloids is derived from two molecules of acetate possibly by way of acetoacetate [44]. Alkaloids related to cocaine retain all four carbons and have a carboxyl substituent, while alkaloids related to hyoscyamine lose the fourth carbon by decarboxylation at some stage. A hypothetical scheme is given in Fig. 5-2. The keto intermediates shown in this scheme are 2-carboxyltropinone and tropinone. The latter has been found as a natural constituent of *Cyphomandra* spp. [13]. Reduction of the ketone to a secondary alcohol would in the latter case produce tropine, which on esterification yields the usual ester alkaloids. Robinson's original suggestion that the C_{3-4} unit might come from citric acid via acetonedicarboxylic acid has been shown to be invalid [61]. Although *Datura* root cultures will convert proline or hydroxyproline into tropane alkaloids, the pathway is believed to be indirect [17,18]. Since glycine-2-[^{14}C] is a poor precursor [15], a pathway involving its conversion to methylamine and

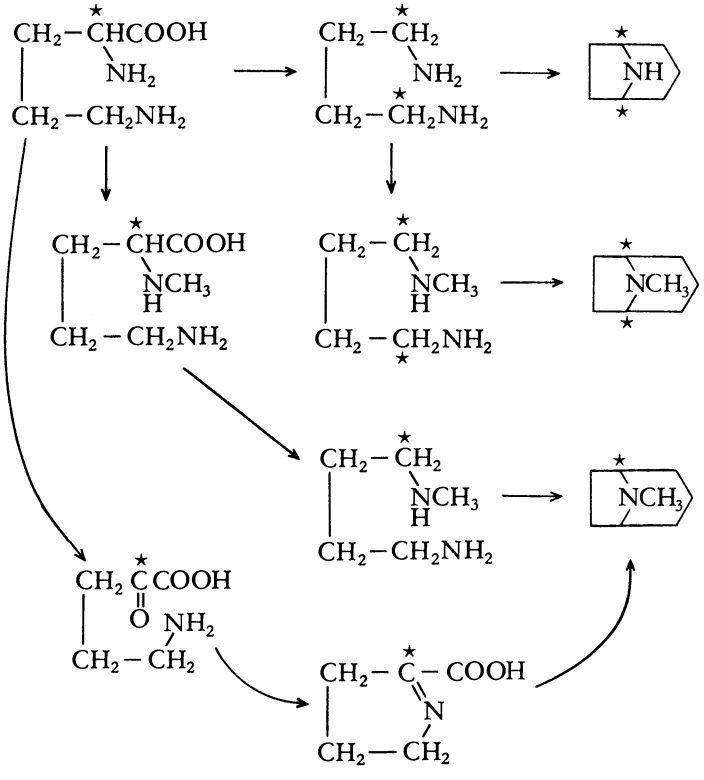


Fig. 5-1. Possible pathways from ornithine to tropane alkaloids. Labeled carbon atoms designated by*

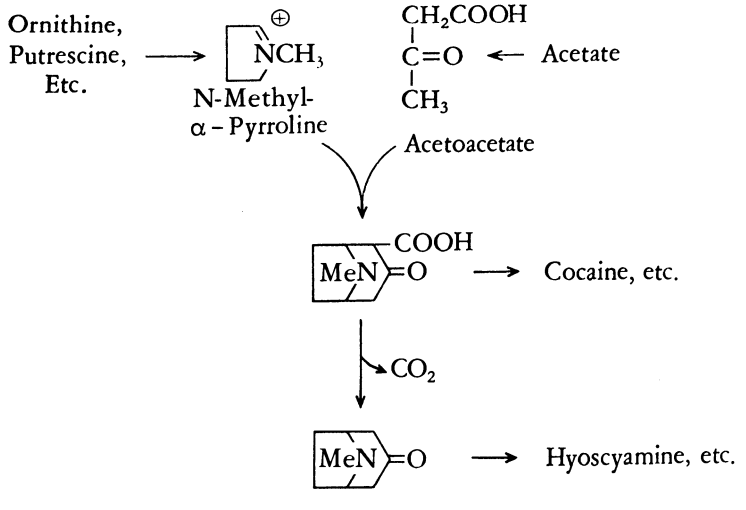


Fig. 5-2. Hypothetical scheme for incorporation of C_{3-4} unit into tropane alkaloids

incorporation of this as an —N—CH_3 group is ruled out. Since plant tissues do not ordinarily contain appreciable amounts of ornithine, the observations of Jindra et al. with *D. stramonium* [29] possibly throw important light on the control of biosynthesis for those alkaloids apparently derived from ornithine. Arginine being more common than ornithine, the level of arginase activity controls the availability of ornithine. In turn, one important factor controlling arginase activity is the concentration of manganese. Thus a mineral deficiency may indirectly decrease the alkaloid concentration of a plant. A similar interrelationship has been found for the lupine alkaloids (Chap. 6).

Most of the tracer work with tropane alkaloids has been applied specifically to hyoscyamine, but some also to scopolamine. The metabolic relationship between these two has been explored to some extent. Isolated root cultures of *Atropa belladonna* and *D. ferox* can synthesize both hyoscyamine and scopolamine [58,62]. Interspecific grafting experiments also indicate that the root is the primary site of formation of the tropane ring, but further transformations take place when the alkaloids are translocated to the aboveground organs [54]. In particular, roots normally contain more hyoscyamine than scopolamine; the reverse is true of aerial portions. Considerable conversion of hyoscyamine to scopolamine must therefore occur in stem and leaves, but excised root cultures can also convert hyoscyamine to scopolamine [67]. The conversion probably proceeds via 6-hydroxyhyoscyamine and 4,7-dehydrohyoscyamine [34]. The first of these two intermediates occurs naturally in *Datura* spp. [65]. Hyoscyamine is converted to its N-oxide by fruits of *Atropa belladonna* [59] and to various products by mature plants of *Datura innoxia* [23]. Earlier views that scopolamine is a precursor of hyoscyamine [77] or that the two are made by parallel routes from different precursors [40] seem to have lost favor (Fig. 5-3).

The place in metabolism of other minor alkaloids of *Datura* and *Atropa* spp. is also speculative. Tigloyl esters translocated from the roots of some species do not accumulate in aerial portions and therefore must be further metabolized [14]. However, in *Datura meteloides* the 3-tigloyl ester meteloidine (4) is found in leaves. In the biosynthesis of meteloidine, the hydroxyl groups at C-6 and C-7 are introduced after esterification at C-3 [38], and the process is evidently one of direct hydroxylation rather than dehydrogenation and hydration [39]. Other minor alkaloids appear attractive as intermediates between hyoscyamine and scopolamine [63].

The tiglic acid portion in some of these alkaloids is derived from isoleucine by loss of the carboxyl group and oxidation. Intermediates are 3-keto-2-methylvaleric acid and 2-methylbutyric acid [2,41,49]. The latter acid is found esterified in an alkaloid of *Datura ceratocaula* [3]. There has been conflicting evidence relating to possible precursors of the tropic acid found in hyoscyamine and scopolamine. The majority opinion now favors phenylalanine as a precursor through a pathway by which C-2 of its side chain becomes C-3 of tropic acid, and C-3 becomes C-2 of tropic acid. Tracer

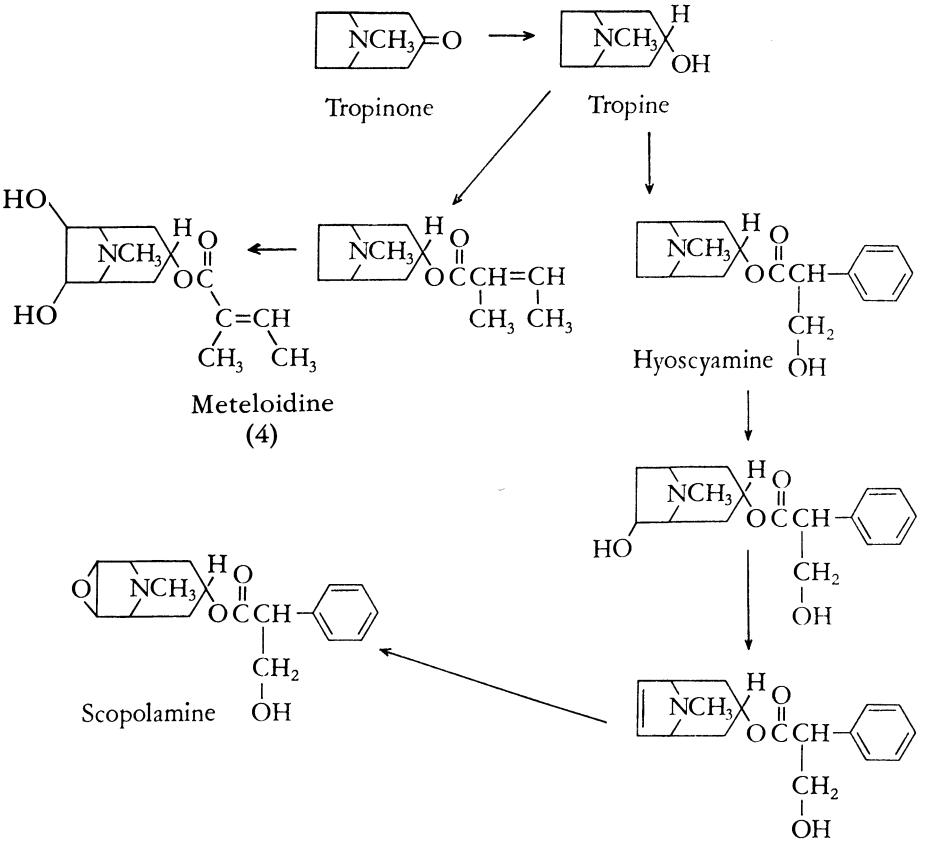


Fig. 5-3. Possible interrelationships of tropane alkaloids

experiments with *D. stramonium* have shown these migrations to occur [36]. A probable intermediate is phenyllactic acid, but the mechanism of its rearrangement to tropic acid is unknown [16,35]. The benzoic acid moiety of cocaine is also derived from phenylalanine [20] and the *m*-hydroxybenzoic acid moiety of cochlearin from *m*-tyrosine [42].

Ontogenetic variations and effects of day length on the concentrations of alkaloids in *Datura* and *Hyoscyamus* spp. have been studied [7,27,48,70]. Total concentration increased at time of flowering [55]. The ratio of scopolamine to hyoscyamine also varies ontogenetically—for instance, it is much higher in old leaves than in young leaves [8]. While scopolamine was always the predominant alkaloid in *Datura metel*, the ratio of scopolamine to hyoscyamine could be influenced by day length—long days and intense light favoring a higher ratio [6,57].

Exposure to ultraviolet radiation also increased alkaloid content [78]. Treatment of plants with certain growth regulators has an effect on alkaloid content. Growth retardants such as CCC and B-nine cause increase in

alkaloid content of *Datura metel* leaves. Gibberellins have the opposite effect [22] with *Datura metel*, but they increase alkaloid production in *Atropa belladonna* [60] and *Datura innoxia* [73]. Indoleacetic acid also increased alkaloid content of *D. innoxia* [74]. Infection with pathogens can also influence alkaloid content [79].

For a long time it has been known that polyploid plants of *Datura* spp. have a higher alkaloid content than diploid plants [69]. More recently it has been possible to obtain haploid plants and to show that they have a lower content than diploid plants [10,31,52,53]. In diploids the content declines after flowering; but since haploids do not flower, this decline is not observed [52,53]. Ploidy also has a small effect on relative amounts of the different alkaloids in *Atropa belladonna*, lower ploidy correlating with increased amount of unesterified tropine [10].

Both callus and cell-suspension cultures have been grown for several plants that produce tropane alkaloids, and in all cases the yield from cultures is much less than from whole plants [5,9,10,11,24,31,75]. The ploidy of cells in culture has little influence on alkaloid content [31]. During serial subcultures, alkaloid content declines and finally disappears altogether in some cases [9]. Addition of various growth regulators to the culture medium has some effect on alkaloid synthesis and accumulation [12,21,68], and there are indications that the factor limiting alkaloid formation in cultures is the availability of tropic acid rather than of tropane ring precursors [25,71,76]. When hyoscyamine was supplied to a callus culture from *Duboisia* spp. that did not contain any normal tropane alkaloids, 6-hydroxyhyoscyamine and scopolamine were produced; so it appears that later stages of this pathway remain active in cultured cells [19].

Enzymatically catalyzed processes involving the tropane alkaloids have been more extensively investigated than those involving most other alkaloid classes. Enzymes involved in the formation and hydrolysis of the ester bond have been found in the root and seeds of *Datura stramonium* [4,28], and some purification of these enzymes has been attained [26,30]. Early steps in the formation of the tropane ring from ornithine have also been studied to a small extent at the enzymatic level. Conversion of ornithine to α-keto-δ-aminovaleric acid is catalyzed by a transaminase present in many plants [72], including *Datura* and *Atropa* spp. [27]. Ring closure of this keto acid to Δ^1-pyrroline carboxylic acid could be one step in the sequence of reactions leading from ornithine to the alkaloids.

For the biosynthesis of cocaine and related alkaloids of *Erythroxylum* spp., experimental data are lacking except for the benzoic acid moiety [20].

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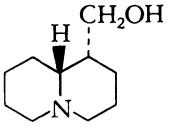
Chapter 6

Lupine Alkaloids

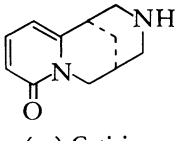
Sweet is the broom flower, but yet sour enough;
And sweet is moly, but his root is ill;
So, every sweet, with sour is tempered still.

Sweet is the Rose, EDMUND SPENSER

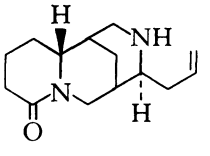
The alkaloids of lupine (*Lupinus* spp.) comprise about 30 different compounds with structural resemblances and, apparently biochemical interrelationships. These compounds, often called "lupine alkaloids," are not completely confined to this genus but are well known in related Leguminosae genera such as *Baptisia*, *Cytisus*, and *Genista* and are also found sporadically in other plant families. All contain one or two quinolizidine ring systems and may therefore be called quinolizidine alkaloids. Some examples are given below to illustrate the major structural types.



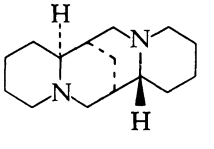
(—)-Lupinine



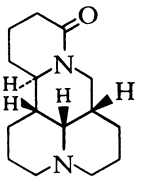
(—)-Cytisine



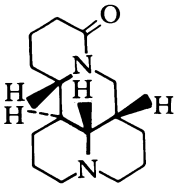
(—)-Angustifoline



(+)-Sparteine



(—)-Matrine



Sophoridine

It will be noted that (−)-cytisine and (+)-sparteine have the same absolute configuration of the methylene bridge. Okuda et al. [39] have pointed out that customarily a series of lupine alkaloids with the same configuration occur together in the same plant. Thus, plants having (+)-sparteine as a rule contain (−)-cytisine, (−)-baptifoline, (−)-lupanine, and (−)-thermopsine. Plants having (−)-sparteine will have the enantiomorphic series. An exception is *Genista aetnensis* where (+)- and (−)-sparteine are found in different parts of the same plant (see below). These observations on co-occurrence presumably indicate that members of the same stereochemical series are also biosynthetically related.

Alkaloidal varieties of lupine may contain 1%–2% of alkaloids in the seeds. "Sweet" varieties, which have been bred for use as forage, have only about 0.01% in total and also somewhat different proportions of the various alkaloids [3,13,15,26,29]. Since the genes for alkaloids are dominant, low alkaloid content is dependent on the presence of several homozygous, recessive genes. Genetic analysis of different lupine varieties with different patterns of alkaloids has been of considerable value in suggesting routes of biosynthesis and confirming pathways suggested by other approaches [29,55]. This is one of the few cases where a genetic approach to higher plant metabolism has given results comparable to those obtained in the much-exploited field of biochemical genetics of microorganisms. Grafting experiments between bitter and sweet lupines have indicated that the upper parts of the plant are the major site of alkaloid synthesis [44]. Alkaloids accumulate in the green parts during vegetative growth and are transferred to seed pods and seeds after flowering [5]. During ontogenetic development there is also a general trend for the more-reduced alkaloids to be replaced by oxygenated structures [29,36,56]. Since the oxygenated alkaloids react more feebly in the usual tests for alkaloids, superficial observation can result in the interpretation that there is a decrease in alkaloid content with development; actually there is only a change in type of alkaloid. The presence of alkaloids in the lupines may account for the fact that livestock avoid feeding on lupines if other forage is available [4]. Nevertheless, lupine poisoning of sheep is a serious problem in some areas [16]. Alkaloids at their usual concentrations do not appear to have any effect on growth of lupine plants containing them, but seeds of sweet varieties are inhibited from germination if treated with alkaloids from bitter varieties [28]. Mostly the lupine alkaloids do not confer protection against attack by microorganisms,

molluscs, or insects [28]; but there is one instance where a high and variable content of alkaloids does appear to defend against larvae of the butterfly *Glaucopsyche lygdamus* [9]. Surprisingly, it has been shown that the sparteine present in *Sarothamnus scoparius* acts as a feeding stimulus for aphids [51]. The aphids accumulate on parts of the plant with the highest content of sparteine and feed on an abnormal host plant if it is painted with a sparteine solution [51]. The toxicity of *Sophora secundiflora* is due not merely to its content of cytisine but also to nonalkaloidal constituents [14].

Correlation of alkaloid content with concentration of free amino acids in different kinds of lupines has been of some interest. Bitter varieties were found to be relatively low in arginine, threonine, and glutamic acid but high in histidine, tyrosine, lysine, and aminobutyric acid [23]. In particular, sweet lupine seeds can have more than three times the arginine content of bitter lupine seeds; and during plant development arginine concentration gradually rises in the sweet varieties, while decreasing in the bitter varieties [41]. This result might suggest that arginine is a normal precursor of the lupine alkaloids, but such a conclusion is not in accord with other findings. Arginine could be regarded rather as an alternative end product for precursors that are genetically blocked in the sweet varieties from going to alkaloids [30].

Tracer feeding experiments have led to the overall conclusion that the carbon skeletons of all the major lupine alkaloids are derived from lysine, probably by way of cadaverine. Lysine-2-[^{14}C] or cadaverine-1,5-[^{14}C] fed to *Lupinus* spp. are good precursors of lupinine, sparteine, and lupanine [22,30,44,46]; 4-hydroxylysine is ineffective [30]. Cadaverine-1,5-[^{14}C] is also a good precursor of sparteine in *Chelidonium majus* [45] and of matrine in *Sophora tetraptera* [44] and *Goebellia pachycarpa* [1,2]. Although total degradations have not been done so that isotope incorporation into each carbon of the alkaloids could be measured, partial degradations have shown incorporations in agreement with the theoretical scheme represented in Fig. 6-1. Those isotope locations that have been measured are indicated in this figure. Since lysine-2-[^{14}C] gives the same labeling pattern as cadaverine-1,5-[^{14}C], and since cadaverine is a more efficient precursor than lysine, decarboxylation of lysine to cadaverine must occur before loss of amino group nitrogen. Results of experiments using lysine doubly labeled with 2-[^{14}C] and α-[^{15}N] as a precursor of sparteine support the hypothesis that such a mechanism exists since some α-amino nitrogen was retained in the alkaloid, and the [^{15}N]/[^{14}C] ratio of the sparteine was in agreement with cadaverine's serving as an intermediate [47]. When the two enantiomers of lysine were compared, L-lysine was found to be the preferred precursor of lupanine in *Lupinus angustifolius*, but D-lysine was used as a precursor of pipecolic acid [22]. Presumably then, there is a decarboxylase for the L-isomer but not for the D-isomer. It is surprising, though, that lysine decarboxylase of plants has not been characterized [12].

Intermediates between cadaverine and lupinine remain hypothetical. It is often assumed that diamine oxidase acts on cadaverine to make an

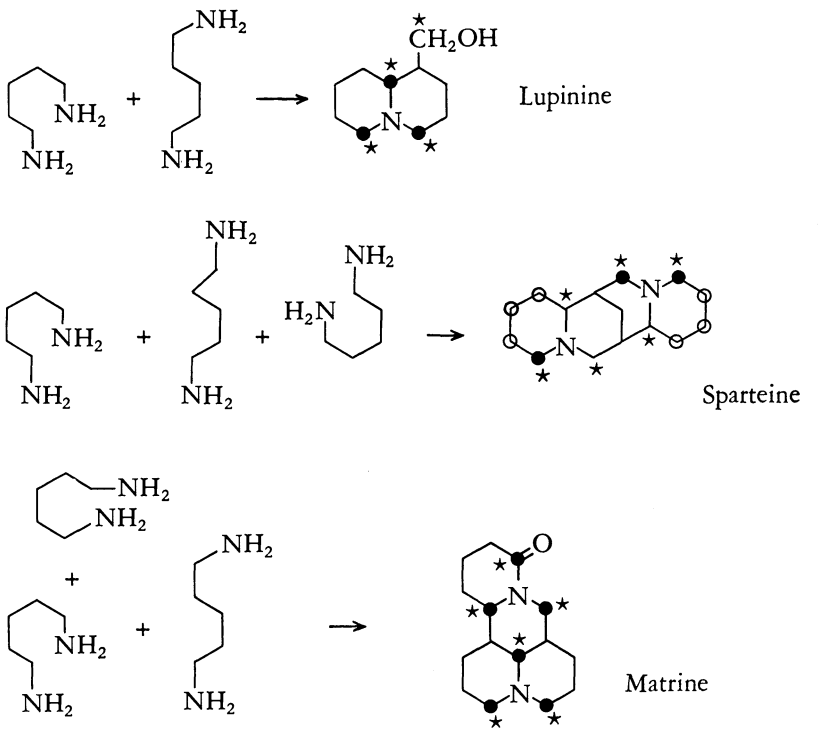
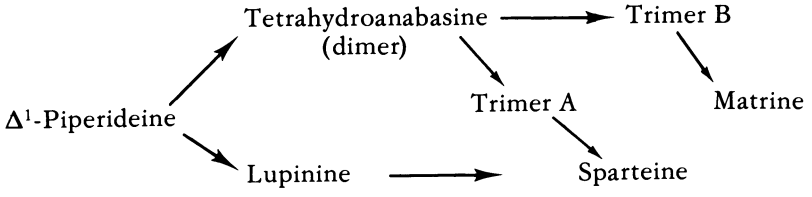


Fig. 6–1. Expected and experimental incorporation of cadaverine-1,5-[^{14}C] units into lupine alkaloids. * = Expected to be labeled; ● = measured radioactive; ○ = measured inactive

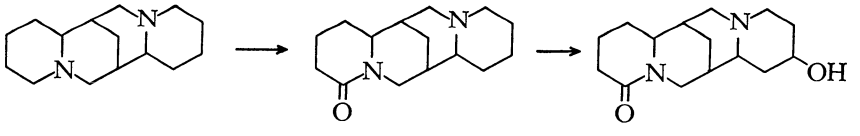
aminoaldehyde that ring-closes to Δ^1-piperideine (see Chap. 2). This reaction is known to occur in some plant extracts and to lead to piperidine alkaloids such as anabasine and tetrahydroanabasine (see Chap. 4). Nevertheless, diamine oxidase activity in homogenates of *Lupinus luteus* has been found to be negatively correlated with alkaloid content of the plant. Maximum alkaloid synthesis is occurring at a period in development when diamine oxidase activity is lowest [50]. Application of diamine oxidase inhibitors to plants did not inhibit alkaloid biosynthesis, but in homogenates they did inhibit the conversion of cadaverine to alkaloids [30]. A transaminase active with cadaverine is present in chloroplast membranes of lupine plants; and it, rather than diamine oxidase, may be responsible for generating 5-aminopentanal [58]. As outlined in Chap. 4, this aminoaldehyde can close spontaneously to Δ^1-piperideine, and the latter can dimerize to tetrahydroanabasine and related compounds. In several plants dipyridyl and lupinine-type structures coexist [10,43], so it appears likely that there is a metabolic connection. Tetrahydroanabasine does not appear to be a precursor of lupinine [12]. However, a different pathway of dimerization for piperideine could lead to the lupinine-type structure and a

trimerization pathway to the sparteine- and matrine-type structures [11]. Since tetrahydroanabasine does serve as a precursor for sparteine- and matrine-type structures in *Ammothamnus lehmannii* [20], it may be that there is a pathway like:



This scheme can be incorporated fairly easily into a more complex metabolic grid presented in [38,55]. The conversion of lupinine to sparteine that is indicated above is supported by experiments in which [^{14}C]-labeled lupinine and sparteine were fed to *L. luteus*. Conversion of lupinine to sparteine was extensive but the reverse reaction slight [34]. In *L. angustifolius*, which normally contains no lupinine and only small amounts of sparteine, the conversion of lupinine to tetracyclic alkaloids such as lupanine was small, and thus the possibility of an alternative pathway was raised [34]. Knowledge of any actual intermediates between lupinine and sparteine is lacking. Piperidyllupinine has been suggested, but tracer feeding experiments have shown that it is a very poor precursor of sparteine [46].

Interrelationships of the tricyclic and tetracyclic alkaloids have been studied by several different approaches. The best-established pathway is the successive oxidation of sparteine to lupanine and hydroxylupanine:

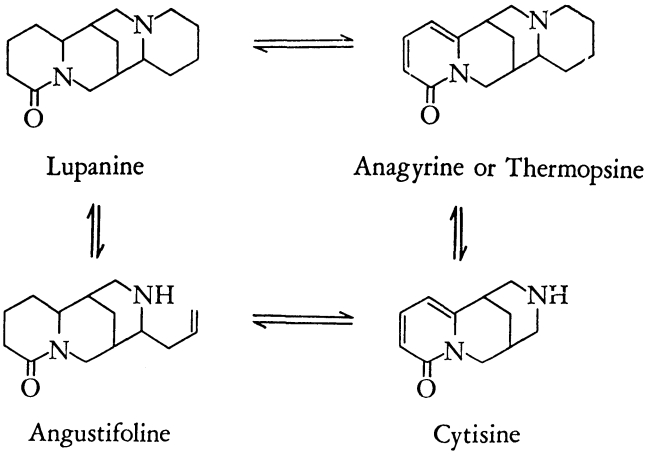


Sparteine Lupanine Hydroxylupanine

The trace amounts of sparteine in *L. angustifolius* become highly radioactive after administration of labeled lysine, supporting the idea that sparteine is an active intermediate on the way to lupanine, the major alkaloid of this species [35]. Sparteine injected into lupine seedlings can be converted to lupanine and hydroxylupanine, but the reverse reactions do not occur significantly [17,27,29,36,37,53]. It is interesting that sparteine is oxidized in the same way if fed to some plants (e.g., *Vicia faba*) which do not contain sparteine [27,53]. Either the oxidizing enzymes are not specific to the alkaloid biosynthesis pathway or, possibly, *V. faba* represents an evolutionary line whose ancestors contained lupine-type alkaloids but which through muta-

tions has lost certain enzymes of the biosynthetic pathway without losing the oxidases [29]. *L. angustifolius* normally contains lupanine as its major alkaloid, with hydroxylupanine secondary. Mutants that contain sparteine rather than either lupanine or hydroxylupanine, or that lack only hydroxylupanine, have been observed. Other mutants have hydroxylupanine as their major alkaloid. These genetic findings can be rationalized in terms of the above pathway by postulating two oxidizing enzymes that vary in their activities from one strain to another [29]. Species of *Lupinus* that do not normally contain lupanine or hydroxylupanine (e.g., *L. luteus* and *L. arboreus*) are similarly believed to lack the necessary oxidizing enzymes [29,31,33]. When young, however, they do contain a small amount of lupanine.

The biosynthetic relationship of the tricyclic alkaloids such as angustifoline and cytisine is not completely resolved. There is some evidence for operation of the following pathway in both directions:



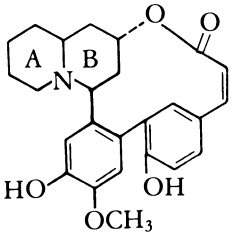
Feeding labeled lupinine or sparteine to *L. luteus* gives rise to labeled cytisine, and feeding cadaverine-1,5-[^{14}C] gives cytisine labeled as would be expected by the forward pathway [48]. However, there is evidence that acetate can be better than lysine as a precursor of ring A of cytisine, and that addition of a C_1 unit to a C_{14} compound like angustifoline can lead to formation of lupanine [30,32]. Transformations of tritiated alkaloids fed to *Ammodendron karelinii* have indicated transfer of tritium in both directions between lupanine and cytisine, but without localization of the tritium it is not certain that transformation of the ring skeletons occurred [21]. The technique of pulse-labeling with [$^{14}CO_2$] has been used to follow the sequence of alkaloid synthesis in *Thermopsis rhombifolia*, and it supports the pathway from lupanine through anagyrine to cytisine [6]. It seems that the reverse pathway, while it may occur to some extent, is quantitatively much less significant than the forward, degradative pathway. Hybridization experiments with *Baptisia* spp. have shown hybrids to be generally less capable than either parent of converting tetracyclic to tricyclic alkaloids [7]. N-

methylcytisine, while not present in *Lupinus* spp., is found in several other genera of the Leguminosae. In *Cytisus laburnum*, its formation from cytisine is considered to be a detoxification, since cytisine inhibits growth and N-methylcytisine does not [40]. However, in *Laburnum anagyroides* there is suggestion of a direct formation of N-methylcytisine from N-methylcadaverine, then demethylation to cytisine, and conversion of some of the cytisine to tetracyclic alkaloids [30]. Studies of changing alkaloid patterns in different parts of *Genista aetnensis* indicate a complex situation where young twigs synthesize (−)-sparteine from three molecules of cadaverine, but blossoms synthesize (+)-sparteine from two molecules of cadaverine by way of the methylcytisine → cytisine → anagyrine pathway [52]. Esters of hydroxylupanine found in *Lupinus* spp. further complicate the picture. Some regard these esters as crucial intermediates in alkaloid transformations [5,57], but others consider them to be side issues [30]. The degradative pathway does not end at cytisine and can proceed as far as nonalkaloidal metabolites, including amino acids that can be reutilized for growth [37].

To some extent it has been possible to study the oxidation of sparteine and some other alkaloid transformations in vitro using homogenates of lupine seedlings. An enzyme preparation from *L. angustifolius* catalyzed transformation of sparteine to a dehydro derivative of unknown structure. Sparteine-N-oxide may be an intermediate in the dehydrogenation. Lupanine was not found as a product [29]. An enzyme preparation from aerial parts of *L. albus* catalyzed transformation of sparteine to a dehydrosparteine, and of lupanine to a dehydrolupanine. Nicotinamide adenine dinucleotide (NAD) was found to accelerate both of these dehydrogenations [25]. Since the products formed in both cases have unknown structures, their places in the normal pathway of alkaloid biosynthesis remain problematical.

For the matrine-type alkaloids, tracer experiments have been done with *Sophora alopecuroides* and *Goebellia pachycarpa*. No intermediates between cadaverine and matrine have been identified for certain, although matridine probably precedes matrine in the sequence [54]. Matrine is further metabolized, first to its N-oxide and then to other more highly oxygenated alkaloids such as sophacarpine and sophoramine [1,2,20,54]. A similar series of reactions takes place with the stereoisomeric ring system of sophoridine, and there is no interconversion of the two configurations [18,19].

Quinolizidine alkaloids are also found in the family Lythraceae, but it appears that on the basis of biosynthesis they are quite distinct from the lupine alkaloids. An example of this group is lyfoline of *Heimia salicifolia*:



Although no precursor is known for ring A, three carbon atoms of ring B derive from the side chain of phenylalanine rather than from lysine [8,42].

There have been a number of unsatisfactory attempts to study the enzymology of lupine alkaloid biosynthesis in cell-free systems. Somewhat off the main path has been the characterization of an enzyme in *Lupinus luteus* that catalyzes the formation of (−)-*trans*-4′-hydroxycinnammyl lupinine from lupinine and *p*-coumaric acid [24]. This ester is a natural constituent of the plant.

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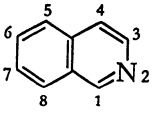
Chapter 7

Isoquinoline Alkaloids

Two Sister-Nymphs to Ganges' flowery brink
Bend their light steps, the lucid water drink,
With playful malice watch the scaly brood,
And shower the inebriate berries on the flood.
—Dim your slow eyes, and dull your pearly coat,
Drunk on the waves your languid forms shall float,

The Loves of the Plants, ERASMUS DARWIN

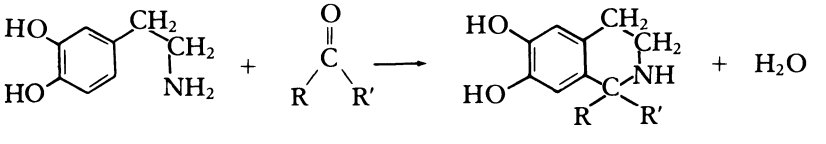
Alkaloids with an isoquinoline (or reduced isoquinoline) nucleus are considerably more common than alkaloids with a quinoline nucleus.



Isoquinoline

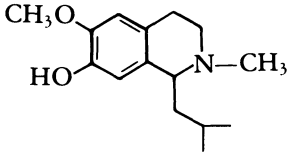
There are general reviews on the chemistry and pharmacology of this group [73,74] as well as on their biosynthesis [76,77]. Some examples of isoquinoline alkaloids are shown in Fig. 7-1. The majority have an asymmetric carbon atom at C-1 and can therefore exist as enantiomorphic pairs. In some cases both enantiomorphs are known to be naturally occurring, and therefore particular configurations are not specified in Fig. 7-1.

The biosynthetic route to all of these compounds begins with the condensation of a phenethylamine derivative, usually dopamine, with an aldehyde or α-keto acid:



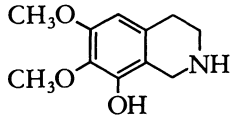
If an α-keto acid is used, R′ is a carboxyl group that eliminates CO_2. Dopamine is not a common constituent of plants, but is presumably derived from tyrosine via dihydroxyphenylalanine (DOPA) [38,46,66].

As expected from the above general scheme, tyrosine has been found to be a precursor of the simple tetrahydroisoquinoline alkaloids that are characteristic of several genera of cacti [49]. The pathway to these compounds is obviously related to that for the simpler phenethylamines found in the same plants (cf. Chap. 3). Dopamine is the precursor of all of them, but specific methylation evidently determines whether ring closure occurs. 3,4-Dimethoxy-5-hydroxyphenethylamine leads to the tetrahydroisoquinolines, while 3,5-dimethoxy-4-hydroxyphenethylamine goes to mescaline [45,53,62]. N- and O-methyl groups come from methionine [26,71]. Pyruvate is the source of the C_2 unit of anhalonidine [50]. Alkaloids of this group with larger 1-aliphatic side chains are also known. In lophocerine, the C_5 unit is derivable from either mevalonic acid or leucine, with 3-methylbutanal likely to be the common precursor [59].

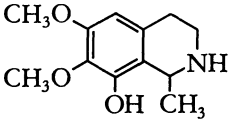


Lophocerine

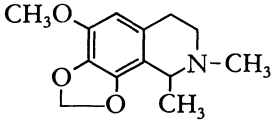
Oxidative coupling of three molecules of lophocerine yields the trimeric alkaloid pilocereine [60].



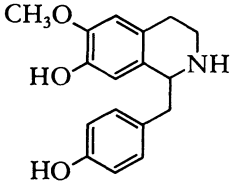
Anhalamine



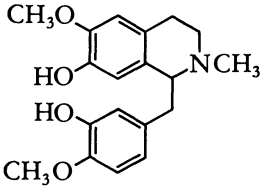
Anhalonidine (1)



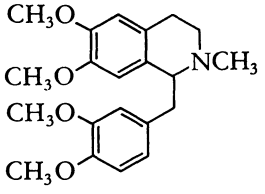
Lophophorine



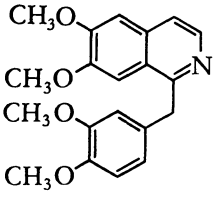
Coclaurine



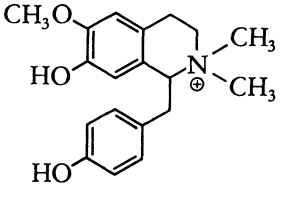
Reticuline (3)



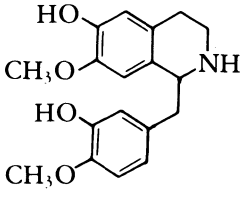
Laudanosine



Papaverine (2)



Magnocurarine

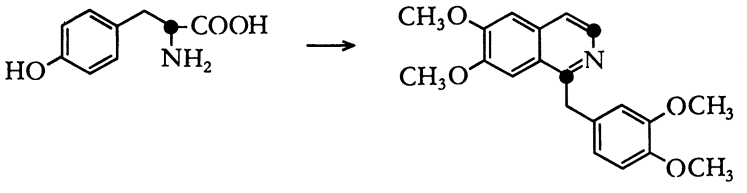


Norprotosinomenine

Fig. 7-1. Some isoquinoline alkaloids

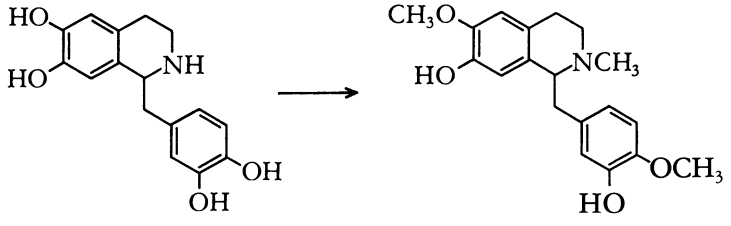
The most familiar isoquinoline alkaloids are the 1-benzylisoquinolines. Biosynthetic routes to many of them have been studied by tracer feeding techniques; and, more recently, there are experiments with cell-free enzyme systems that can carry out certain steps of the synthesis.

When tyrosine-2-[^{14}C] was fed to *Papaver somniferum*, 0.16% of the label was incorporated into papaverine (2), and degradation of this product showed the following pathway [9]:



Papaverine (2)

When (±)-norlaudanosoline was fed, it was converted to reticuline (3) with a (+)/(−) ratio at first 1:1 as would be expected, but changing with age to 6:1 as a consequence of more rapid use of (−)-reticuline in further reactions [4]:



(±)-Norlaudanosoline (tetrahydropapaveroline)

(±)-Reticuline (3)

With the finding that reticuline is a precursor of morphine and berberine alkaloids, tracer experiments that have been done to elucidate biosynthesis of these other alkaloids from simple precursors can now be interpreted as giving information about biosynthesis of reticuline and related isoquinolines (see below and Chap. 8). For instance, although both aromatic rings of the benzylisoquinolines can come from tyrosine, they are derived via different routes, with ring A going through dopamine and the benzyl group through dihydroxyphenylpyruvic acid. As shown in Fig. 7-2, the earliest identifiable condensation product is norlaudanosoline-1-carboxylic acid. Tracer experiments with L-DOPA have shown that 78% of this amino acid is decarboxylated to dopamine and 11% is deaminated to dihydroxyphenylpyruvic acid before the two halves are joined [11,82]. The decarboxylation of DOPA to dopamine is catalyzed by an enzyme present in poppy latex [67], but it is not known whether the deamination reaction is catalyzed by a transaminase or an oxidase. Norcoclaurine, which has only a single 4-hydroxyl group in the benzylic portion, probably comes from 4-hydroxyphenylpyruvic acid [64]. The condensation reaction occurs in high yield nonenzymatically [82], and so the only requirement for the appearance of 1-

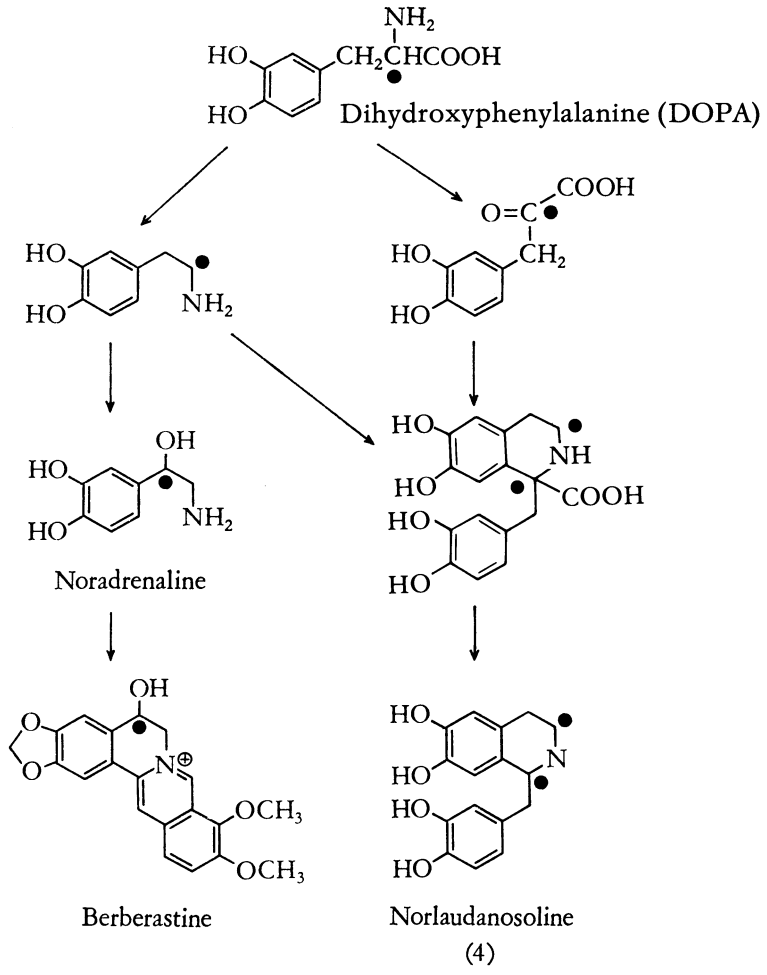


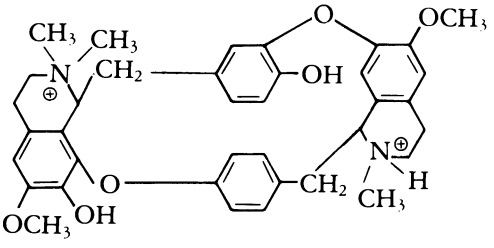
Fig. 7-2. Pathways to isoquinoline alkaloids

benzylisoquinolines in plants may be the co-occurrence of high concentrations of precursors. The first-formed 1-carboxylic acids can be decarboxylated by the action of peroxidase [30].

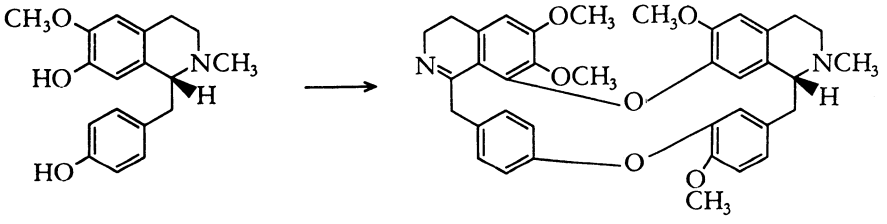
Further transformations leading to other alkaloids are methylations and oxidations. In the conversion of norlaudanosoline to reticuline O-methylation occurs before N-methylation in *Litsea glutinosa* plants [20]. Further methylation of reticuline leads to such alkaloids as isocorydine in *Annona squamosa* [63] and papaverine in *Papaver somniferum* [24]. For papaverine, dehydrogenation is also a necessary step; and studies with stereospecifically tritiated compounds have shown that a pro-S hydrogen is removed from C-3 followed by nonstereospecific loss of H from C-4 [13]. A beginning has been made in studying methylation of these alkaloids in crude plant extracts [44].

Bis-benzylisoquinoline alkaloids have two benzylisoquinoline nuclei joined together through one, two, or three ether bridges. A review of these

complex compounds tabulates nearly 200 different structures [36]. One of the most important for its pharmacological use is (+)-tubocurarine:



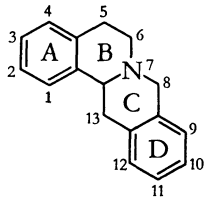
There is tracer evidence for biosynthesis of several bis-benzylisoquinoline alkaloids. Tyrosine-2-[^{14}C] fed to *Stephania japonica* was incorporated to 0.17% into epistephanine and labeled each benzylisoquinoline half equally. (−)-N-methylcoclaurine labeled with tritium was incorporated into only the right half of the epistephanine molecule. The left (imine) half is possibly derived from either (+)- or (−)-coclaurine [3].



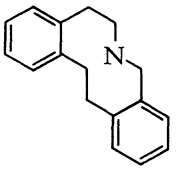
(—)-N-Methylcoclaurine Epistephanine

Coclaurine and N-methylcoclaurine are clearly the monomeric precursors of several bis-benzylisoquinoline alkaloids in several different plants [17–19]. It is interesting that callus tissue cultures of *Stephania cepharantha* produce a different mixture of bis-coclaurine alkaloids than is found in the original plant [1]. Many isomeric bis-benzylisoquinoline alkaloids are obviously possible, depending upon which hydroxyl groups are converted to ether bridges. Chemical oxidation with ferricyanide produces some coupled products [43,81], as does oxidation using peroxidase [41,42]. How the enzymatic oxidation is controlled in vivo to produce specific products is unknown.

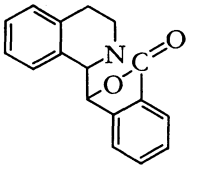
A large number of very widely distributed alkaloids have the benzylisoquinoline structure with one additional carbon atom. Three skeletons of this type are known:



Protoberberine Type



Protopine Type



Phthalide-Isoquinoline Type

The structures appear related, and tracer experiments suggest a common origin for all of them, with (+)-reticuline (3) the closest known precursor. Tyrosine-2-[^{14}C] fed to *Papaver somniferum* [12] or *Hydrastis canadensis* [34] labeled the two carbon atoms which would be predicted in narcotine (5), berberine (6), or hydrastine (7). The finding that C-3 of hydrastine contained more label than C-1 suggested a different route to each "half" of the molecule, with dilution by a nonradioactive precursor occurring on the pathway to the benzyl residue. Further experiments showed that dopamine labeled only C-3 [12]. These findings are incorporated into Fig. 7-3. The "extra" C_1 unit of these alkaloids can be derived from formate [12,75] but more efficiently from the methyl group of methionine [37]. This has been established for narcotine in *P. somniferum* [12,46] and for berberine and hydrastine in *H. canadensis* [37]. The proposed mechanism for addition of the C_1 unit is methylation of nitrogen in a benzylisoquinoline, oxidative cyclization to a protoberberine-type skeleton, and finally oxidation to a phthalideisoquinoline [2]. A cell-free preparation from *Macleaya microcarpa* catalyzes the conversion of (+)-reticuline to (−)-scoulerine, and the latter to berberine. Molecular oxygen is required [23], and the 3′-hydroxyl group of reticuline is also essential for the reaction to go. Tracer experiments with *Chelidonium majus* have indicated that the free hydroxyl at C-7 of reticuline is a requirement for this pathway to occur, inasmuch as protosinomenine (which has the positions of hydroxyl and methoxyl on ring A interchanged from reticuline) is not a precursor of berberine [2]. The methyl and methylenedioxy groups of hydrastine and berberine were also efficiently labeled from methionine [37]. (±)-Reticuline labeled in the N-methyl group fed to *H. canadensis*, *Dicentra spectabilis*, and *P. somniferum* gave rise respectively to berberine, protopine (8), and narcotine labeled in the expected positions [2,6]; but it was not a precursor of hydrastine in *H. canadensis* [2]. When multiply labeled (+)- and (−)-reticulines were fed to *Chelidonium majus*, the (+) form was found to be utilized as a unit for the synthesis of protopine and stylopine (9) [7]. It is interesting that the (+)-reticuline was a much more efficient precursor than the (−) [2]. This argues for an enzymatic mechanism and contrasts with other pathways, which preferentially use (−)-reticuline (see Chap. 8). Many experiments have shown that (−)-scoulerine is the crucial branch-point intermediate for this group of alkaloids in both *C. majus* and *P. somniferum* plants [5]. Several trans-

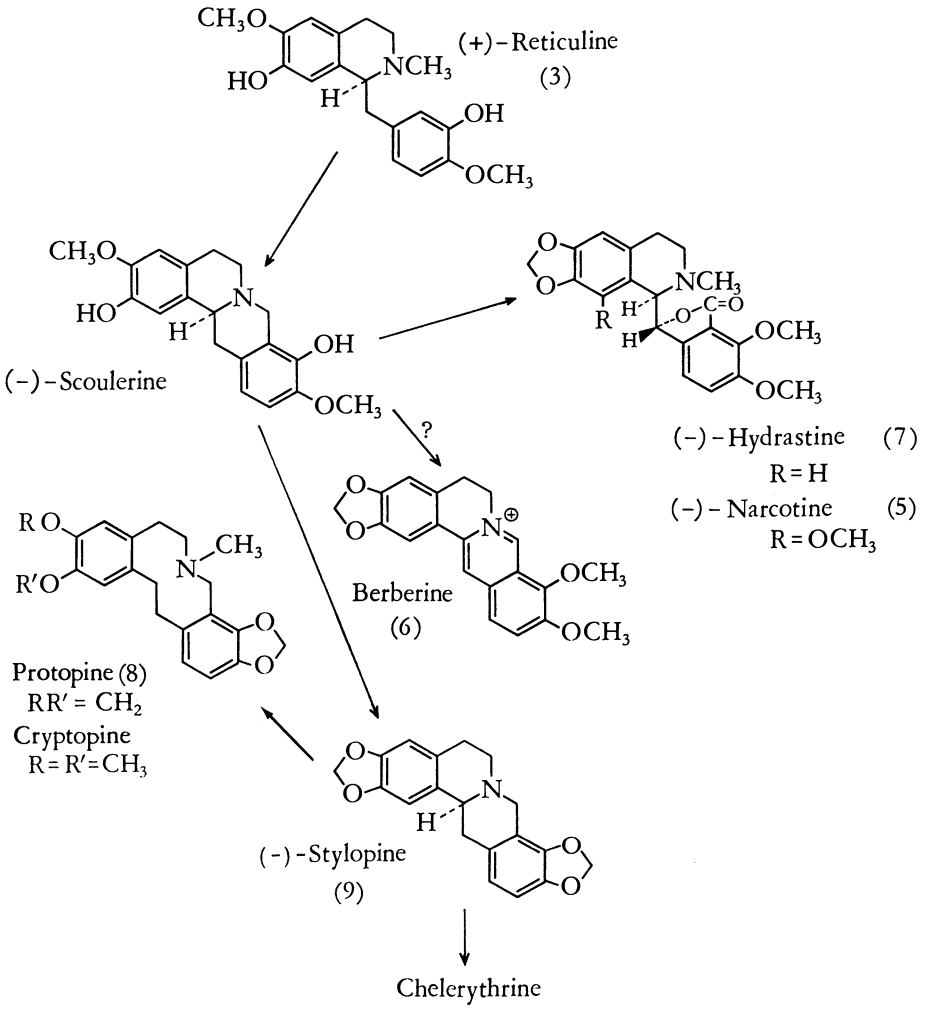


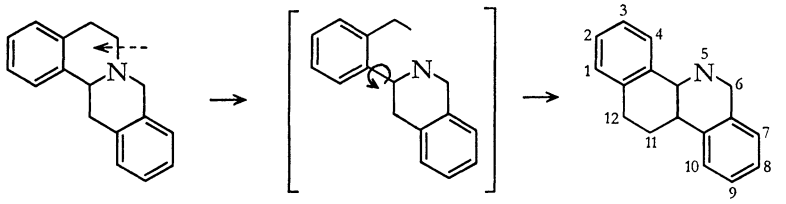
Fig. 7-3. Biosynthetic pathways of benzylisoquinoline alkaloids

formations depend on reaction at C-13. Corydaline is methylated at this position [21]; ophiocarpine, hydrastine, and narcotine are hydroxylated [6,40]; and for protopine the ring is opened at this point. Stereospecific labeling experiments have shown that in the biosynthesis of narcotine it is the pro-S hydrogen at C-13 that is removed [15], but for ophiocarpine it is the pro-R hydrogen [40]. In the ring-opening reaction, quaternary N-metho salts may be intermediates [16,78,79].

Berberastine, a minor alkaloid of *H. canadensis*, is interesting for having a hydroxyl group at C-4 of the quinoline ring. Dopamine-1'-[^{14}C], when fed to these plants, labeled C-3 of berberastine as it does berberine. However, berberine was found not to be a precursor of berberastine, and nor-

adrenaline-2'-[^{14}C] labeled C-4 of berberastine without labeling other alkaloids [56]. These findings show that the C-4 hydroxyl group is introduced before condensation to benzylisoquinoline structure (Fig. 7-2).

Alkaloids with a benzophenanthridine skeleton are found mostly in plants of the Papaveraceae and often in association with alkaloids of the structural types discussed above. This association and resemblances in structural features led to postulation of a possible transformation of the protoberberine skeleton to a benzophenanthridine skeleton by ring opening, rotation of ring A, and ring closure [25,48].



Protoberberine Type Benzophenanthridine Type

When tyrosine-2-[^{14}C] was fed to *C. majus*, it gave rise to labeled chelidonine (10) and sanguinarine (11). Degradation of the chelidonine showed label in the positions in which it would be expected by such a mechanism [48,51]. Similarly, multiply labeled (+)-reticuline served as a good precursor of chelidonine, and degradation established conversion of reticuline as a single unit to chelidonine. (−)-Scoulerine is somewhat better than (+)-reticuline as a precursor of chelidonine and, like (+)-reticuline, gives higher incorporation into chelidonine (0.61%) than into (−)-stylopine (0.28%). Incorporations from (−)-scoulerine into protopine and chelidonine were similar (0.92% and 0.61%, respectively) [10]. The initial stages in the pathway from (+)-reticuline to the benzophenanthridines are shown in Fig. 7-3, but with the formation of (−)-stylopine a branch point is reached that can go either to the protopine-type structure or to chelidonine [14,16,52,78]. Stylopine-6-[^{14}C] (9) is converted (0.7% incorporation) to chelidonine-11-[^{14}C] by *C. majus* plants [52]. The finding that (+)-reticuline is incorporated to 0.5% into chelidonine but only to 0.19% into protopine and 0.06% into stylopine [7] is difficult to reconcile with stylopine being an intermediate between reticuline and chelidonine, but there is so much other evidence for this pathway that the discrepancy must be explained in some way. One possibility is that the real precursor is not stylopine itself but its N-metho salt and that the latter, while it may be made from stylopine, is more rapidly derived from the N-metho salt of (−)-scoulerine. The observation that pyridone alkaloids such as oxysanguinarine (12) and oxynitidine co-occur with the corresponding N-metho salts (e.g., sanguinarine and nitidine) has led to the suggestion that the pyridone alkaloids may be derived by enzymatic oxidation of the N-metho salts [68]. Several possible interrelationships among the benzophenanthridine alkaloids are summarized in Fig. 7-4.

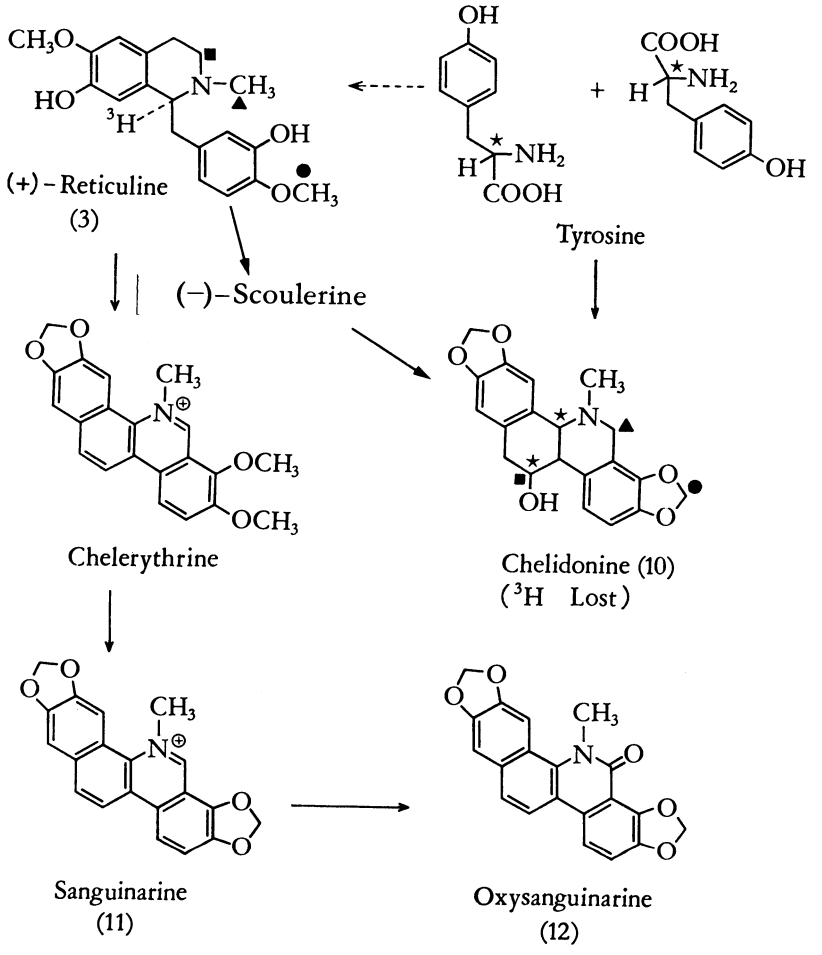
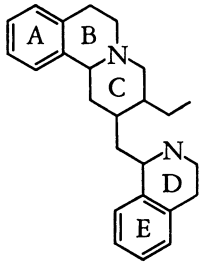


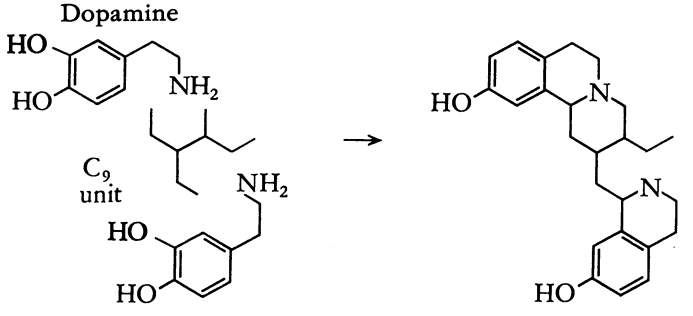
Fig. 7-4. Biosynthesis of chelidonine and related alkaloids. Symbols indicate labeling sites in different experiments

The ipecacuanha alkaloids, found in several species of the Rubiaceae and in *Hedera helix* [54], have two isoquinoline rings attached by a C_7 unit between their C-1 positions:

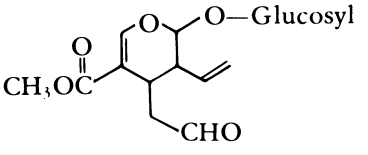


Ipecacuanha Type Skeleton

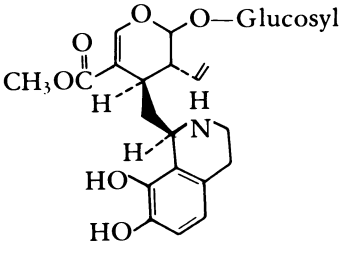
Biosynthetically, these compounds are made of two molecules of dopamine linked by a C_9 unit that comes from the monoterpenoid, geraniol [8]. Addition of a C_9 unit to two dopamine units may be formulated as follows:



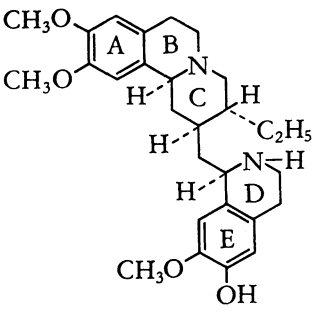
The immediate monoterpenoid precursor is secologanin:



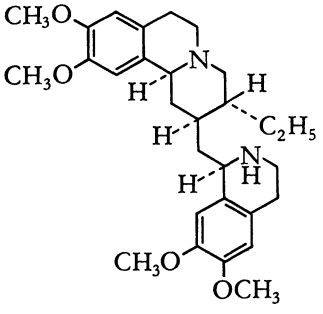
It first condenses with one dopamine unit, to form desacetylisoipecoside:



The remaining steps involve loss of ester group and glucose units, addition of a second dopamine unit, and methylation reactions so that the characteristic alkaloids cephaeline, emetine, and others are produced [58]:

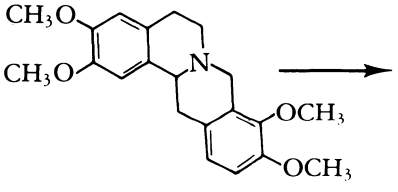


Cephaeline

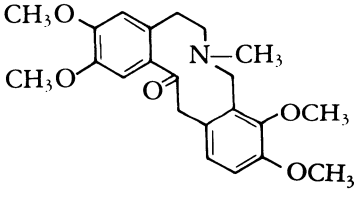


Emetine

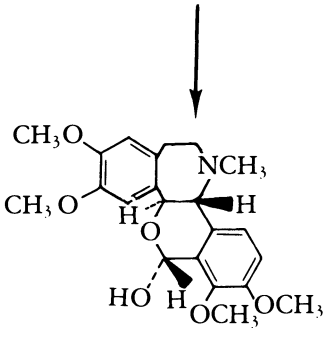
Besides the aporphine and morphinan alkaloids, which are discussed in Chap. 8, there are several less-known groups of alkaloids that are biosynthetically related to the benzylisoquinolines. In the formation of alpiniginine and rhoeadine of *Papaver* spp., ring B is expanded to become seven-membered, incorporating what was C-13 of muramine, a protopine-type precursor [69,70,80]. It is interesting that older *Papaver bracteatum* plants lose the ability to make alpiniginine from reticuline, but can make it if they are supplied with the tetramethoxylated compound tetrahydropalmatine [22]. Thus they have evidently lost some capacity for O-methylation.



Tetrahydropalmatine



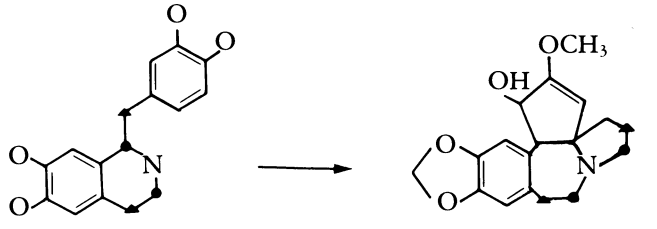
Muramine



Alpiniginine

The alkaloids of *Cephalotaxus* spp. are also derived from benzylisoquinolines via a complex sequence of reactions in which the B ring is expanded to seven

members while the benzyl group is cleaved and its atoms redistributed [32,61,72]:

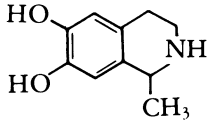


Cephalotaxine A

Ochotensimine of *Corydalis ochotensis* has a spirane structure that may be derived by rearrangement of a protoberberine [38]. Other alkaloids that appear related to the benzylisoquinolines are the cularines and dibenzopyrrocolines, but there is no biochemical evidence for the relationship.

Callus tissue cultures from various plants of the Papaveraceae do make alkaloids; but until some differentiation occurs, the alkaloid patterns are not the same as in the parent plants [33,39].

An interesting sidelight on the biochemistry of isoquinoline alkaloids is the proposal that their aberrant synthesis in humans may account for some symptoms of alcoholism and phenylketonuria. In its barest form, the suggestion with regard to alcoholism is that acetaldehyde resulting from ethanol oxidation condenses with dopamine to produce salsolinol:



A further elaboration proposes that the oxidation of dopamine successively to 3,4-dihydroxyphenylacetaldehyde and then 3,4-dihydroxyphenylacetic acid is inhibited at the aldehyde stage by acetaldehyde, and this aldehyde as well as acetaldehyde can condense with dopamine. The resulting isoquinolines, salsolinol and norlaudanosoline, might themselves produce some symptoms or might be precursors of more complex and more active alkaloids [28,31,55]. In addition, other related products could be made from catecholamines other than dopamine with still other aldehydes [35]. There is little doubt that under appropriate conditions mammalian tissues can synthesize isoquinolines, but their relevance to alcoholism remains controversial [65]. One report says that the injection of norlaudanosoline into rat brains produced an addiction for ethanol [47]. In the case of phenylketonuria, it has been observed that children suffering from the disease excrete into their urine 3',4'-dideoxynorlaudanosolinecarboxylic acid. This isoquinoline derivative would result from a condensation of dopamine with

phenylpyruvic acid. The fact that it is an inhibitor of dopamine-β-hydroxylase could account for the low levels of normal catecholamines in phenylketonurics [47]. Still another appearance of isoquinoline alkaloids in mammalian metabolism is in the urine of patients who are being treated with L-DOPA for parkinsonism. Presumably as a consequence of high dopamine levels in their tissues these patients produce not only simple isoquinoline derivatives but also protoberberines [27,29]. Possibly some of these compounds contribute to the therapeutic effect of L-DOPA.

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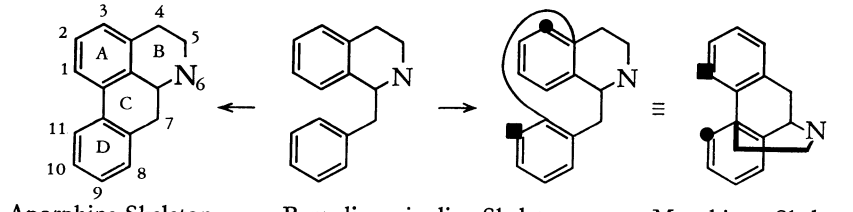
Chapter 8

Aporphine and Morphinan Alkaloids

Not poppy, nor mandragora,
Nor all the drowsy syrups of the world,
Shall ever medicine thee to that sweet sleep
Which thou ow'dst yesterday.

Othello, SHAKESPEARE

The aporphine and morphinan alkaloids can be conveniently considered together because they often occur together in the same plant, and also because in terms of structure they can both be derived from a benzylisoquinoline skeleton by additional ring closures:

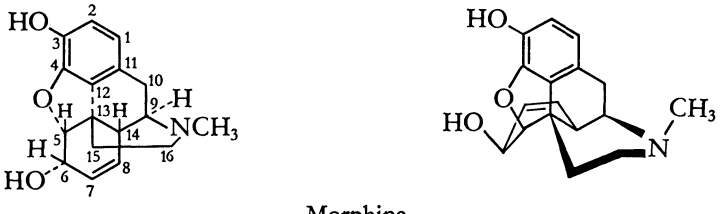


Aporphine Skeleton Benzylisoquinoline Skeleton Morphinan Skeleton

More than 200 aporphine alkaloids are known, distributed among about a dozen plant families and 40 genera [63]. The morphinan alkaloids are more limited in numbers and distribution. There are fewer than a dozen of them distributed in the Papaveraceae genus *Papaver* and several genera of the Menispermaceae. Because of the commercial value of many of these alkaloids there has been much examination of their taxonomic distribution, particularly within the genus *Papaver*. *P. somniferum* is the only significant producer of morphine, although a few other species contain small amounts. In contrast, *P. orientale* has mostly oripavine and thebaine [82,86,87]. *P. pseudo-orientale* has salutaridine, isothebaine, and macrantaline as prominent alkaloids [82,87]. *P. bracteatum* is noted for its accumulation of thebaine [4,43,82,96]. An interesting correlation is the common occurrence of

meconic acid in plants that make morphinan types but not in plants characterized by aporphines [54]. The morphinans present in Menispermaceae have the opposite absolute configuration to those of the Papaveraceae [95].

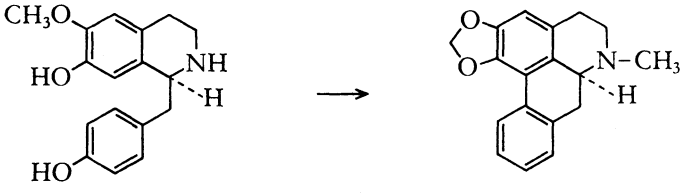
Early tracer feeding experiments showed tyrosine-2-[^{14}C] to be a precursor of aporphine and morphinan alkaloids in poppy seedlings, with the label appearing at C-9 and C-16 of morphine [22,60,73].



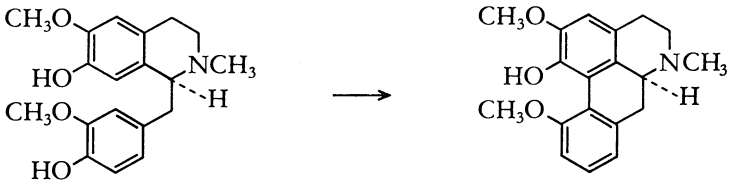
Morphine

It has since been shown that there is an asymmetry between C-9 and C-16 in their incorporation of label. Tyrosine-2-[^{14}C] labels C-9 at a greater rate than C-16. A C-9 would correspond to C-1 of a benzylisoquinoline precursor [78]. Labeled carbon dioxide also was found to label C-9 more rapidly than C-16 [80]. 3,4-Dihydroxyphenylethylamine-1-[^{14}C] injected into poppy capsules gave rise to morphine labeled at C-16 but not C-9 [21]. This asymmetric labeling is similar to that found for some benzylisoquinoline alkaloids (see Chap. 7), except that there the dopamine "half" going to C-3 became labeled faster than the phenylacetaldehyde "half." Both tyrosine and *p*-hydroxyphenylpyruvic acid have been found to occur free in *P. somniferum* and therefore can be considered as natural precursors [17,69].

More recent experiments have definitely shown that benzylisoquinolines are natural precursors of aporphine alkaloids. Tritium-labeled (+)-coclaurine or N-methylcoclaurine fed to *P. dubium* was a good precursor of (+)-roemerine, the major alkaloid of this plant [7]. Feeding tritium-labeled (+)- and (−)-orientalines, (±)-orientaline-3-[^{14}C], or doubly labeled orientaline to *P. orientale* gave a preferential conversion of (+)-orientaline to isothebaine with no loss of methoxyl groups [19]. Other experiments have shown reticuline to be the precursor of some aporphine alkaloids (e.g., magnoflorine, bulbocapnine, and boldine) and norprotosinomenine to be the precursor of others [24,31,32,35]. These various results, in which the only difference between precursor benzylisoquinolines is the pattern of O-methylation, illustrate an important principle—the positions that are methylated early in the sequence have a controlling influence on the future direction of the pathway.



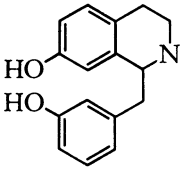
(+)-Coclaurine (+)-Roemerine



(+)-Orientaline (+)-Isothebaine

The benzylisoquinoline alkaloids norlaudanosoline, norlaudanosoline dimethyl ether, and reticuline (in increasing order of effectiveness) were good precursors of morphinan alkaloids when injected into capsules of *P. somniferum* [16]. Quadruply labeled (+) and (−) forms of reticuline were both found to be precursors of morphine. Although the (−) form has the correct stereochemistry and is the direct intermediate, it exists in equilibrium with the (+) form through the 1,2-dehydro compound, which is also a good intermediate. Results with norlaudanosoline indicate that the (−) form is a better precursor of morphine that the (+) form; but since (+)-norlaudanosoline has the same absolute stereochemistry as (−)-reticuline, a 1,2-dehydro intermediate must intervene between (−)-norlaudanosoline and (−)-reticuline [20]. A quintuply labeled reticuline fed to *P. somniferum* was found to be converted to thebaine with no degradation [13]. The role of reticuline as a major intermediate is also supported by short-time experiments with labeled carbon dioxide. After 2.5 h of exposure of poppy seedlings to [$^{14}CO_2$], reticuline accounted for 80% of the radioactivity in the phenolic alkaloids. Reticuline also had 10 to 20 times the specific activity of thebaine and a greater rate of turnover [74].

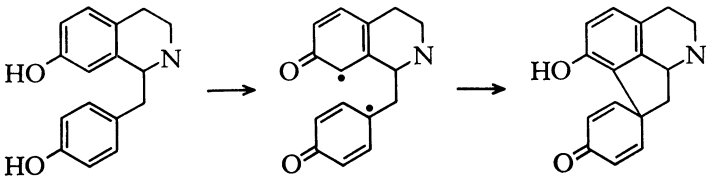
Considerable attention has been given to understanding the mechanism by which benzylisoquinolines condense into aporphine and morphinan structures. The general view proposed by Barton [5,6] is that phenolate free radicals are formed by oxidation and then couple in pairs to give cyclohexadienone intermediates, which may undergo further reactions of various types. In most general terms, this sequence is shown in Chap. 2, p. 15. An implication of this scheme is that the presence of free hydroxyl groups is essential for condensation and that their locations determine the directions in which ring closures may occur. Thus, the following structure is minimal for formation of aporphine or morphinan rings:



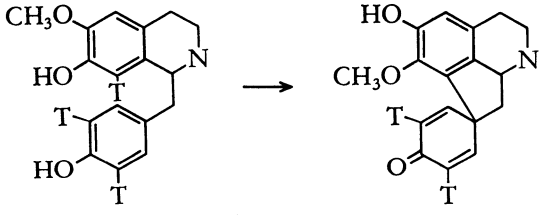
and could go to either, depending on whether the A ring goes to an *ortho*- or a *para*-quinonoid intermediate. However, benzylisoquinolines with methoxyl rather than hydroxyl at C-7 (e.g., laudanidine, protosinomenine) or at C-3′ (e.g., orientaline) cannot give intermediates leading to a morphinan skeleton, but only intermediates leading to an aporphine. Steric hindrance

can also have a directive effect. This reasoning has been confirmed by tracer experiments, described above [19], showing that orientaline was converted well to isothebaine but not at all to thebaine. Thus there appears to be no direct conversion of isothebaine to thebaine as was once proposed [60].

If ring C has a hydroxyl group only at C-4′, neither aporphine or morphinan structures are directly approachable. Instead, the following reaction could be expected:

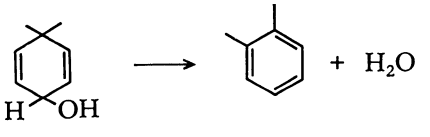


That this actually occurs in nature is shown by the conversion of (+)-coclaurine to crotonosine, the major alkaloid of *Croton linearis*, and to alkaloids of related structure in *C. sparsiflorus* [8,28]. Fed (+)-coclaurine labeled with tritium at C-8, C-3′, and C-5′ was converted to crotonosine with retention of tritium on ring C but loss of



Coclaurine Crotonosine

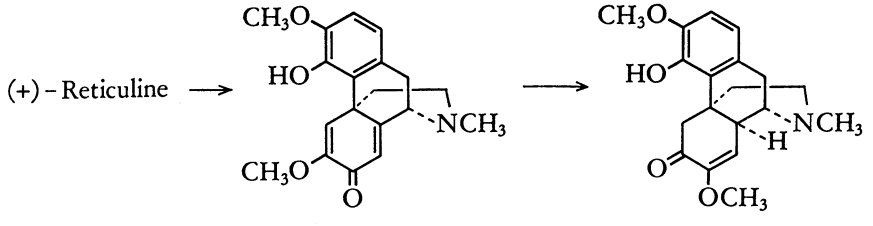
the C-8 tritium. Demethylation and remethylation occurred somewhere during the process [8]. Several alkaloids of the genus *Croton* have this type of structure, known as "proaporphine" [55]. Some of them by rearrangement are precursors of aporphines not only in *Croton* but also in *Papaver* spp. The conversion of coclaurine to roemerine described above [9] is presumed to occur by way of a crotonosine-type structure which rearranges with elimination of oxygen from the D ring. This is a general type of reaction known as the dienol-benzene rearrangement,



and may play a role in other biosyntheses wherever there is a 4′-hydroxyl in the precursor benzylisoquinoline and no hydroxyl at this position in the

final product. Another example is the conversion of orientalinol to isothebaine in *Papaver orientale* [18].

There is now excellent evidence for the occurrence of cyclohexadienone alkaloids and for their participation as intermediates in the biosynthesis of other alkaloids. Sinomenine and related alkaloids of *Sinomenium acutum* have been shown by tracer feeding experiments to be synthesized from (+)-reticuline by the following route [12]:



Sinoacutine Sinomenine

All compounds of this pathway are enantiomorphic to the morphine alkaloids at C-9, C-13, and C-14. In other species there are other morphinandienones that have the same configuration as the more familiar morphinan alkaloids [70,91]. Tracer experiments have shown that (±)-reticuline is a precursor of several of them [27,93] and coclaurine of others [64].

The pathway shown in Fig. 8-1 for the morphine alkaloids has also been confirmed by extensive tracer evidence. The places of salutaridine and salutaridinol in this pathway are supported by the finding that traces of salutaridine normally present in poppies readily acquire label from fed norlaudanosoline [13]. Synthetic salutaridine-1-[^{3}H] or 16-[^{14}C] and one of the corresponding isomeric salutaridinols labeled at C-1 and C-7 with tritium or C-16 with [^{14}C] went efficiently to thebaine without degradation and to codeine with loss of tritium from C-7 [9,13]. The direction of the pathway thebaine → codeine → morphine has been shown by [$^{14}CO_2$] feeding experiments in which label appeared consecutively in these compounds [80], and by actually feeding the labeled alkaloids to the plant to show that this sequence of conversion occurs [89]. The conversion of codeinone to codeine is irreversible in vivo [23]. Codeinone rather than codeine methyl ether has been shown to be intermediate between thebaine and codeine. Fed codeine methyl ether was incorporated into codeine, but it could not be demonstrated as naturally present, and its conversion was less efficient than that of codeinone [33]. By using thebaine-6-[^{18}O] it has been possible to show that this oxygen atom is retained in the conversion of thebaine to codeine by *Papaver somniferum* [67]. It is surprising that tobacco plants, which do not contain any compounds of related structure, are able to bring about the conversion of thebaine to codeine and morphine in vivo [76]. Callus tissue cultures of *Phaseolus vulgaris* convert exogenous thebaine to unknown alkaloids but not to codeine [88].

Chemical oxidation of benzylisoquinoline structures to aporphines or morphinans had been attempted for many years without success until the

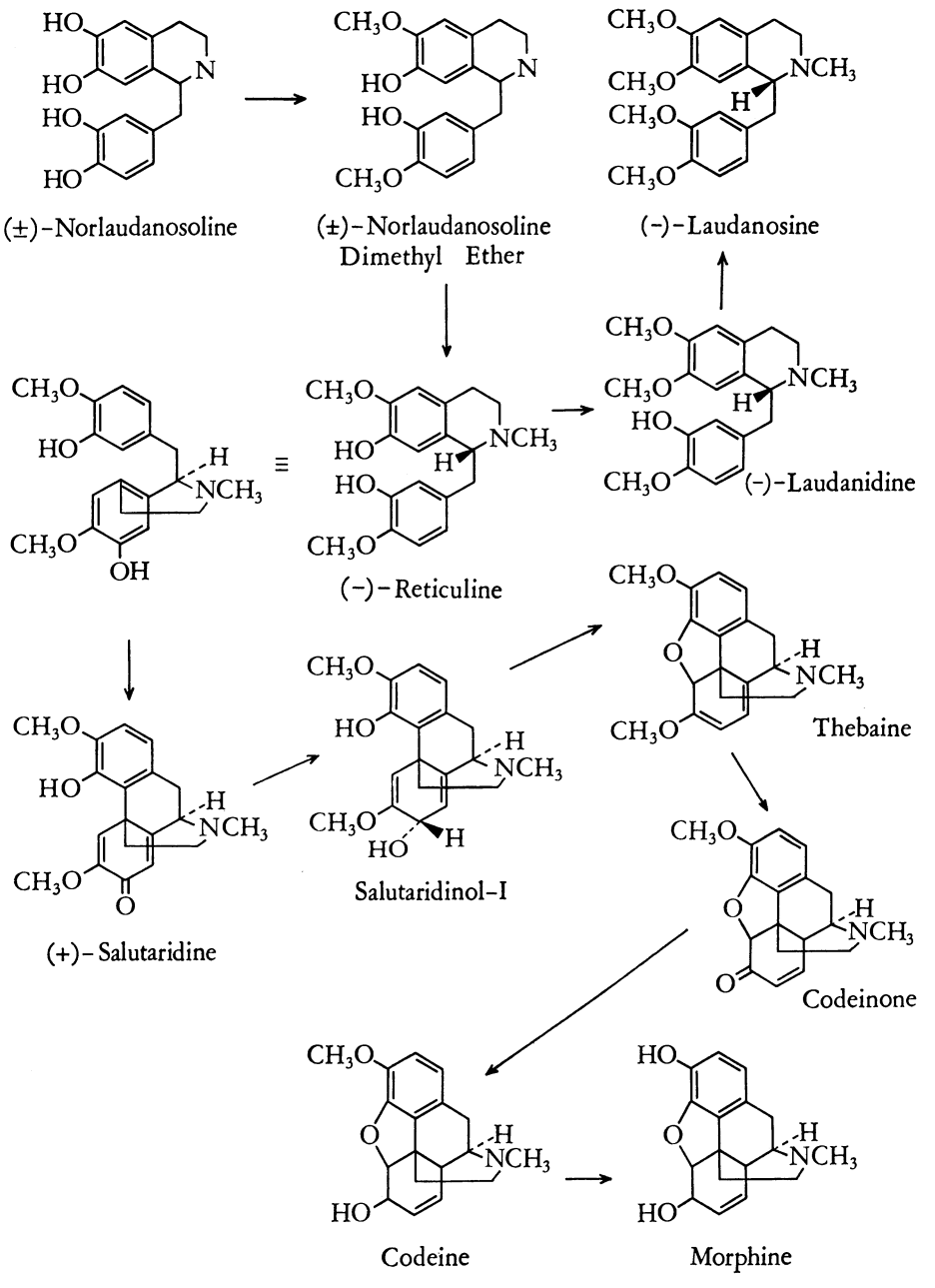
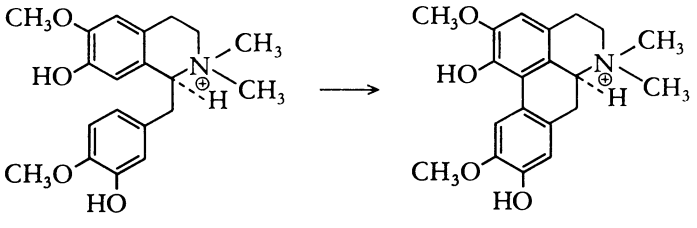


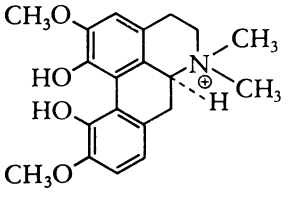
Fig. 8-1. Pathways of morphine alkaloid biosynthesis

discovery was made that it was necessary to quaternize the nitrogen or to complex *o*-diphenol groups in order to avoid competing reactions [56,57]. Thus chemical oxidation of (+)-tembetarine gives (+)-laurifoline. These two alkaloids occur together in *Fagara* spp. (family Rutaceae) [72]; so the

chemical pathway may reflect the biological one [1]. The product of *ortho-ortho* coupling would be magnoflorine, but chemical oxidation does not produce it [38].



(+)-Tembetarine (+)-Laurifoline



(+)-Magnoflorine

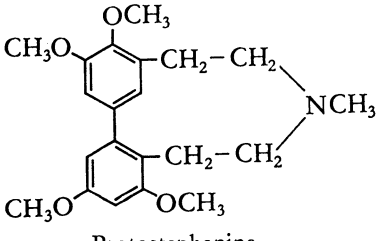
Attempts to bring about these ring closures enzymatically have had limited success although one might expect peroxidases or laccases to catalyze them. Peroxidase activity has not been found in poppy latex, and phenol oxidizing preparations from this source are inactive toward reticuline and salutaridinol [81]. Still, the overall phenol oxidase activity of *P. somniferum* does correlate with the content of morphinan alkaloids at various stages of development of the plant [68], and phenol oxidizing enzymes have been used to catalyze conversion of (+)-laudanosoline to an aporphine [37,58] or morphine to its dimer pseudomorphine [83].

The latex of *P. somniferum* has been found capable of completing the synthesis of morphine from tyrosine, although at a low rate, perhaps limited by a slow conversion of tyrosine to DOPA [34,45]. Methylating and demethylating enzymes have been studied in the latex. They can use S-adenosylmethionine as a substrate in methylation reactions and can also catalyze demethylation of thebaine to codeine [2,3]. Vesicles that can be sedimented not only contain most of the alkaloids of the latex but are an efficient site of synthesis from DOPA [42,44,49]. Presumably waiting to be characterized in these vesicles are specific methylases and oxidases that, acting together, determine the alkaloid structures that are made. Enzymes of *P. bracteatum* latex have also been investigated [77] but less extensively than those of *P. somniferum*.

Although thebaine, codeine, and morphine are normally present simultaneously in *P. somniferum*, morphine is considered to be the real end product, which simply accumulates in inert storage. The findings of Fairbairn and others [46,51–53] regarding the rapid turnover of morphine alkaloids come,

therefore, as a surprise. By feeding tyrosine-U-[^{14}C] to *P. somniferum* and sampling the various alkaloids for radioactivity at increasing time intervals, these workers have shown the morphine is continuously being broken down to other products even though the total amount of morphine increases with time. During the course of a day considerable variation in the pattern of alkaloids was observed, plants sampled at noon showing a high content of thebaine and codeine but very little morphine. If radioactive morphine was fed to plants, more than 90% of the radioactivity had left the alkaloidal fractions after 3–5 days. Seed extracts containing 3%–6% of the fed label showed 90% of the radioactivity present to be located on chromatograms as three ninhydrin-positive compounds which were not identified. Several weeks after feeding radioactive morphine there was radioactivity present in insoluble fractions of the plant [51]. Morphine-U-[^{14}C], when supplied to phloem, was rapidly absorbed into the latex and there converted to polar, nonalkaloidal substances that were translocated to other parts of the plant and metabolized further to CO_2 or methanol-insoluble material [46]. The complex nature of these bound alkaloids is indicated by the fact that acid hydrolysis of a polysaccharide fraction releases some [98], but pepsin digestion is also effective at release [47]. Although the content of free morphine and codeine in commercial, edible poppy seed is very low, acidic hydrolysis can release additional amounts [61]. During seed germination these bound alkaloids are also released [47]. In addition to being linked to polymers, morphine is also metabolized by other pathways, e.g., being irreversibly demethylated to normorphine [75]. N-oxides of the major morphinan alkaloids are also made and may be intermediates in the demethylation process [50,79]. The same latex vesicles that function in biosynthesis also function in these degradative reactions [48]. Thus morphine can serve as an intermediate of plant metabolism rather than an end product, which is not metabolized further, but how quantitatively important it is in this role remains to be seen.

There are a few other types of compounds found in plants which appear to be biochemically related to the aporphine-morphinan group of alkaloids, although they have received much less study. *Stephania japonica* contains, in addition to several aporphine and morphinan alkaloids, the only naturally occurring dibenzazonine compound:

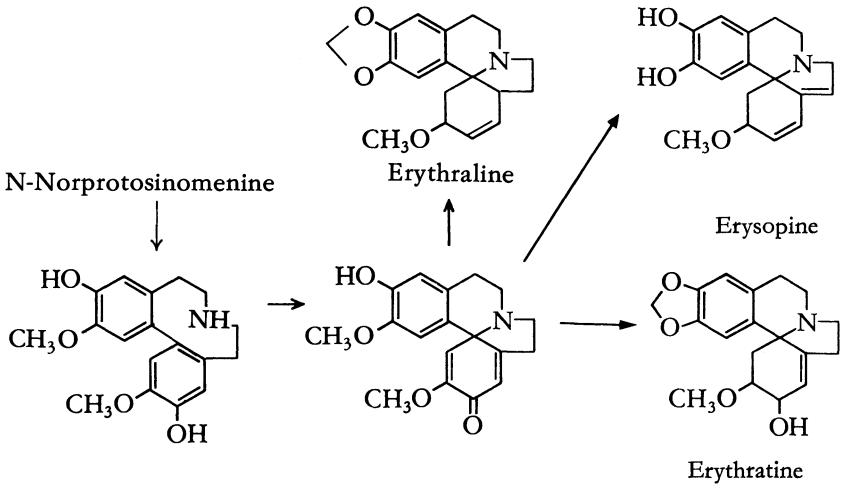


Protostephanine

This structure appears to be formed from two tyrosine residues, and tracer experiments have shown that a key intermediate is a benzylisoquinoline that

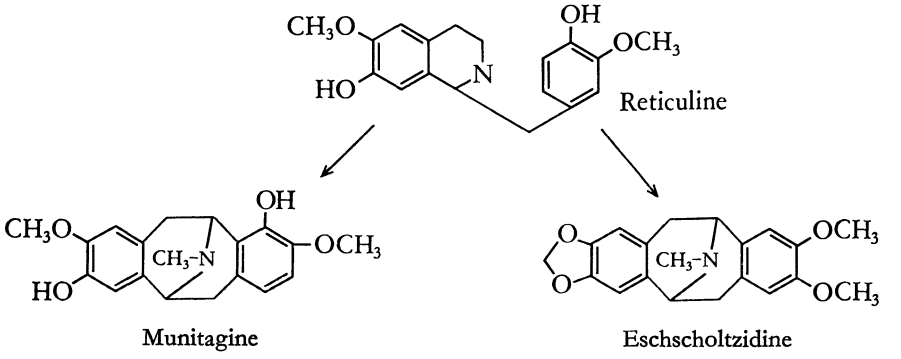
undergoes *para-para* coupling in contrast to the *ortho-ortho* coupling that makes morphinans. Breaking of the bond between the original A ring and C-1 then produces the dibenzazinone skeleton [25]. The lower aromatic ring in the above structure corresponds to the original A ring.

Erythrina species (family Leguminosae) are distinguished for containing, among other types of alkaloids, compounds with the erythrinan ring system, such as erythratine and erythraline. It appears that such structures are derived from the benzylisoquinoline (+)-N-norprotosinomenine as follows:



Radiotracer experiments have confirmed many aspects of this pathway [10,11,14,29,30]. In addition to alkaloids, with the normal erythrinan ring system there are others that are obviously related via cleavage, expansion, and contraction of some rings [15,97].

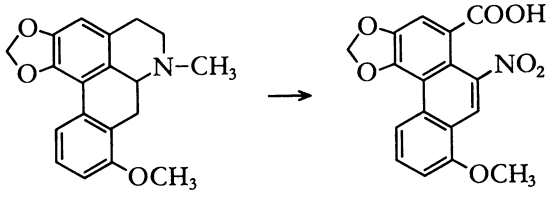
Alkaloids of *Eschscholtzia* and *Argemone* spp. are dibenzocyclooctane derivatives presumed to be derived from reticuline or related compounds by coupling of the benzyl ring to the 3-position of the quinoline ring, possibly through a $\Delta^{2,3}$-iminium intermediate [90]:



Tracer experiments for this group are not yet available. The tetracyclic ring system is called "pavine."

Just as there are dimeric benzylisoquinoline alkaloids (Chap. 7), there are also a small number of dimers in which one or both of the monomeric units has cyclized further to an aporphine or pavine structure. The ether bridge connects C-9 of one aporphine unit with various positions on the other unit [62].

It has been suggested for some time that the unusual nitrophenanthrene compounds found in *Aristolochia* spp. could be derived from oxidation of aporphine alkaloids contained in these same species [65]. This possibility has been tested by feeding several labeled alkaloids to *A. sipho* and finding support for such a relationship, with stephanine the probable precursor of aristolochic acid [41,48]. Noradrenaline-2-[^{14}C] labeled exclusively the carboxyl group, indicating possible introduction of oxygen at this location even before assembly of a benzylisoquinoline precursor [41].



Stephanine Aristolochic Acid

Besides the general environmental effects on alkaloid biosynthesis that are discussed in Chap. 1, some special effects on alkaloids of the aporphine-morphinan group seem worthy of mention here. Effects of day length and light intensity on the alkaloids of *Papaver somniferum* are complex. Although it is not surprising that long days and high light favor alkaloid synthesis, it is noteworthy that these conditions also increase the proportion of methylated to demethylated alkaloids—e.g., codeine/morphine [26]. An observation bearing on the hypothesis that alkaloids act as protective compounds is that injury to the stem of *Liriodendron tulipfera* stimulates the synthesis of several aporphine alkaloids that are normally not present in the sap. These are unusual aporphines having a carbonyl group at C-7 and being colored green or violet [40,85].

The genetic control of alkaloid metabolism has been examined at several taxonomic levels. At the species level the difference between *P. somniferum* and *P. bracteatum* can be attributed to a genetic difference in demethylating systems. The former plant has the complete pathway to morphine but the latter cannot demethylate endogenous thebaine to codeinone or exogenous codeine to morphine. It converts exogenous thebaine to unknown products [36,66]. At the subspecies level, a serrate-leaf variety of *Croton flavens* has flavinine and norsinoacutine while an entire-leaf variety makes the corresponding N-methyl derivatives, flavinantine and sinoacutine [39]. In *Croton linearis*, which is dioecious, there is even a difference in alkaloid

metabolism between the male and female plants. Female plants are 10 times more efficient than male plants in converting linearisine to crotonosine [92]. Even more subtle mechanisms can be seen at work in comparison of the alkaloids produced by tissue cultures with the composition of parent plants that should be genetically identical. Callus tissue cultures from *P. somniferum* do not make morphinan alkaloids because they evidently lack the specific *ortho-para* phenol oxidizing system that is required to act on (−)-reticuline. They do convert (+)-reticuline to protopine-type alkaloids and can convert exogenous codeinone to codeine. Recently, cell-suspension cultures have been found that produce codeine but not either thebaine or morphine [59,94]. Cell cultures from *P. bracteatum* are similar to cultures from *P. somniferum* in that they also make thebaine only in negligible amounts but, rather, use the (+)-reticuline pathway to stylopine and protopine. At an early stage these cultures make some thebaine, and its synthesis can be increased somewhat by the addition of substrate (L-DOPA) and growth regulators [71]. It will be very interesting in all of these cases to discover just how regulation is effected.

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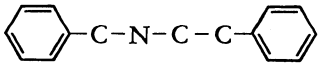
Chapter 9

Amaryllidaceae Alkaloids and Colchicine

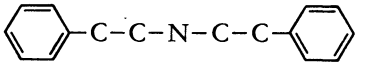
Fair daffodils, we weep to see
You haste away so soon;
As yet the early-rising sun
Has not attained his noon.

To Daffodils, ROBERT HERRICK

About 100 alkaloids are known from the family Amaryllidaceae. In their route of biosynthesis they bear several resemblances to the morphine alkaloids. In each type there are two six-membered rings derived from aromatic amino acids; and the additional ring closures are apparently produced by a mechanism of phenolate free-radical coupling. The carbon-nitrogen skeletons of the presumed precursors in the two groups differ from each other by one carbon atom:

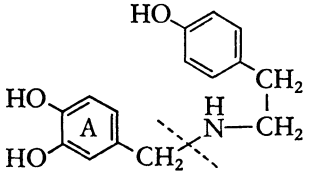


→ Amaryllidaceae Alkaloids



→ Morphine Alkaloids

The key intermediate for alkaloid biosynthesis in the Amaryllidaceae is presumed to be norbelladine, although this compound has never been isolated from a plant:



Tracer experiments have shown the origin of the portion of the molecule to the right of the dotted line from a molecule of tyrosine [5,31]. The left-hand portion, however, does not obtain label from administered tyrosine but does from phenylalanine or from such nonnitrogenous precursors as cinnamic acid, hydroxycinnamic acids, or protocatechuic aldehyde [1,34]. These findings suggest the route shown in Fig. 9-1. Simple O-methylation of the norbelladine leads to belladine. This methylation is one of the few reactions in alkaloid biosynthesis which has been studied at the enzymatic level [22]. A preparation from *Nerine bowdenii* catalyzes it with S-adenosyl-methionine as the methyl donor. The enzyme was found to be nonspecific, active with other catechols as well.

Appropriate ring closures of norbelladine lead to the skeletons of the other major Amaryllidaceae alkaloids. The ring positions on norbelladine that can participate in phenolate free-radical coupling are starred in Fig. 9-2, and three of the possible ring systems resulting are realized in actual alkaloid structures [1,10]. Fed to *N. bowdenii* plants, norbelladine labeled in carbons adjacent to the nitrogen atom was found to be incorporated into belladine, lycorine, and crinamine to the extent of 2.64%, 0.07%, and 0.0009%, respectively [30]. Norpluviine and caranine are sequential precursors of lycorine, the commonest alkaloid of the family [16].

Other alkaloids are clearly related to this pathway [28], and for some of them (e.g., haemanthamine, galanthine, narcissidine, and norpluviine) the relationship has been confirmed by tracer experiments [5,14].

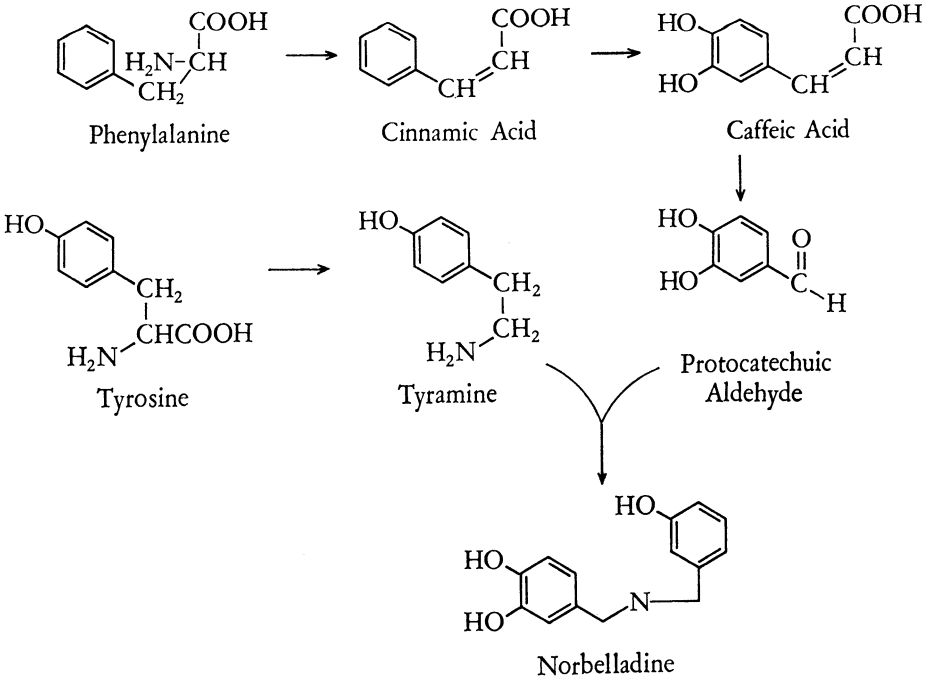


Fig. 9-1. Probable pathways to norbelladine

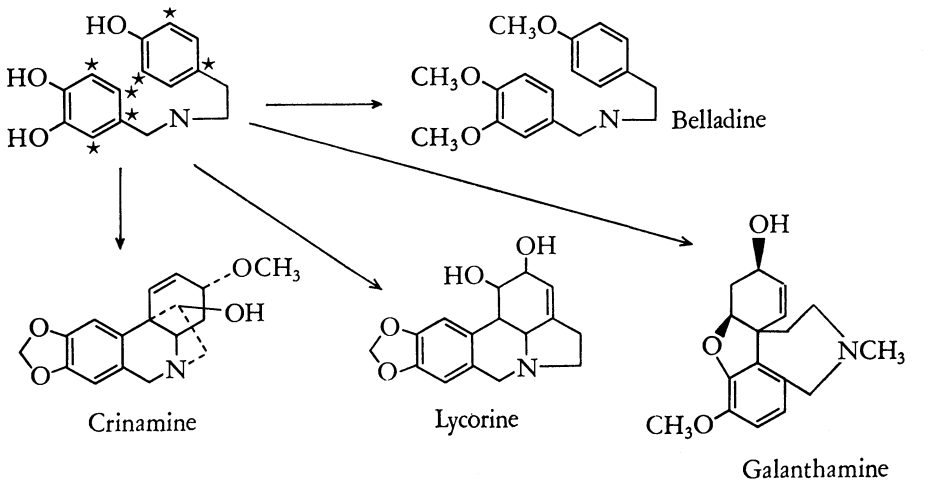
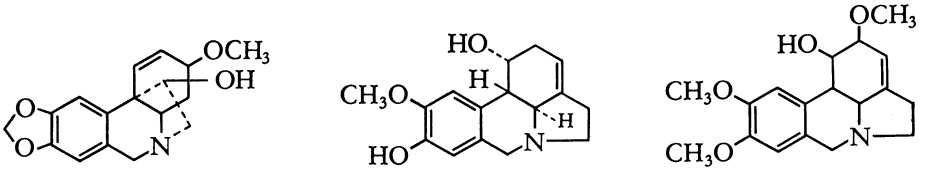
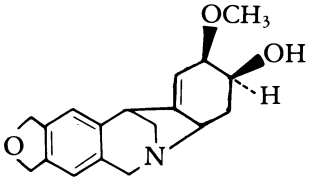


Fig. 9-2. Pathways from norbelladine to other alkaloids (* = possible coupling position)



Several alkaloids appear to result from *para-para* coupling of the two aromatic rings followed by elimination of the C_2 bridge. Although the sequence of these reactions is not completely known, tracer experiments have shown that a pathway something like that of Fig. 9-3 is functional [12,15,18]. Montanine (and a few other Amaryllidaceae alkaloids) are peculiar in being made by a route in which there is no coupling of the two aromatic rings. Although 4'-O-methylnorbelladine is a precursor, the mechanism of ring closure is not obvious [13,32].



In addition to the four structural types whose relationship to norbelladine has been outlined, two other types of Amaryllidaceae alkaloids, which might be classed on superficial examination with the indole alkaloids, are encountered. However, it appears that their reduced-indole rings are derived

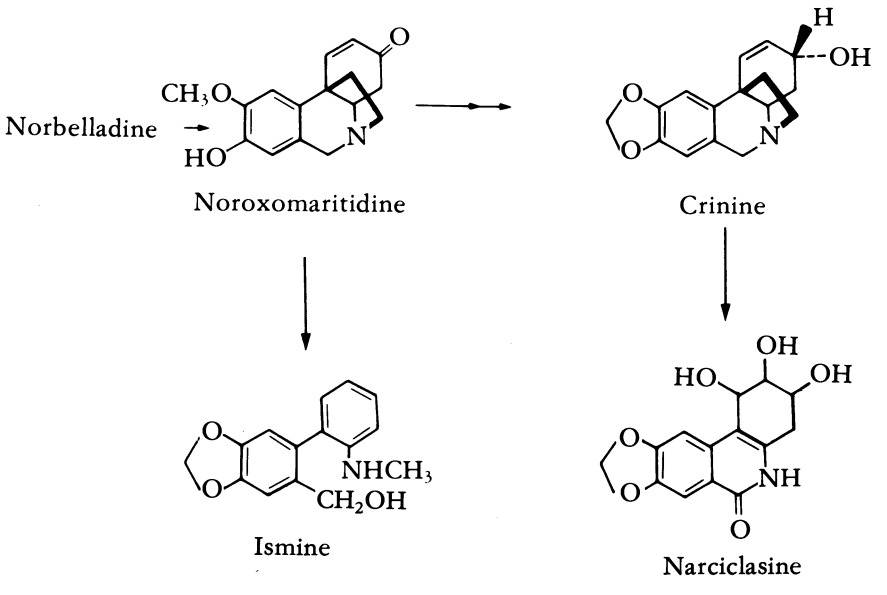
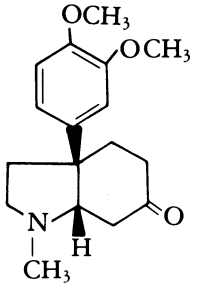


Fig. 9-3. Pathway in which C_2 bridge is lost

by secondary reactions from the more common Amaryllidaceae structures. Thus, by pathways involving oxidative splitting of ring B, homolycorine and lycorenine could be derived from pluviine, or tazettine from haemanthamine (Fig. 9-4). There is tracer evidence for these pathways [11,17,20], although tazettine is probably an artifact of isolation formed by rearrangement of pretazettine [29]. The general principle that methylene dioxy groups are derived by an oxidative cyclization of a methoxy group and a neighboring hydroxyl has been confirmed by a tracer experiment for haemanthamine, and presumably applies to other methylene dioxy groups in these alkaloids [9].

A group known as "mesembrine alkaloids" is found in several species from the family Aizoaceae. Tracer experiments have shown a biosynthetic resemblance to the alkaloids of Amaryllidaceae in that tyrosine and phenylalanine are the units of construction. However, the details of biosynthesis remain obscure [19].



Mesembrine

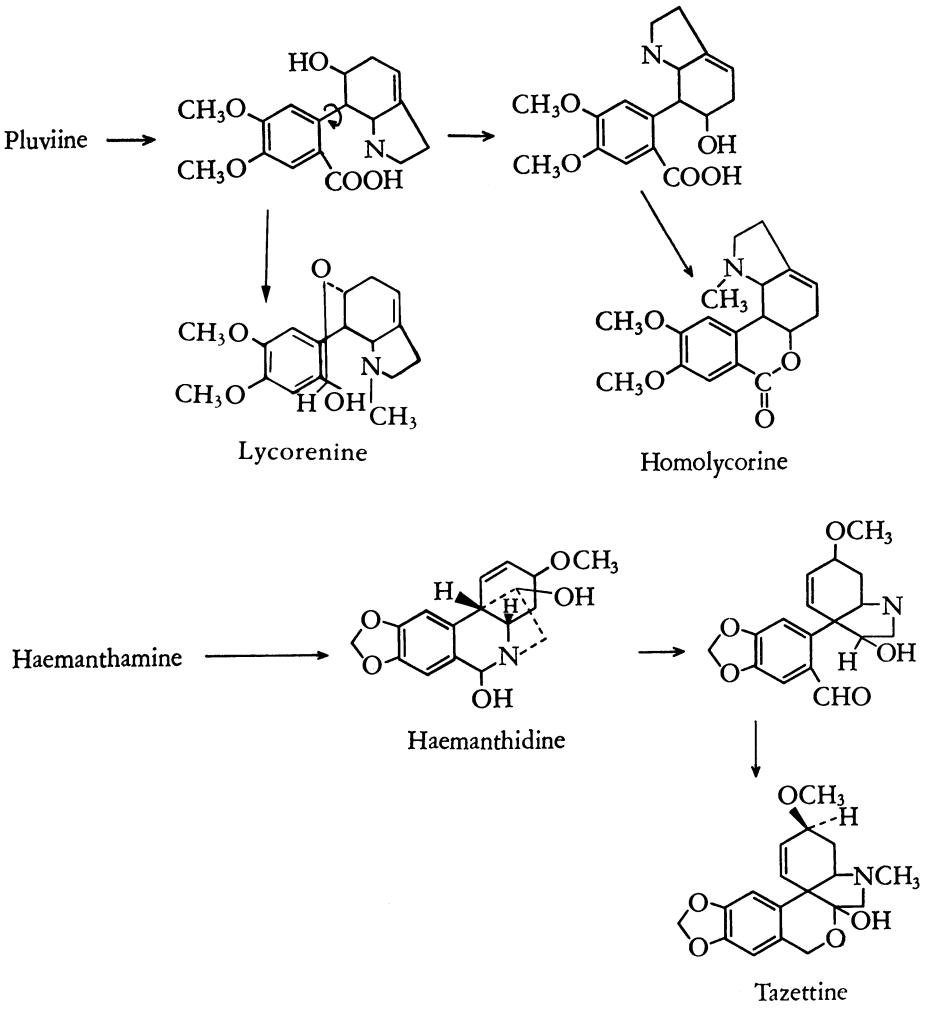


Fig. 9-4. Pathways to homolycorine and tazettine

Colchicine and similar alkaloids found in *Colchicum* spp. and related genera of the Liliaceae bear little structural resemblance to alkaloids of the Amaryllidaceae [33]. Nevertheless, tracer feeding experiments have also shown for them an origin from one molecule of tyrosine plus one molecule of phenylalanine or cinnamic acid. Tyrosine-3'-[^{14}C] puts label into C-12; tyrosine 4-[^{14}C] labels C-9; while phenylalanine gives rise to ring A and C-5, C-6, and C-7 [2,21,27]. With the finding of androcymbine, a dienone structurally related to colchicine in the same plant, additional steps in this biosynthesis became reasonable. The feeding of [^{14}C]-labeled phenethylisoquinolines and tritium-labeled O-methylandrocymbine to *Androcymbium methanthiodes* gave good yields of labeled colchicine, in support of the pathway shown in Fig. 9-5 [6,7].

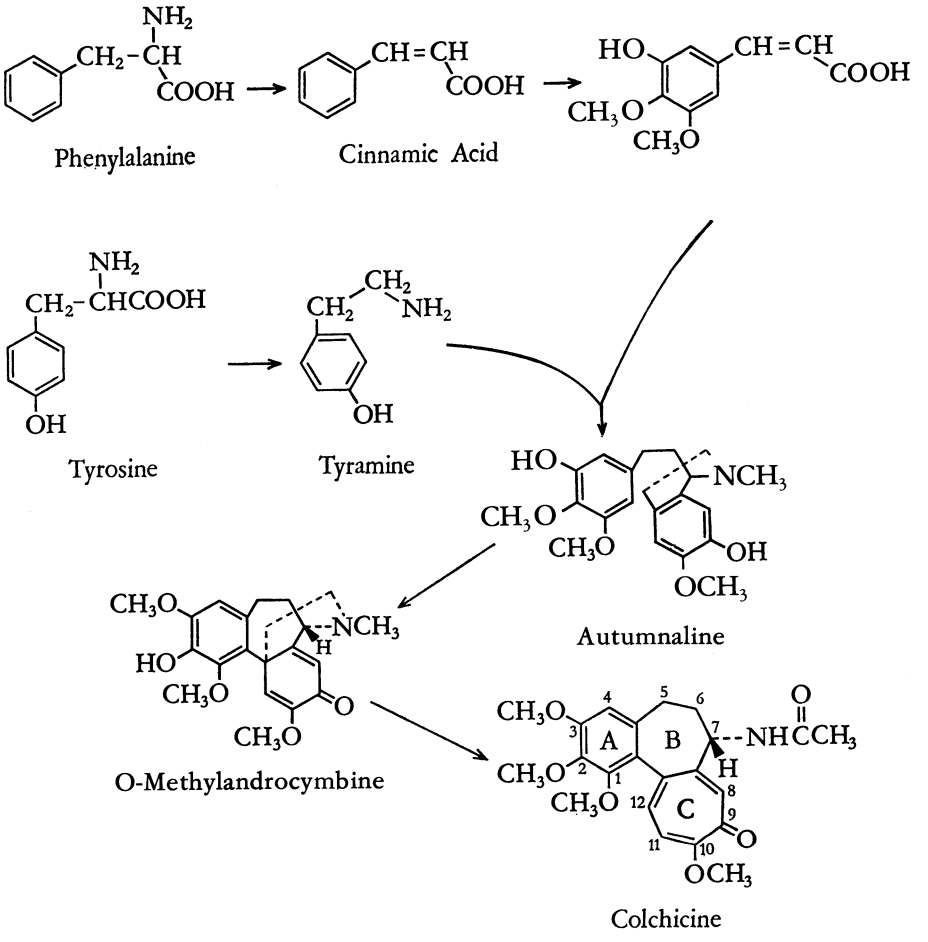
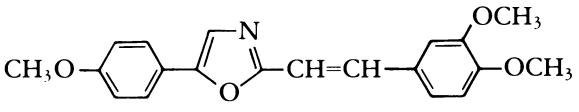


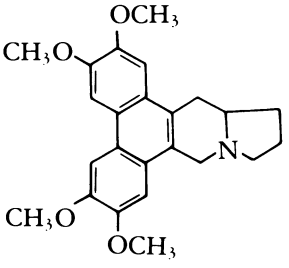
Fig. 9-5. Colchicine biosynthesis

Demecolcine, which occurs together with colchicine, differs from colchicine only in having N-methyl rather than N-acetyl. When it was labeled with tritium at C-1 and in O-methyl groups and fed to *C. autumnale*, it was incorporated 13.8% into colchicine [8]. It is therefore a likely intermediate following androcymbine. Noradrenaline has been proposed as an intermediate between tyrosine and ring C [26], but as yet there is no tracer evidence on this point.

Finally, there are a few other alkaloids whose fundamental structures are derived from one molecule of tyrosine plus one molecule of phenylalanine. Information is not extensive, but two examples seem to be annuloline of *Lolium multiflorum* [25] and tylophorine of *Tylophora asthmatica* [23,24].

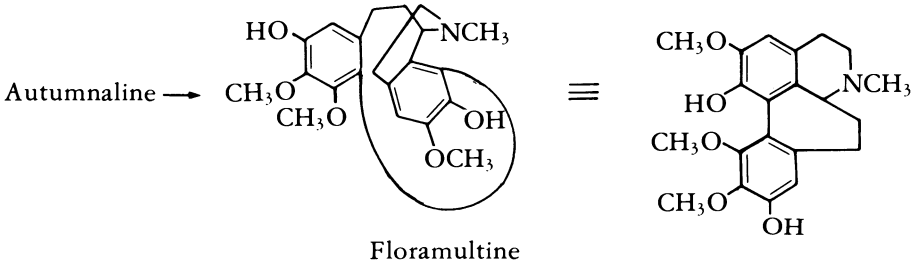


Annuloline



Tylophorine

The homaporphine alkaloids of *Kreysigia multiflora* are derived from autumnaline by an *ortho-para* coupling reaction in the same way that the aporphine alkaloids (Chap. 8) are derived from 1-benzylisoquinolines [3,4].



Floramultine

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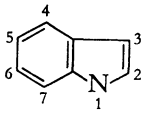
Chapter 10

Indole Alkaloids

Q for Quinine which children take
With Jam and little bits of cake
Moral
How idiotic! Can Quinine
Replace Cold Baths and Sound Hygiene?

A Moral Alphabet, HILAIRE BELLOC

The alkaloids containing an indole (or reduced indole) nucleus



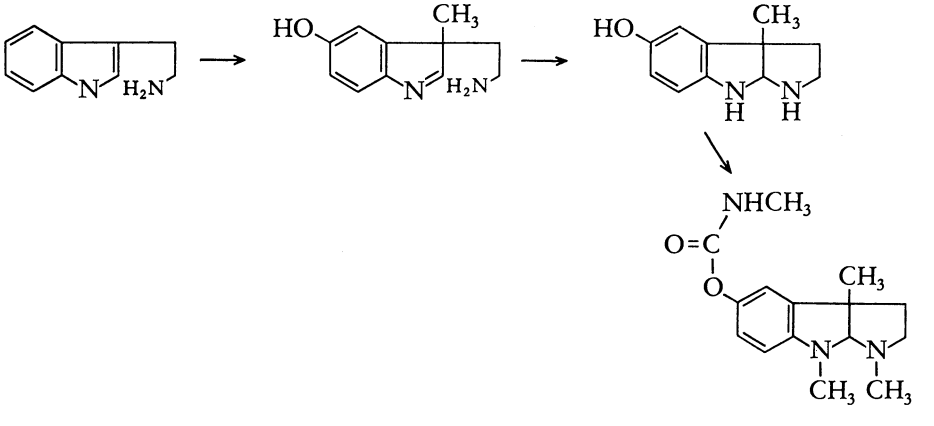
Indole

make up an extensive and complex group. A rough estimate is that more than one-quarter of all known alkaloids are indoles. However, other aspects of their structure are so diverse that superficially there appears to be little unity in the group. As knowledge of biosynthetic pathways has developed, though, a few generalities have been noted which make it possible to arrive at many of the natural structures by starting with only a few simple precursors and proposing some generalized types of reactions. Knowledge of biosynthetic pathways has also resulted in the grouping of certain alkaloids that do not have an indole nucleus with the indole alkaloids because of similarities in precursors and biosynthetic pathways (e.g., quinine, discussed below).

While the distribution of indole alkaloids is broad, certain plant groups are noted for containing them. Among the seed plants, the family Apocynaceae has been a very rich source of indoles. In this family, the genera *Rauwolfia*, *Catharanthus*, and *Aspidosperma* have been particularly well-investigated [112]. Another important source is the fungal genus *Claviceps* (ergot), which is known to contain more than two dozen different indole alkaloids.

All of the usual approaches to clarifying pathways of alkaloid metabolism have been applied to the indole alkaloids; and, perhaps because of their rich complexity, these compounds seem to have been endowed with a greater variety of hypothetical metabolic pathways than any other group. One of the earliest proposals, at least, has stood the test of time and indeed has never really been questioned. This is the suggestion of Pictet [110] that the indole nucleus is derived from the amino acid tryptophan. An aminoethyl residue attached to the β-position of the indole ring in most of these alkaloids is also considered to come directly from the corresponding atoms in the tryptophan side chain. Tracer evidence for this much of the pathway has been provided in the cases of chimonanthine [123], serpentine [74], rutaecarpine [157], vindoline [76], lysergic acid [95], ibogaine [81], and ajmaline [73]. Intermediates between tryptophan and the alkaloids are still in doubt. Tryptamine and N-methyltryptamines have often been considered. Where the alkaloids are hydroxylated in the indole portion, a derivation from hydroxytryptophan has been proposed rather than hydroxylation late in the sequence. Indole-3-acetic acid and related plant hormones known to be derived from tryptophan have also been implicated as possible alkaloid precursors [142]. In a few cases the alkaloid structures are best interpreted as resulting from incorporation of the parent compound, indole, since the aminoethyl residue does not appear at all in the final structure. There is only slight experimental evidence for or against any of these possibilities; such evidence, where available, will be cited under consideration of the individual alkaloid groups. Simple derivatives of tryptophan have been discussed as protoalkaloids (Chap. 3). Reviews on indole alkaloid biosynthesis are [34,68]. A proposal for classification of indole alkaloids on the basis of their biosynthetic pathways is [65].

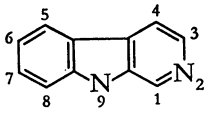
Physostigmine, the chief alkaloid of the calabar bean (*Physostigma venenosum*), and related compounds are apparently derived from tryptamine by a pathway such as the following:



Physostigmine

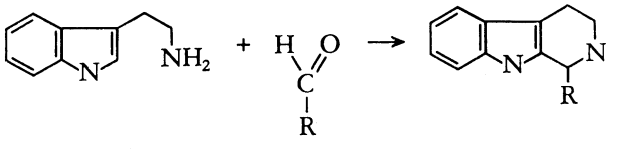
Questions needing attention concern the stage at which hydroxylation and methylation occur, the course of the three methyl groups, and the source of the methylcarbamoyl group. Experimental approaches to these questions are thus far lacking.

Simple derivatives of β-carboline



β-Carboline

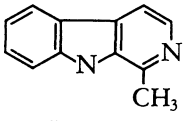
are readily synthesized in vitro by the reaction of tryptamine with aldehydes [59]:



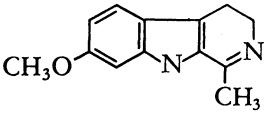
Tryptamine Aldehyde β-Carboline Derivative

Such reactions are believed to account for the biosynthesis of such alkaloids as harman, harmaline, etc. Simple acid hydrolysis of tryptophan-containing proteins gives rise to harman and derivatives of it [147]. About a dozen alkaloids of this type are known from nine different plant families. One tracer experiment has suggested that the C_2 unit of harman, rather than coming from acetaldehyde, is produced by the reduction of N-acetyltryptamine [91,129]. Cell-suspension and callus tissue cultures of *Peganum harmala* also produce alkaloids, but compartmentation problems limit their usefulness in studying biosynthetic pathways [99,100]. Tissue cultures do metabolize exogenously supplied alkaloids by hydroxylation and glycosylation. Auxins inhibit total alkaloid production, but allow the later transformations; so that, for instance, they will convert harmaline into harmine and β-glucosides of 8-hydroxyharmaline and harmine [98–100]. In whole plants of *Passiflora edulis* no metabolism of harman seems to occur [33]. A curious observation is that cell-suspension cultures of the common bean, *Phaseolus vulgaris*, produce harman when the nutrient is supplemented with tryptophan [149,150]. Thus the synthesis of an alkaloid may depend on availability of substrate rather than activity of special enzymes. In this same vein β-carbolines can be detected in mammalian brain tissue, where they may be formed by reaction of tryptamine with methylenetetrahydrofolic acid [8,9,108,127]. When humans or rats ingest large amounts of ethanol, harman and tetrahydroharman are excreted in the urine. The alkaloids are

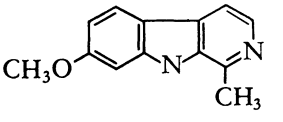
presumably made by condensation of surplus acetaldehyde with endogenous tryptamine [118]. Since these alkaloids are hallucinogens, inhibitors of ion transport and of monamine oxidase, these mammalian results may have important pharmacological implications (cf. Chap. 15).



Harman



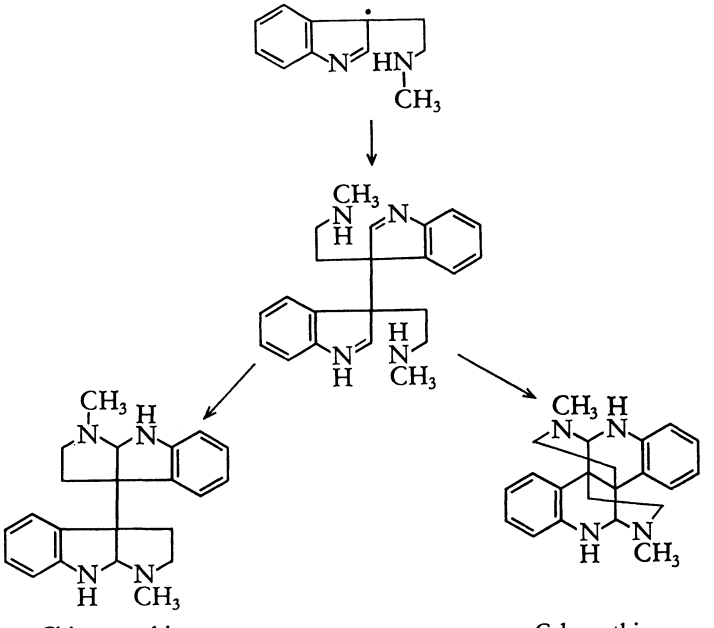
Harmaline



Harmine

The condensation between a tryptamine derivative and an aldehyde is regarded as a starting point for the biosynthesis of other, more complex, indole alkaloids where the presence of additional functional groups on the aldehyde portion leads to further reaction possibilities [59].

Calycanthus floridus contains indole and quinoline alkaloids that have been shown by tracer experiments to be derived from tryptophan [64,91, 101,123], as was predicted earlier. The proposed biosynthetic scheme involves formation of an indolenine radical that on coupling with itself generates an intermediate that can go to alkaloids of either type. By labeling C-2 of tryptophan or tryptamine with tritium it could be shown that there was no oxindole intermediate in the formation of chimonanthine by *Chimonanthus fragrans* [64].

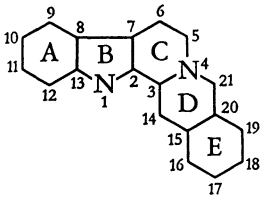


Chimonanthine

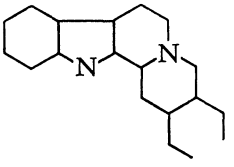
Calycanthine

The alkaloids of *Sceletium strictum* have a reduced indole nucleus that is not derived from tryptophan. They are probably related biosynthetically to the Amaryllidaceae alkaloids (cf. Chap. 9).

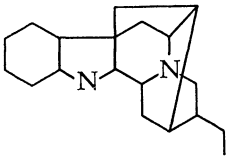
Large numbers of indole alkaloids with obviously related structures are found in many plants of the families Apocynaceae, Loganiaceae, Rubiaceae, and Euphorbiaceae. For clarity, the carbon-nitrogen skeletons of the four major groups are given below. In all, at least 10 parent ring systems have been distinguished [146]:



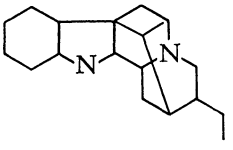
Yohimbine Group



Corynantheine Group



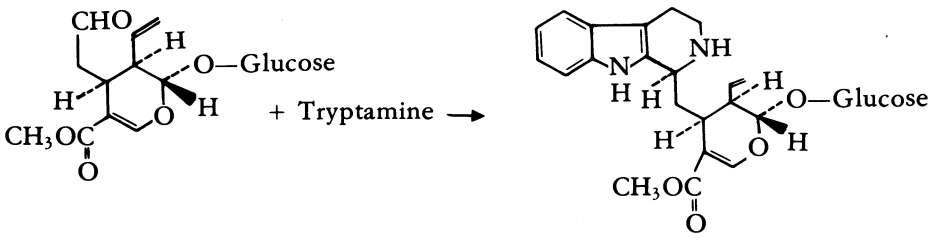
Ajmaline Group



Picraline Group

Many of these alkaloids have another carbon atom attached to C-16. Thus there is in addition to the indole unit derived from tryptophan a C_9 or C_{10} unit whose origin remained obscure until recently. Previous proposals were that the C_{9-10} unit derived from an aromatic amino acid [156], prephenic acid [154], or acetate [32] and involved splitting a six-membered ring corresponding to ring E of yohimbine (1) to give the other three skeletons. Isotope experiments have not confirmed any of these proposals. A terpenoid origin for this part of the molecule was suggested in 1961 [143,155] and now has been confirmed in many different ways [14–16, 50,78,83,140]. The pathway from the common terpenoid alcohol, geraniol (2), through loganin (3) to various forms of the monoterpenoid unit is shown in Fig. 10-1. Structures of some well-known indole alkaloids with the monoterpenoid unit emphasized are shown in Fig. 10-2. Figure 10-3 shows some typical conformational structures.

As a result of both radiotracer and enzymatic experiments, many details are now known about the nature of the immediate monoterpenoid precursor and its subsequent transformations. For many of these alkaloids the initial steps are the cleavage of loganin to secologanin and condensation of the latter with tryptamine:



Strictosidine

Except for the unusual aldehyde reactant, this condensation is no different from what occurs in making the simpler β-carbolines described earlier in this chapter. Therefore, the significant metabolic difference in those plants that are noted for complex indole alkaloids may well be their production of

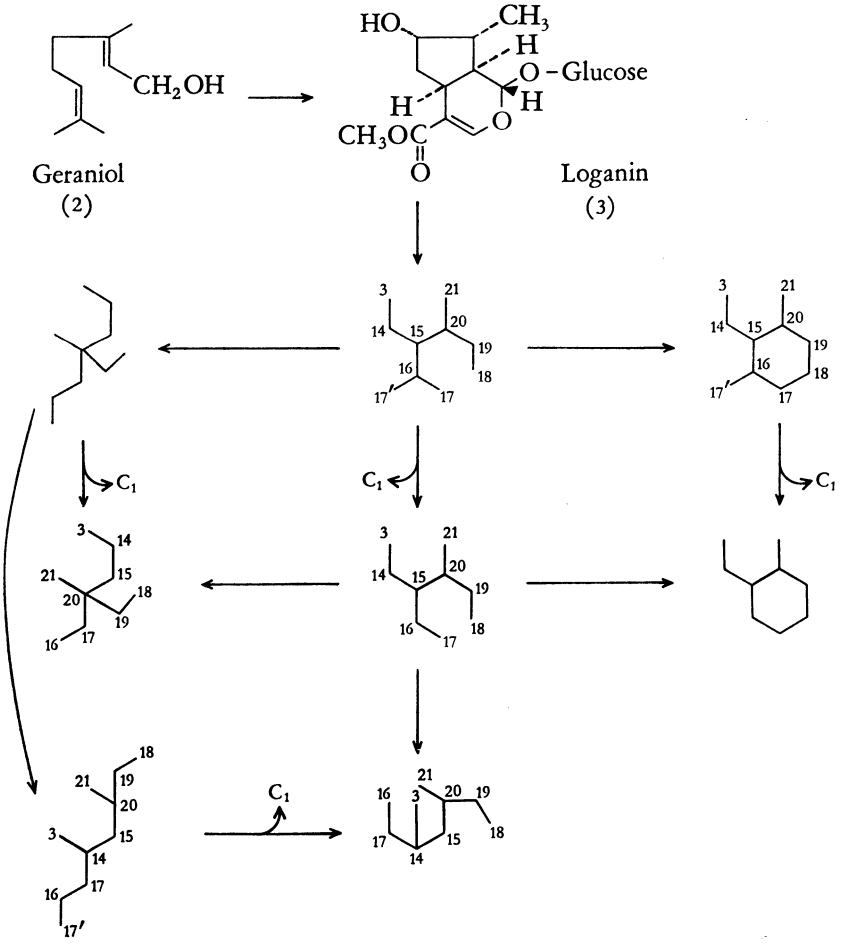
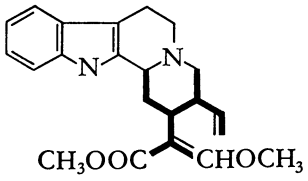
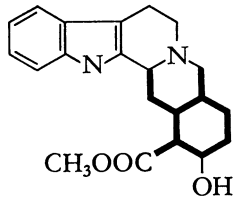


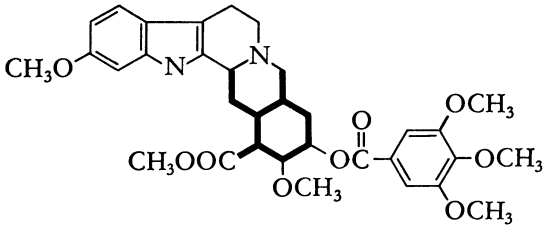
Fig. 10-1. Numbering and possible rearrangements of monoterpenoid unit in indole alkaloids



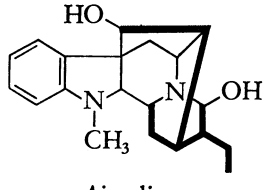
Corynantheine
(8)



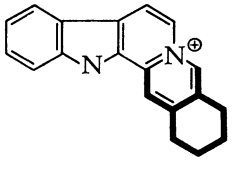
Yohimbine
(1)



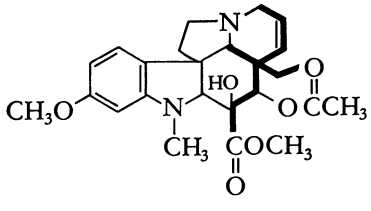
Reserpine
(5)



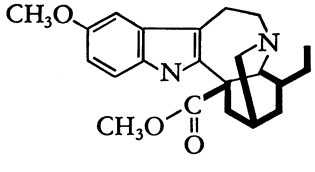
Ajmaline
(7)



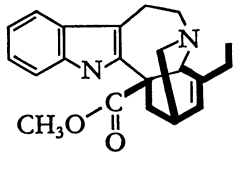
Sempervirine



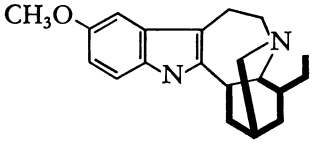
Vindoline
(6)



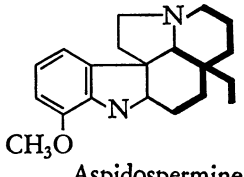
Voacangine



Catharanthine
(4)

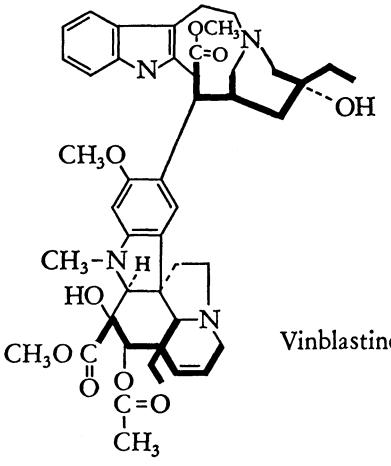


Ibogaine



Aspidospermine

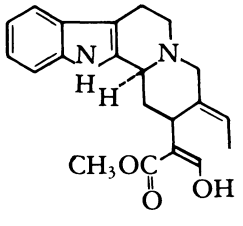
Fig. 10-2. Some indole alkaloids showing monoterpenoid units (*heavy lines*) incorporated into their structures



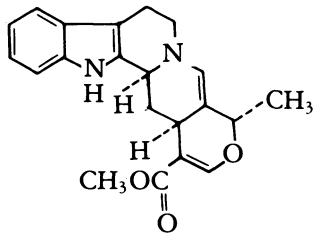
Vinblastine

Fig. 10-2. (*Cont.*)

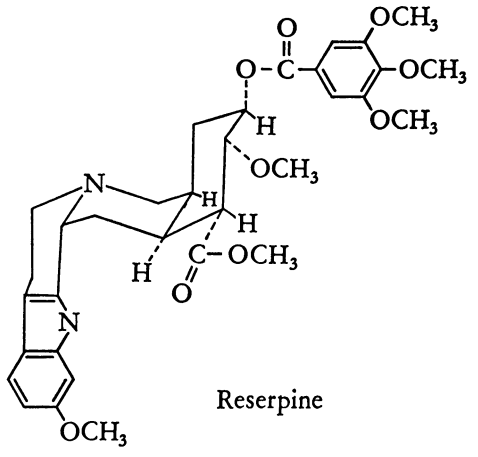
secologanin and the required condensing enzyme [145]. For several years the stereochemistry and nomenclature of the initial β-carboline product was confused. As shown above, strictosidine has the α or (S) configuration at C-3. Its isomer, vincoside, is β or (R) at this center [18,134,144]. While strictosidine is the preferred precursor in all cases where the comparison has been made [96], some plants make both isomers, and vincoside might be a precursor of certain alkaloids [27]. It is just as possible that any alkaloids having a 3β configuration are derived from later epimerization rather than the initial condensation [135,93]. The action of a β-glucosidase on strictosidine produces a hemiacetal that rearranges, and at this point there is controversy over the product(s) formed. Likely candidates are geissoschizine and cathenamine. They are interconvertible and both can be precursors of the later products [60,72,131,132]:



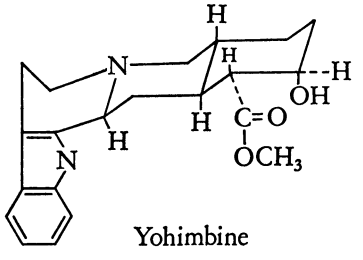
Geissoschizine



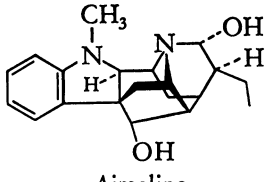
Cathenamine



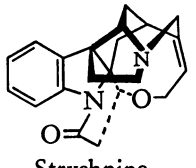
Reserpine



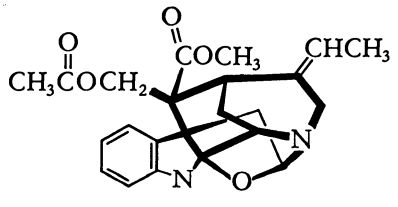
Yohimbine



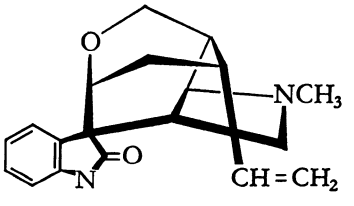
Ajmaline



Strychnine



Picraline



Gelsemine

Fig. 10-3. Conformational structures of some typical indole alkaloids

Enzymatic reduction of cathenamine at the 20,21-double bond with NADPH produces ajmalicine (20β) or tetrahydroalstonine (20α) [72,131, 135]. Similar reduction of geissoschizine gives isositsirikine [60]. Rearrangements of the ajmalicine skeleton can explain the structures of many other indole alkaloids. An outline of such transformations is presented in Fig. 10-4.

The pathway, as far as ajmalicine, has been clarified not only by tracer experiments with whole plants of *Catharanthus roseus* but also with cell-free extracts from the plants or from cell cultures. A few highly purified enzymes

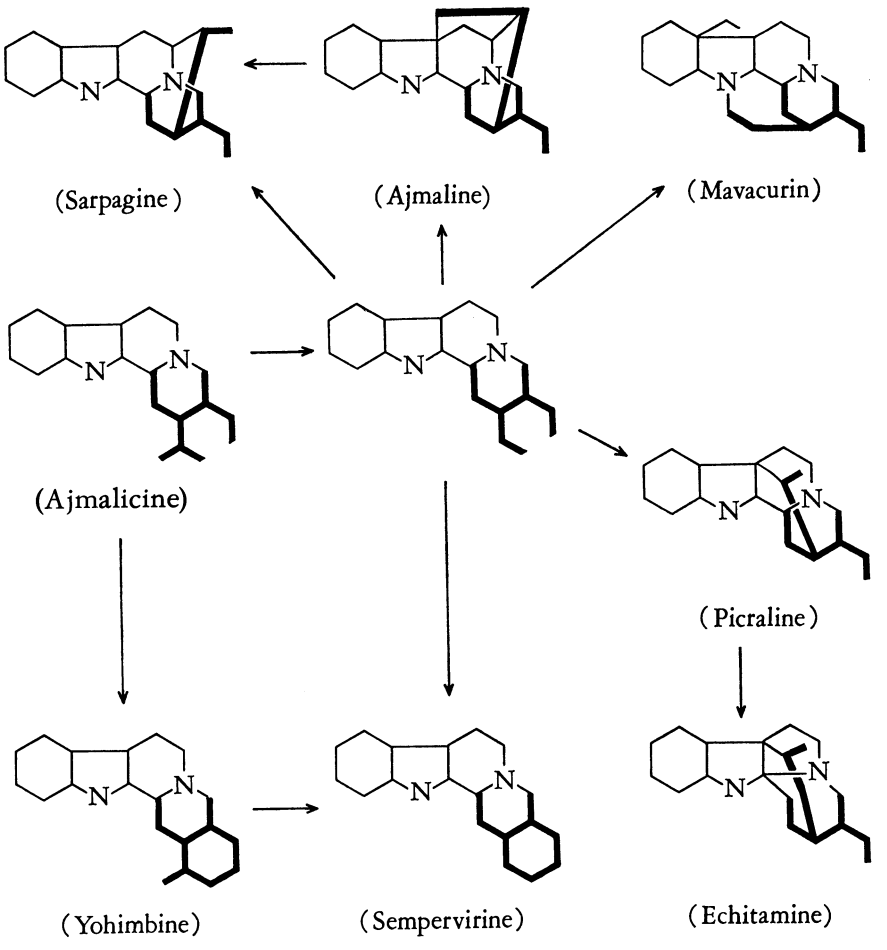


Fig. 10-4. Possible interconversions of alkaloids derived from ajmalicine. Names of particular alkaloids having the skeletons are given *in parentheses*. Monoterpenoid unit shown by *heavy lines*

have also been studied [26,84,93,135]. In addition to those already mentioned, there is a terpenoid hydroxylase responsible for conversion of geraniol to 10-hydroxygeraniol, a precursor of loganic acid [57]; and there is a methylase that catalyzes conversion of loganic acid to loganin, or secologanic acid to secologanin [84]. Both of these two enzymes are subject to feedback inhibition by alkaloids further down the pathway [84,90]. Another interesting aspect of the enzymology is that the strictosidine-condensing enzyme will utilize unnatural analogues of tryptamine to produce unnatural products [144]. Although most research has used extracts of *Catharanthus roseus*, a similar system that produces cathenamine is also obtainable from *Rauwolfia verticillata* [58].

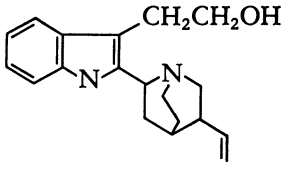
After the general pathways that have been described, different groups of plants have specialized pathways that produce their distinctive alkaloids. In the periwinkle (*Vinca* and *Catharanthus* spp.) these pathways are extraordinarily elaborate, leading to about 120 different alkaloid products [89,142]. Some of these, such as reserpine (5) and ajmaline (7), are also found in other genera of the Apocynaceae, but some are evidently peculiar to the periwinkles. Vindoline (6) and catharanthine (4) originate along the general lines already discussed [14,16,50,77,78]. The branch point to the special periwinkle alkaloids probably comes at geissoschizine, which rearranges to give the three major classes [17,125]. Dimeric indole alkaloids of *C. roseus* have been extensively investigated because of their usefulness in treating certain neoplasms. Similar dimers are found in other plants as well, for example, toxiferine I of calabash curare (13) [47]. Vinblastine is an unsymmetrical dimer apparently made from vindoline and a modified catharanthine. Leurocristine differs from it only in having N-formyl rather than N-methyl on the vindoline unit. Tracer studies have indicated that the monomers are, in fact, precursors of the dimers [20,56] and that 20′-hydroxylation is the last step in vinblastine biosynthesis [55].

Because of the commercial importance of periwinkle alkaloids, much has been done to try to increase their yield in plants or to develop cell cultures that would synthesize them efficiently. Several workers have noted the variation in alkaloid content during the course of development. The highest concentration is found in roots just before flowering [114]. Diploid callus tissue contains less than whole plants and haploid callus tissue still less. Roots redifferentiated from diploid callus tissue have at least as much alkaloid as stock roots, but redifferentiated haploid roots have less [2]. There are wide differences in the concentrations of alkaloid produced by different strains in tissue culture and also clonal differences in response to growth conditions. Light, low sucrose concentration, low protein concentration, and a kinetin/auxin ratio of 10:1 are generally favorable to alkaloid production [29,36]. Indoleacetic acid is more effective than 2,4-D at stimulating growth, but the latter is better at stimulating alkaloid production [48]. Vitamin supplements affect the pattern of alkaloids—perhaps reflecting the need for NADPH in reductive steps or flavines in oxygenation steps [29]. Some cell-suspension cultures are stimulated to alkaloid production by high tryptophan or tryptamine concentrations, but others are inhibited [67,124]. Alkaloids supplied exogenously to cell-suspension cultures undergo some metabolism to other alkaloids [30]. Whole plants and cuttings show a rapid turnover of vindoline and catharanthine, but the products made from them are unknown [35].

Not much detailed work has been done on the alkaloids of *Rauwolfia* spp., but enough to see that the pathway through ajmalicine applies to them [58]. Closure of a fifth ring in one direction leads from ajmalicine to reserpine-type structures and in another direction to ajmaline-type structures. An intermediate on the pathway to ajmaline is 21-deoxyajmaline [12].

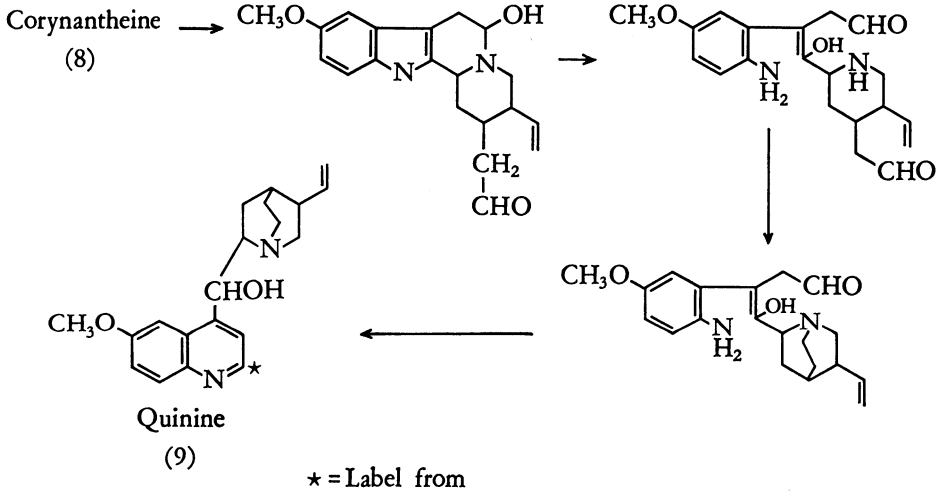
Some alkaloids of *Cinchona* (family Rubiaceae) are indoles that appear

likely to be derived from an ajmalicine-type structure by opening ring C and closing an additional ring across ring D. Thus cinchonamine has the structure:



Cinchonamine

Other *Cinchona* alkaloids, such as quinine (9), do not contain an indole ring, but are derived by secondary transformation of indole alkaloids [75,156]. Thus quinine could be formed from coryantheine through a pathway like the following:



Quinine
(9)

★ = Label from
Tryptophan–α–[^{14}C]

In support of such a pathway it has been found [66] that tryptophan-α-[^{14}C] fed to *Cinchona succiruba* gave rise to quinine with all label at C-2′ (starred position in above formula). Tryptophan labeled in the indole ring with [^{15}N] and [^{14}C]-2 gave rise to quinine with [^{15}N] in quinoline ring and [^{14}C] at the carbinol carbon atom [80]. The C_{10} unit was shown to be derived from geraniol or loganin [19,79].

Oxindole alkaloids found in the families Rubiaceae and Loganiaceae may also be derived by rearrangement of a corynantheine skeleton involving intermediates with skeletons possibly like those shown in Fig. 10-5. Another possible route to these alkaloids shown in Fig. 10-5 would be through the alkaloid caracurine-VII (Wieland-Gumlich aldehyde) (10). Nothing is

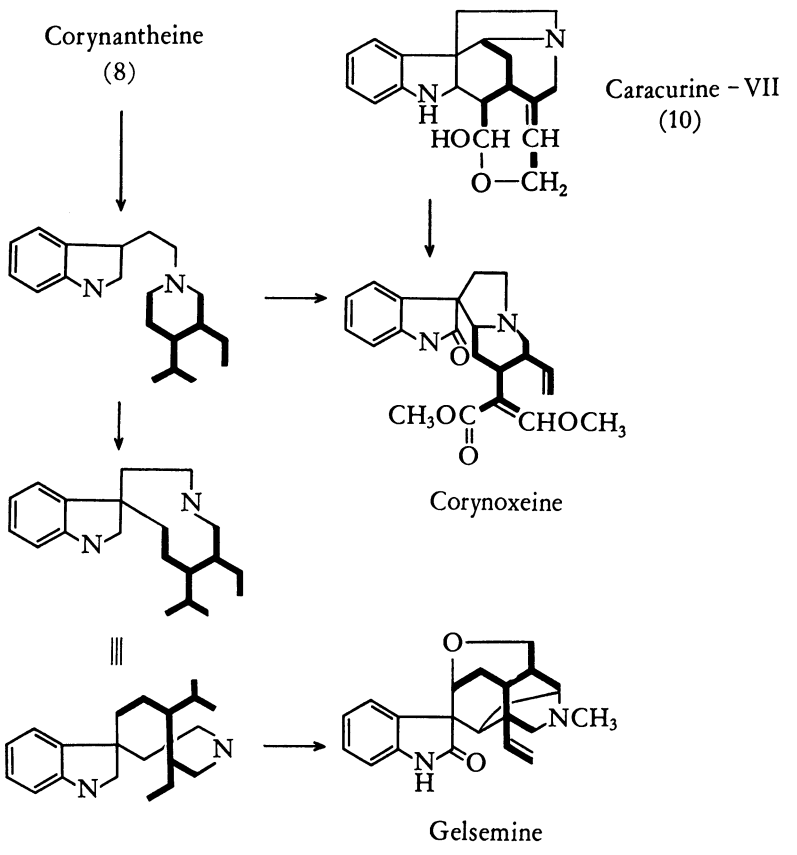
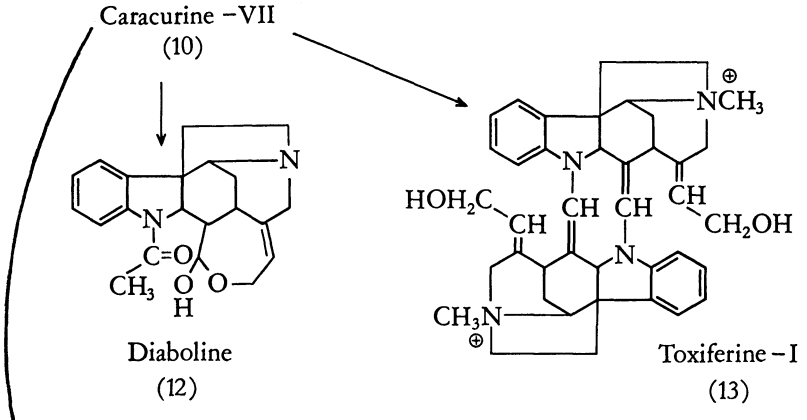
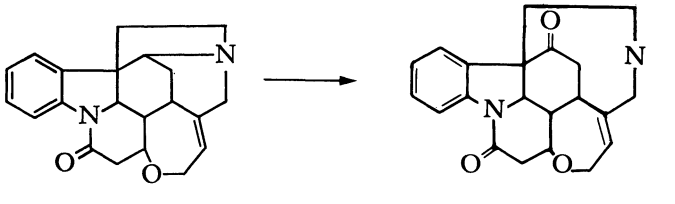


Fig. 10-5. Two plausible routes to oxindole alkaloids. Possible skeletons of intermediates shown in outline

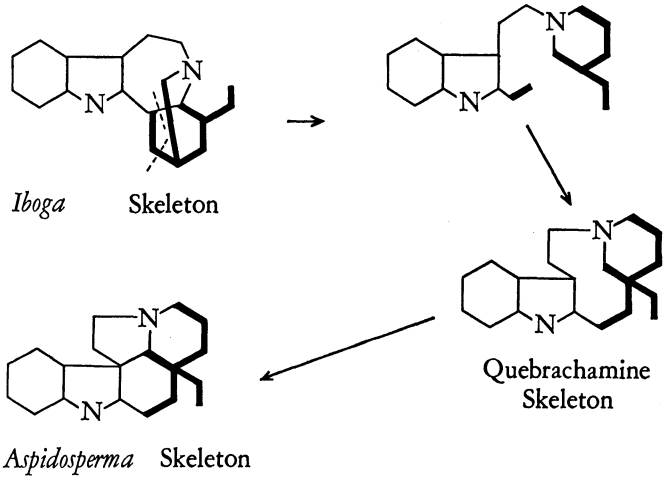
shown about the biosynthesis of caracurine-VII, but examination of its structure reveals a tryptamine plus a monoterpenoid unit.

Strychnine (11) and related alkaloids of *Strychnos* spp. seem to be composed of tryptamine plus a C_{11} unit, which can be viewed as derived from one of the C_9 monoterpenoid-type units plus acetate. Since N-acetyl alkaloids such as diaboline (12) occur naturally in *Strychnos* spp., it seems likely that condensation of the acetate with the C_9 unit occurs after both are joined in an alkaloid structure. However, diaboline itself is probably not a direct precursor of strychnine, while geissoschizine and caracurine-VII are [57]. Caracurine-VII is also considered to be a precursor of the still more complex calabash-curare alkaloids, such as toxiferine-I (13), found in South American species of *Strychnos* [31]. Strychnine is metabolized further in the roots of *Strychnos nux-vomica* plants to the so-called seco-alkaloids, such as icajin [54].



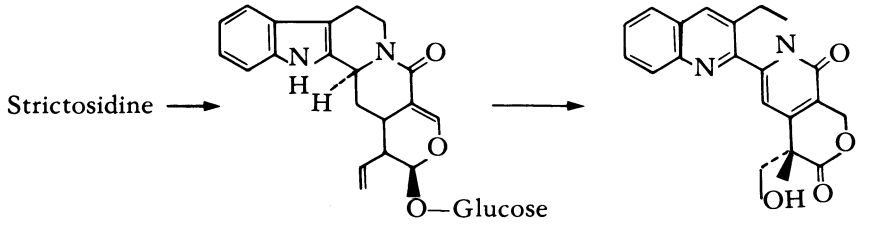


The *Iboga* and *Aspidosperma* alkaloids are both found in plants of the family Apocynaceae but do not occur together in any genus. Both types can be dissected into a tryptamine moiety and a C_9 unit. The reason for considering them together is that an acid-catalyzed rearrangement of the *Iboga*-type skeleton is known to produce the *Aspidosperma*-type skeleton [69]. This transformation is outlined below, with the C_9 unit emphasized:



Structures of ibogaine, voacangine, and aspidospermine obtained respectively from *Iboga*, *Voacanga*, and *Aspidosperma* species are included in Fig. 10-2. The close similarity in structure between catharanthine and voacangine will be noted, as well as the resemblance between vindoline and aspidospermine. Nevertheless, these similar structures have not been found to occur together. Two unusual indole alkaloids of *Aspidosperma pyricollum* are apparicine and uleine. While the former seems to be derived from tryptophan, the latter is not; and neither has an evident monoterpenoid unit [70].

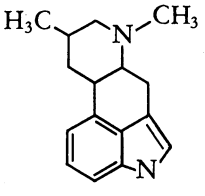
Camptothecin of *Camptotheca acuminata* has been found to have therapeutic value. Although its structure has no indole ring, it is in fact derived from strictosidine by a rearrangement in which the pyrrole ring expands as the piperidine ring contracts. The lactam formed from strictosidine is intermediate in the sequence [62,126].



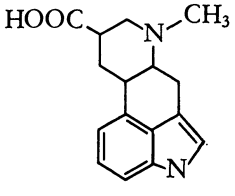
Lactam Camptothecin

Ergot Alkaloids

The ergot fungus (*Claviceps* spp.) produces about 30 different indole alkaloids. These alkaloids, long thought to be exclusively fungal products, are now known to be present in higher plants of the family Convolvulaceae [111,130,137,148]. Two groups of ergot alkaloids are distinguished by their structures. In the first group (the clavines) are simple derivatives of 6,8-dimethylergoline (14); in the second are amide and peptide derivatives of lysergic acid (15). Biosynthesis of these compounds is reviewed in [40,43].



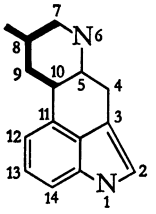
6,8-Dimethylergoline (14)



Lysergic Acid (15)

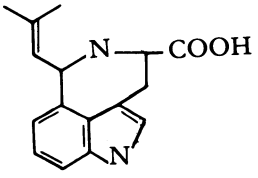
The common ring skeleton of all these alkaloids is derived from tryptophan and a C_5 hemiterpenoid unit. The C_5 unit is emphasized in the skeleton

structure below:



Ergoline Skeleton

The first reaction of the pathway is addition of a dimethylallyl group from dimethylallyl pyrophosphate to the 4-position of tryptophan [71]. Isopentenyl pyrophosphate can also serve as an alkyl donor in cell-free extracts, probably because isomerase is also present. N-6 is then methylated, one 4'-methyl group is hydroxylated, and the carboxyl group is lost to produce chanoclavine-I. This order of these three steps may not be obligatory [88,106,109]. Under anaerobic conditions hydroxylation cannot occur and dimethylallyltryptophan accumulates [116]. At high pH under aerobic conditions the normal alkaloid pathway is aborted, and clavicipitic acid is made [117,120]:



Clavicipitic Acid

The conversion of chanoclavine-I (20) to agroclavine (16) is not as simple as it appears to be (Fig. 10-6). An aldehyde intermediate and a *cis-trans* isomerization of the double bond intervene before the fourth ring is closed [41,45,97]. The E-hydroxymethyl group of chanoclavine-I ends up as C-7 of agroclavine [88]. It will be noted from Fig. 10-6 that after agroclavine there are branches in the pathway that leads to peptide derivatives of lysergic acid. There is also the possibility of an alternate pathway in which agroclavine is not an obligatory precursor of elymoclavine (17) [7,102, 104]. The enzyme that converts chanoclavine-I to agroclavine is found only in strains of ergot that produce alkaloids saprophytically, and it is induced by the presence of tryptophan. Strains that produce alkaloids only when living parasitically may, instead, make elymoclavine in a different way [37]. The oxidation of agroclavine to elymoclavine depends on a specific enzyme present in *Claviceps* spp. and a few other organisms [23,61]. The $\Delta^{9,10}$-8-hydroxyergoline derivatives, such as setoclavine (18) and penniclavine

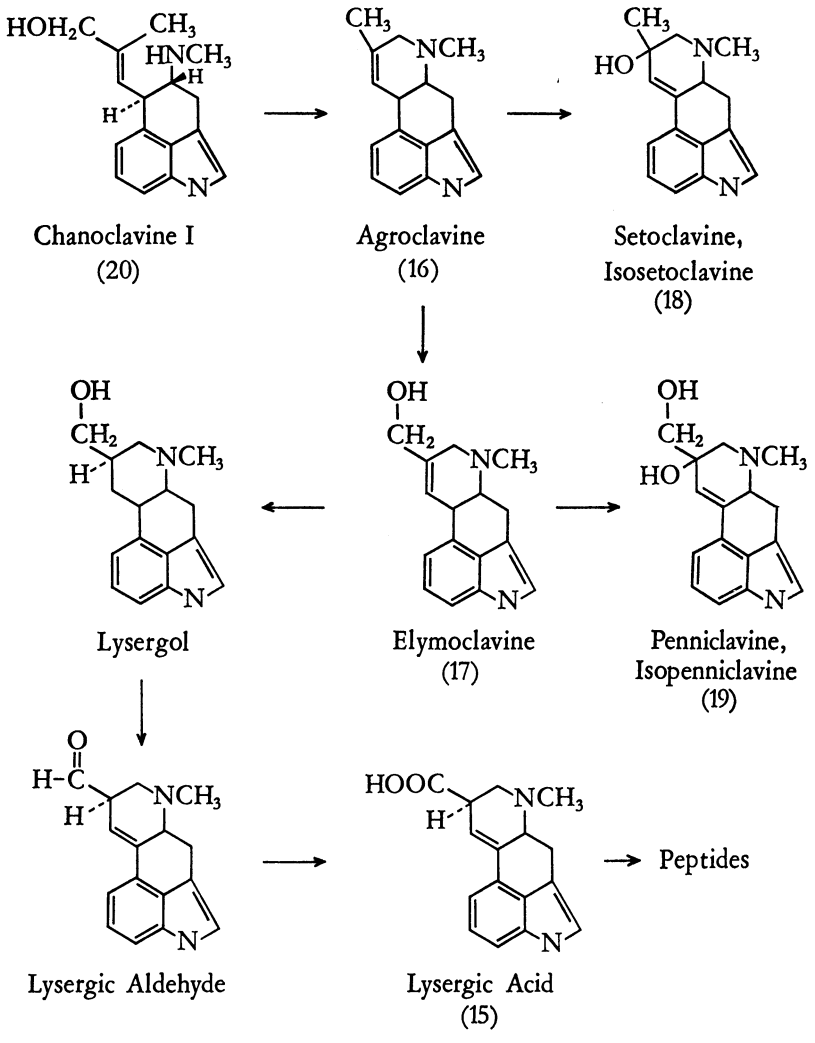


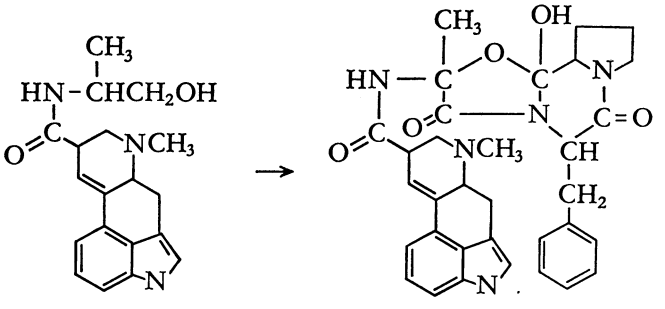
Fig. 10-6. Pathways of ergot alkaloid metabolism

(19), represent alternative pathways and do not serve as lysergic acid precursors [3,44]. The ability to hydroxylate at C-8 with concomitant shifting of the double bond has been found widespread not only among fungi [23] but also in higher plants [138]. This oxidation is catalyzed by a peroxidase and involves intermediate formation of 10-hydroxy derivatives [128,139]. Possibly some of the confusion about pathways of these compounds may result from insufficient attention to enzyme stereospecificity. In some steps, mixtures of the C-8 isomers are produced, but in other steps perhaps only one of these isomers is transformed [39].

In any case, it is usually found that both agroclavine (16) and elymoclavine

(17) are good precursors of lysergic acid derivatives [3,4,44]. It is not certain, though, whether lysergol lies on this pathway [103].

More than a dozen different peptides of lysergic acid (15) are known. Probably the most familiar of these is ergotamine (21), which contains residues of α-hydroxyalanine, L-phenylalanine, and L-proline. The others all have proline as the last amino acid residue; α-hydroxyvaline or α-hydroxyaminobutyric acid as the first; and phenylalanine, leucine, or valine in the middle. Ergometrine (ergonovine) (22) with a single peptide bond acted as a precursor of ergotamine when it was fed to ripening ergot sclerotia [151]. This same conversion could be brought about in a solution extracted from dried sclerotia [152]:



Ergometrine (22) Ergotamine (21)

However, it is now clear that ergometrine is not a normal precursor of the peptide alkaloids [49,92]. Some results suggested that the peptide chain was constructed as a whole starting at the proline end and then combined with lysergic acid [1,86]. It now seems that starting with lysergyl-CoA the amino acids are added one at a time in a multienzyme complex that retains all peptides of intermediate length [42,46,85,87]. The second and third amino acid residues in all the peptide alkaloids exist joined to each other as a reduced lactam (cyclol), and a lactam ring is probably formed before release from the multienzyme complex [85,136]. After release, the amino acid residue next to lysergic acid is hydroxylated on its α-carbon, and the five-membered ring then forms [13,24].

Alkaloid synthetic ability varies greatly from one strain of a *Claviceps* spp. to another [25,52,148]. A peculiar "marker" for alkaloid-producing cultures is the presence of ricinoleic acid [153]. Extensive work has been done to define the optimal nutritional requirements for alkaloid production in saprophytic cultures, but many contradictions remain to be explained [119]. Peptide alkaloid production is evidently more sensitive to culture conditions than is production of the clavine group. In some cultures addition of tryptophan increases the yield, but in others it has no effect [28,53,115]. Where tryptophan has a positive effect, it does so by acting as an inducer for a rate-limiting enzyme—not only as a substrate [28,115]. The rate-limiting

enzyme may be the one converting chanoclavine-I to agroclavine [37]. Tryptophan also binds to the P-450 oxygenase of *Claviceps purpurea* and could have an effect on the activity of this enzyme that catalyzes hydroxylation reactions at several steps in the pathway [5,6]. Alkaloid production is associated with rapid protein turnover, and tryptophan released from proteins may be used preferentially for alkaloid synthesis [38,63]. Addition of tyrosine causes increased synthesis of 4-dimethylallyl-tryptophan [82]. Increased phosphate inhibits alkaloid production [115]. 5-Fluorotryptophan inhibits alkaloid production even in the presence of enough tryptophan to allow normal growth rates [121]. *p*-Chlorophenylalanine can replace phenylalanine in peptide alkaloids that incorporate this amino acid [21]. Mixed cultures of *Claviceps* with rye callus produce a different pattern of alkaloids than is produced in the same *Claviceps* growing alone [22]. As a final aspect of regulation, it has been observed that the anthranilate synthetase of *Claviceps* is inhibited by certain clavine alkaloids. Since anthranilic acid is a precursor of tryptophan, this may be an example of a large feedback loop [122].

Although most work with the ergot alkaloids has been done using *Claviceps* spp., it should be mentioned that other quite different species of fungi appear to produce the same types of alkaloids by similar biosynthetic pathways. For examples, such reactions have been found in *Sphacelis sorghi* [11], *Aspergillus fumigatus* [113], and *Penicillium concavo-rugulosum* [105]. Roquefortine of *Penicillium roqueforti* is also derived from tryptophan and mevalonic acid but incorporates an additional residue from histidine [10].

Seed plants of the genera *Ipomoea* and *Rivea* (morning glories) contain both clavine and peptide alkaloids in their seeds. During the course of development of seeds from the fertilized ovule in *Ipomoea violacea*, it was found that the alkaloid concentration was highest at 20 days, and chanoclavine was then most abundant. Thereafter the concentration decreased and the ratio of lysergic acid amide to chanoclavine increased. Mature seeds contained two to three times as much lysergic acid amide as combined clavines [49]. These results further support the view that the clavines are precursors of lysergic acid derivatives. Although the leaves do not ordinarily contain alkaloids, it has been shown by using detached leaves and grafting techniques that they are the site of synthesis and that alkaloids are translocated from them to the seeds [51,94]. Elymoclavine provided to leaves is converted to penniclavine (19) and other unknown products [51]. In *Rivea corymbosa* plants it is surprising to find that the main sequence of reaction proceeds from elymoclavine to chanoclavine-I—just the reverse of the normal fungal pathway [107].

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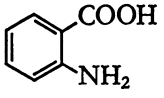
Chapter 11

Quinolines and Other Alkaloids Related to Anthranilic Acid

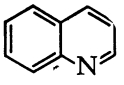
Excellent herbs had our fathers of old—
Excellent herbs to ease their pain—
Alexanders and Marigold,
Eyebright, Orris, and Elecampane—
Basil, Rocket, Valerian, Rue,
(Almost singing themselves they run)
Vervain, Dittany, Call-me-to-you—
Cowslip, Melilot, Rose of the Sun.

Our Fathers of Old, RUDYARD KIPLING[2]

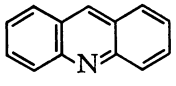
Several alkaloids are derived from anthranilic acid. These include some rather simple substituted anthranilic acids, quinolines, and some with condensed ring systems of various types. Some of the parent nuclei of these alkaloids are as follows:



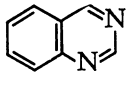
Anthranilic Acid



Quinoline



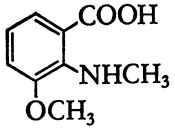
Acridine



Quinazoline

The place of anthranilic acid in intermediary metabolism seems well-established, since anthranilic acid is a necessary intermediate in the biosynthesis of tryptophan from shikimic acid. Anthranilic acid is also found esterified with certain diterpenoid alkaloids (Chap. 13), and its methyl ester is a constituent of some essential oils. Its role as an alkaloid precursor is reviewed in [10].

Damascenine, isolated from *Nigella* spp., has the structure:

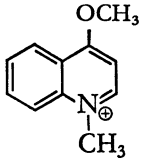


Damascenine

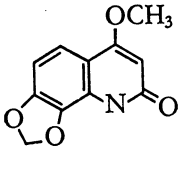
[2]Reproduced by kind permission of Doubleday & Co. Inc., New York, and A.P. Watt & Son, London.

It is therefore a methylated derivative of 3-hydroxyanthranilic acid. Since 3-hydroxyanthranilic acid is a degradation product of tryptophan in animal metabolism, the first experiments on damascenine biosynthesis were done to test the possibility of tryptophan as a precursor. When tryptophan labeled at C-7 or C-3 was fed to *Nigella damascena* plants, it contributed no radioactivity to isolated damascenine [21,22]. This result was one of the first indications that higher plants do not follow the animal pathway of tryptophan degradation. Later tracer studies on damascenine have shown it to be formed from shikimic acid or anthranilic acid, with the methyl groups derived from methionine [27]. It has also been shown that plants have an enzyme that catalyzes oxidation of anthranilic acid to 3-hydroxyanthranilic acid [28]. Other studies on damascenine formation in entire plants as well as graft combinations and root cultures have shown seeds to be the primary site of synthesis and storage, with none being synthesized or stored in the roots. Damascenine fed to leaves or young, developing seeds was rapidly converted to unknown products [34].

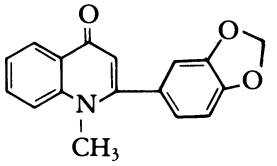
Several simple derivatives of quinoline are found in the family Rutaceae as well as in the Compositae genus *Echinops*. Quinoline itself has been reported to occur in several plants. Some examples of these simple quinoline alkaloids are shown below:



Echinorine (1)

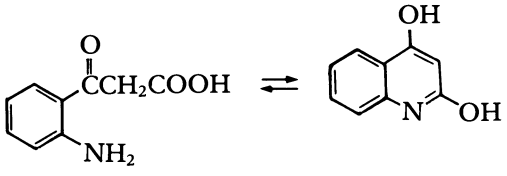


Casimiroine (2)



Graveoline (3)

It is assumed that such compounds are made by condensation of anthranilic acid with another molecule to give the quinoline ring [10]. In the case of echinorine (1) or casimiroine (2), a C_2 unit such as acetate would be involved. The synthetic compound 2-aminobenzoylacetic acid exists in a pH-dependent equilibrium with 2,4-dihydroxyquinoline:



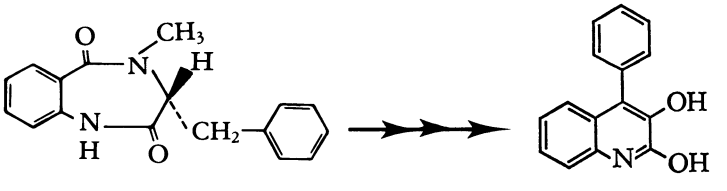
Therefore all that is needed to produce the quinoline ring is a biosynthetic condensation of anthranilic acid and acetic acid, presumably with one or both as coenzyme-A derivatives [5]. For graveoline (3) a condensation between anthranilic acid and a derivative of cinnamic acid could be the starting point, and such a pathway has been indicated by tracer experiments with *Ruta angustifolia* [2]. Tissue cultures of *Echinops ritro* produce echinorine

and related alkaloids. It has been reported that the yield of alkaloids is increased if the growth medium is supplemented with tryptophan, phenylalanine, and methionine; but there are no tracer experiments to establish an actual precursor-product relationship [20].

Once the quinoline ring has been produced by the kinds of condensation suggested above, further reactions lead to more complex alkaloids. Tracer experiments with such plants as *Skimmia japonica, Ruta graveolens*, and *Dictamnus albus* have been used to establish the pathways discussed below.

It is a general rule in the biosynthesis of secondary plant constituents that furan rings are derived from isoprenoid-substituted compounds [1], and this is clearly the case for the furoquinoline alkaloids. Tracer experiments have confirmed that their quinoline portions derive from anthranilic and acetic acids [25,26,30]. After 2,4-dihydroxyquinoline has been formed, a dimethylallyl group is added at C-3 and then modified further to derivatives that include the furoquinolines [3,5–8,13–15]. It is possible that the dimethylallyl group is first added to the C-4 oxygen and later migrates to C-3 [4]. If N-methylation of a quinoline intermediate occurs, closure of the furan ring is prevented, and quinolone alkaloids like edulinine are the result [3,33]. In some alkaloids, closure to a pyran ring rather than a furan ring occurs [15], and in others a second dimethylallyl group is attached to the benzene ring directly or as an ether [13,30]. Some of these pathways are outlined in Fig. 11-1.

The quinoline derivatives produced by several species of the fungus *Penicillium* are derived from one molecule of anthranilic acid, one molecule of methionine, and one molecule of phenylalanine that react first to form the cyclic peptide cyclopeptine. A complex series of oxidative reactions and rearrangements converts cyclopeptine to viridicatine [23]:



Cyclopeptine Viridicatine

All the reactions of this sequence are enzymatically catalyzed. Several of the enzymes have been well-characterized; and they are controlled in a coordinated manner, increasing during hyphal development. The study of regulatory aspects of this system is the most detailed in the area of alkaloid biochemistry, and it offers examples of three or four different types of control mechanisms [24,35].

The acridine alkaloids are most prominent in the family Rutaceae. All but one are derivatives of acridone rather than of acridine. A peculiarity of this group is that some of them occur as glucosides—a rare situation among the

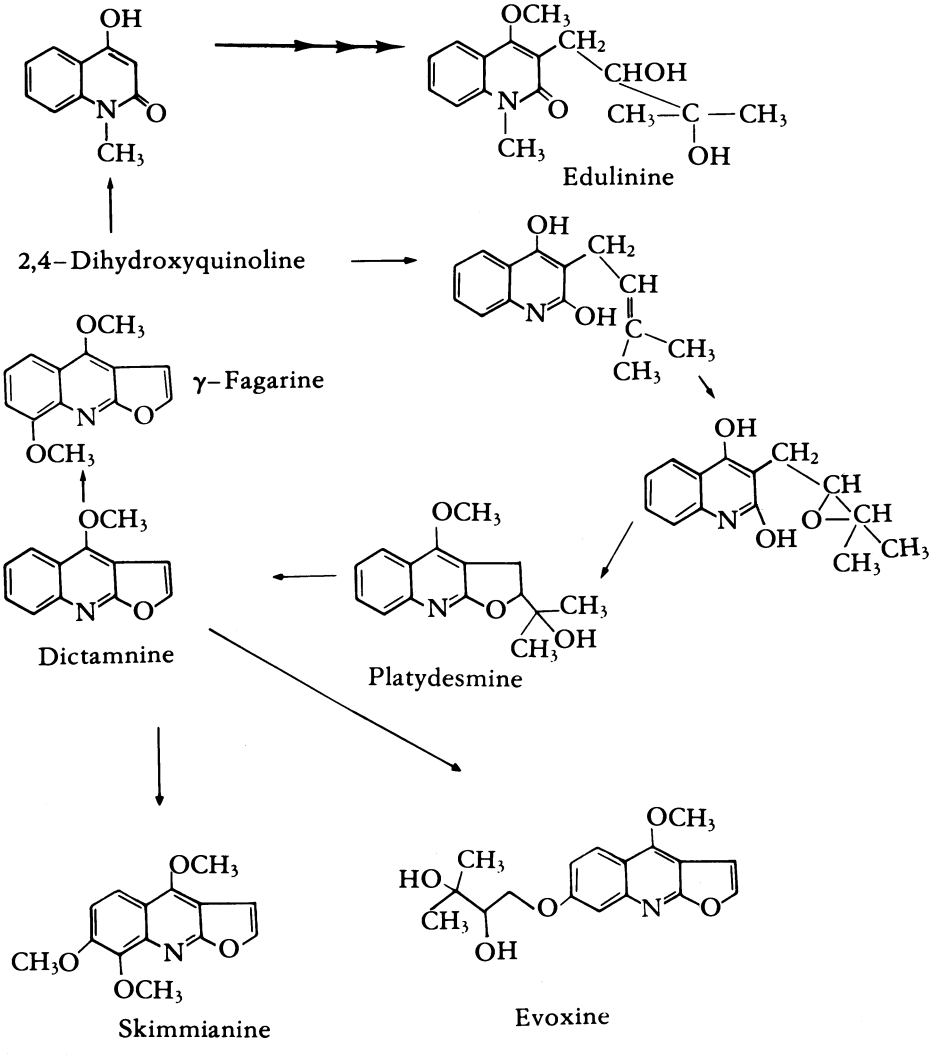
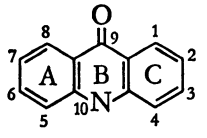


Fig. 11-1. Alkaloids derived from anthranilic acid

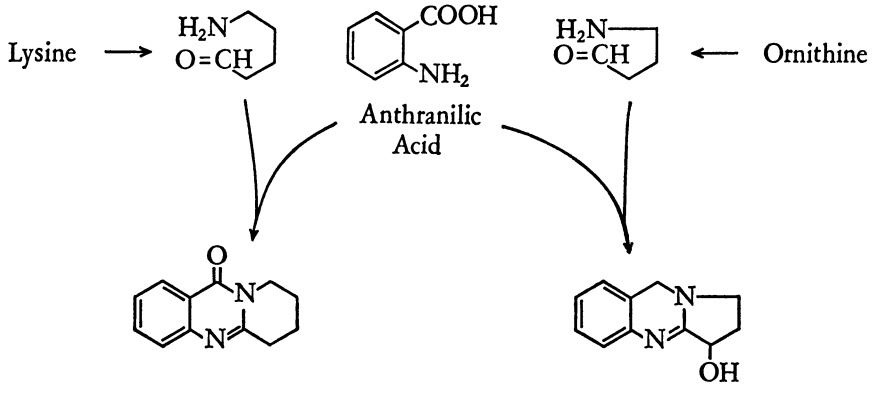
alkaloids [32]. Another peculiarity is that they do not have the basic properties of most other alkaloids and therefore may be missed in common extraction procedures.



Acridone

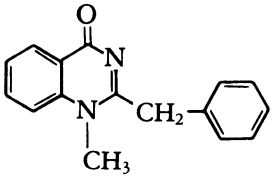
A combination of acetate units with anthranilic acid leads to these alkaloids, and the early steps are probably the same as for the quinoline alkaloids discussed above [16,31]; but two more acetate groups are added at the 2-aminobenzoylacetic acid stage, and ring closure to the acridone then follows. Such a mechanism would be expected to introduce oxygen substitution at C-1 and C-3; and this is usually found in the alkaloids, although additional oxygenation is not unusual.

Alkaloids with the quinazoline (more often quinazolone) nucleus are distributed among several plant families, and also occur in the bacterial genus *Pseudomonas*. About 30 of them are known [18]. They are believed to be derived from anthranilic acid plus units derived from other amino acids. Thus combination with an ornithine derivative would give vasicine-type alkaloids, and combination with a lysine derivative would give the homologous *Mackinlaya* alkaloids [9]:

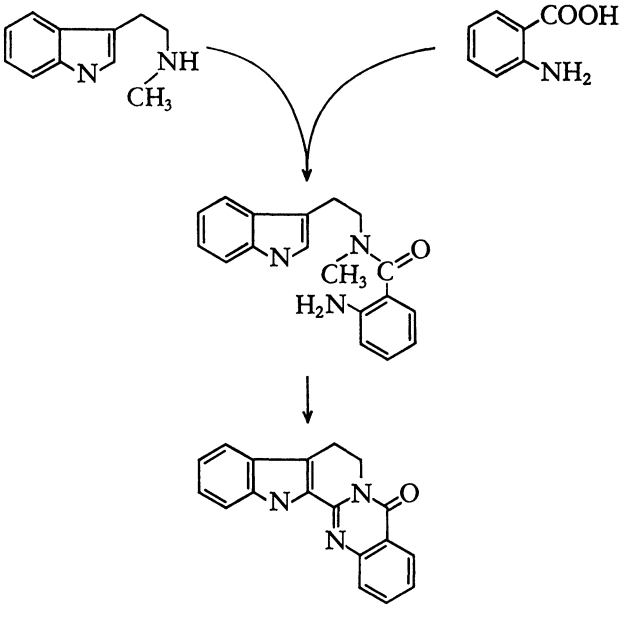


Vasicine (Peganine)

For vasicine the concept of such a route has been supported by feeding anthranilic acid—[^{14}COOH] to rooted leaves of *Adhota vasica* and finding that the vasicine synthesized had all its radioactivity at the predicted atom [12]. Although ornithine looks like a reasonable precursor for the pyrrole ring of vasicine, and is incorporated to some extent [23], other data suggest that its role is indirect and that the pyrrole ring is constructed of an acetate unit plus the middle two carbons and the nitrogen of aspartic acid [17,19,36]. The quinazoline alkaloids of *Glycosomis arborea* have a benzyl group attached to a quinazolone structure; and they are derived from a combination of anthranilic acid with phenylalanine [11,29], e.g., arborine:



Rutaecarpine and other alkaloids of the Rutaceae are characterized by a carbon skeleton that appears derivable from anthranilic acid plus N-methyltryptamine in the following way:



Rutaecarpine

This group of alkaloids, therefore, could be placed as well with the indoles. Experiments with feeding labeled precursors to unripe fruits of *Evodia rutaecarpa* have confirmed that rutaecarpine originates from tryptophan plus anthranilic acid. The additional C_1 unit is derived formate or methionine [37].

Several other alkaloids having a quinoline structure are considered to be derived by rearrangement of an indole rather than from anthranilic acid. They are therefore placed in Chap. 10.

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Chapter 12

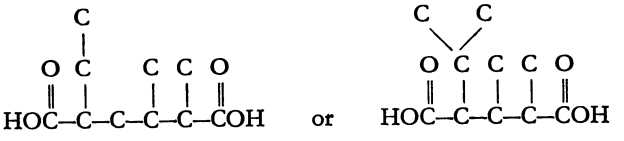
Some Miscellaneous Alkaloids

Ei! wie schmeckt der Coffee süsse
Lieblicher als tausend Küsse,
Milder als Muskatenwein.
Coffee, Coffee muss ich haben;
Und wenn jemand mich will laben,
Ach, so schenkt mir Coffee ein!

Coffee Cantata, C.F. HENRICI

Pyrrolizidine Alkaloids

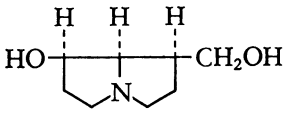
Pyrrolizidine alkaloids are based on diols called necines which are esterified at one or both hydroxyl groups with a number of different acids, mostly dicarboxylic acids known as necic acids. Some examples of necines and necic acids are shown in Fig. 12-1. Structural variation among the necines is slight. Only about a dozen are known, and they differ from one another mostly in stereochemistry or degree of hydrogenation. Most of the necic acids have 10 carbon atoms arranged as follows:



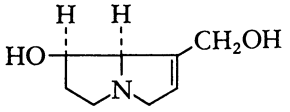
The acids of *Crotolaria* spp. differ in having only six or eight carbon atoms, and a few other exceptions are known [10].

Because of their first discovery and extensive occurrence in the genus *Senecio* (family Compositae), the pyrrolizidine alkaloids are often called *Senecio* alkaloids. However, they have also been found in other genera of the Compositae as well as in several genera of the Boraginaceae and the genus *Crotolaria* of the Leguminosae [10].

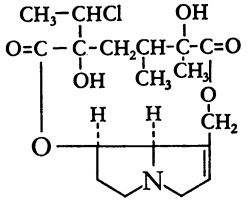
Since the pyrrolizidine nucleus consists of two fused pyrrolidine nuclei, similarities in biosynthesis between the pyrrolizidine and pyrrolidine alkaloids might be expected. From results of feeding ornithine-2-[^{14}C] to excised stems of *Crotolaria spectabilis*, it was concluded that the pyrrolizidine



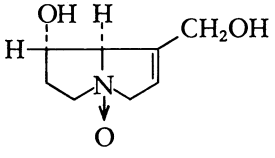
Platynecine



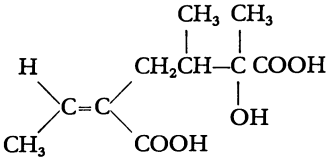
Retronecine



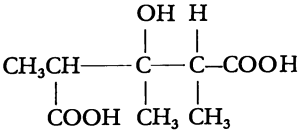
Jaconine



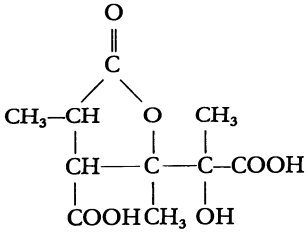
Heliotridine N-Oxide



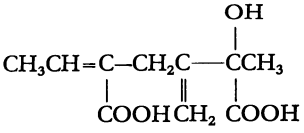
Senecic Acid



Monocrotalic Acid



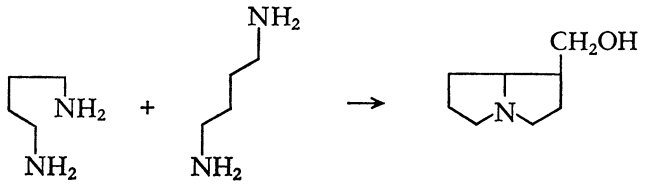
Grantianic Acid



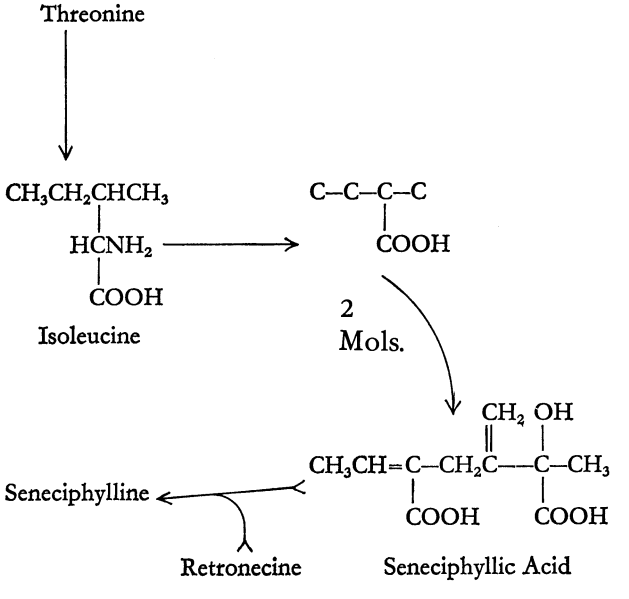
Seneciphyllic Acid

Fig. 12-1. Some necines and necic acids occurring esterified with each other in the pyrrolizidine alkaloids

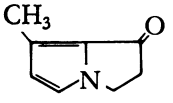
nucleus of monocrotaline is probably derived from two molecules of ornithine [40]. Similar experiments with *Senecio* spp. also implicated ornithine as a precursor of retronecine, with a symmetrical intermediate like putrescine probably involved [4,7,45]. A biosynthesis analogous to the formation of quinolizidine alkaloids from cadaverine seems likely. It could be formulated as follows:



No details as to possible intermediates are available. The N-oxides occasionally found among pyrrolizidine alkaloids are presumably made by a direct enzymatic oxidation of the parent compound as other naturally occurring N-oxides are [15,42]. Proposals and some experimental evidence bearing on the biosynthesis of the necic acids are also available. Although certain of the acid structures resemble terpenoids, feeding experiments have shown that mevalonate is not a precursor, while acetate is a fairly good one [26,40]. On the other hand, both acetate and mevalonate were found to be poor precursors of seneciphyllic acid in *S. douglasii*, while threonine and isoleucine were good precursors [16]. Evidently, two C_5 units derived from L-isoleucine condense to form a C_{10} intermediate that is further modified to the necic acids [12,17]. Threonine is active because it is a precursor of isoleucine [14], and a likely intermediate between isoleucine and the C_{10} compounds is 2-amino-3-methylenepentanoic acid [3]. The alkaloid strigosine is esterified with 2,3-dihydroxy-3-methylpentanoic acid, a compound that is actually on the pathway from threonine to isoleucine [18]. The above results are summarized below, but there are also other possibilities for different types of esterifying acids [16,35].

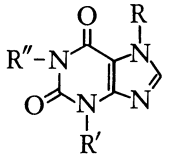


An interesting sidelight on the biochemistry of pyrrolizidine alkaloids is the observation that certain butterflies and moths feed on plants containing the alkaloids. Some of these insects store the alkaloids unchanged, but others metabolize them further and use the products as pheromones [2,20,21]. One of these pheromones, for example, has the structure [37]:



Purine Alkaloids

Purine derivatives are, of course, ubiquitous in plants combined as nucleic acids or nucleotides. The free purines, adenine and guanine, have also been reported to occur in plants, but they are not usually classed as alkaloids and do not usually accumulate to the high concentrations characteristic of alkaloids. Thus the class of purine alkaloids is usually restricted to the methylated derivatives of xanthine and uric acid (Fig. 12-2). Caffeine,



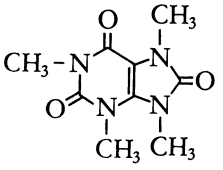
R=R′=R″=H: Xanthine

R=CH_3, R′=R″=H: Heteroxanthine

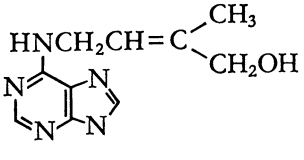
R=H, R′=R″=CH_3: Theophylline

R=R′=CH_3, R″=H: Theobromine

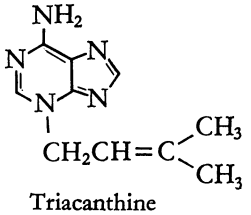
R=R′=R″=CH_3: Caffeine



Tetramethyluric Acid



Zeatin



Triacanthine

Fig. 12-2. Some purine alkaloids

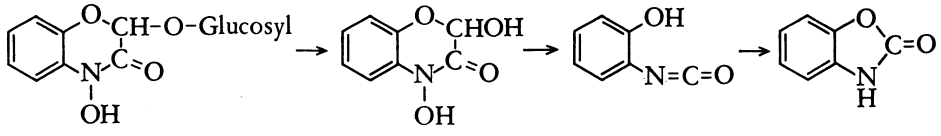
perhaps the best known of all alkaloids, falls into this group and has as objective support for its familiarity the fact that of all the alkaloids, it occurs in the largest number of plant families [58]. The other methylated xanthines are considerably more restricted in occurrence. Another purine that might be classed as an alkaloid is triacanthine, found in leaves of *Gleditsia triacanthos* [32]. The functioning of similar adenine derivatives as cytokinins makes this compound particularly interesting. In fact, zeatin, a cytokinin isolated from corn (*Zea mays*), has a structure very similar to that of triacanthine [33], and related structures have been isolated from a hydrolysate of soluble RNA [11,25].

It has been demonstrated that in a broad sense the purine alkaloids have the same precursors as purines of nucleic acids. The tracer feeding work has been applied to caffeine of *Coffea arabica* and *Camellia sinensis* (coffee and tea). Tissue cultures as well as intact plants of these species are active in synthesis of the alkaloid [22,30,41]. The ring carbons originate from glycine plus C_1 units, and the methyl groups from methionine [1]. Alkylated purines occur as such in certain ribonucleic acids [13]. Hydrolysis and oxidation could then give rise to methylated xanthines without any methylation of free xanthines occurring. It is more likely, however, that caffeine is derived from a pool of free nucleotides than from nucleic acids [52]. With methionine-methyl-[^{14}C] as a source of methyl groups, label is acquired more rapidly by caffeine than by methyl groups of tRNA [52–54]. Between the nucleotide pool and methylated xanthines a likely intermediate is 7-methylxanthosine. This can be hydrolyzed to 7-methylxanthine (heteroxanthine) and the latter methylated further to theobromine and caffeine [5,44]. Cell-free extracts are able to catalyze the latter two methylations using S-adenosylmethionine as a substrate [44,51]. Theophylline must arise from a branched pathway starting from 1-methylxanthosine rather than 7-methylxanthosine [51,52]. A methyl transferase from tea seedlings is known to add a methyl group from S-adenosylmethionine to the 1-position of an adenine residue of tRNA [53]. This suggests that theophylline might be derived from RNA degradation even though the other methylated purines are not. In line with recent findings concerning other alkaloids is the observation that caffeine in *C. arabica* has a short half-life and is therefore an active metabolite rather than an inert end product [28,50]. In tea callus tissue, however, its rate of turnover is very slow [41]. The breakdown products of methylated purines include trimethyluric acid, allantoin, allantoic acid, and urea [6,57]. Some of the enzymes of this degradative pathway have been studied in vitro [39,55].

Benzoxazoles

Benzoxazolinone and 6-methoxybenzoxazolinone have been isolated from crushed plants belonging to several species of the Gramineae and are of interest because of their fungistatic activity. They are actually secondary

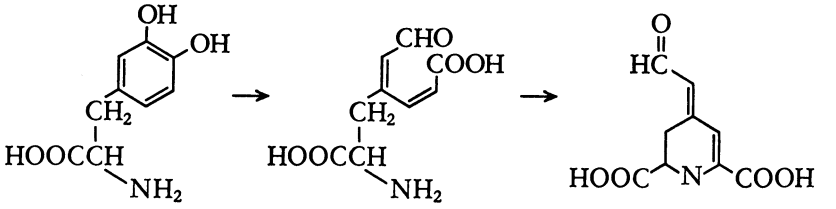
products formed by enzymatic hydrolysis and rearrangement of naturally occurring glucosides according to the following scheme [8,29,56]:



Neither the benzoxazolinones nor their precursors are typical alkaloids, but from consideration of structure there is a possible relationship to anthranilic acid and 3-hydroxyanthranilic acid, which are precursors of other alkaloids (see Chap. 11). Tracer feeding experiments have shown the origin of the C_2 unit to be C-1 and C-2 of ribose, and it has been proposed that condensation of ribose with an aromatic amine leads to their formation [43].

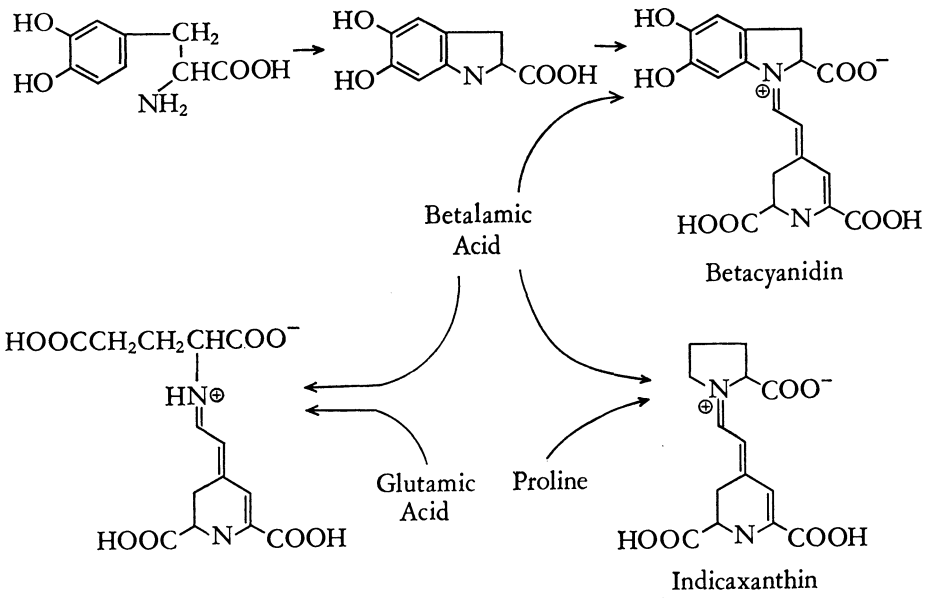
Betalains

The red pigments of beet roots have been known to be nitrogen-containing compounds for many years, but only comparatively recently have their structures been elucidated and experiments carried out to clarify their place in metabolism [31,34]. Yellow pigments of a similar nature have been found to occur along with the red betacyanins; they have been named betaxanthins [31,34]. The betacyanins are glucosides, and their aglycones are known as betacyanidins. Betacyanins all contain an indole unit condensed with betalamic acid. The betaxanthins show more diversity, having various other types of units condensed with the betalamic acid. On the basis of tracer feeding experiments with *Opuntia ficus-indica* and partial degradations of the products it has been concluded that dihydroxyphenylalanine (DOPA) is the precursor of betalamic acid according to a scheme like the following [14,38]:



DOPA Betalamic Acid

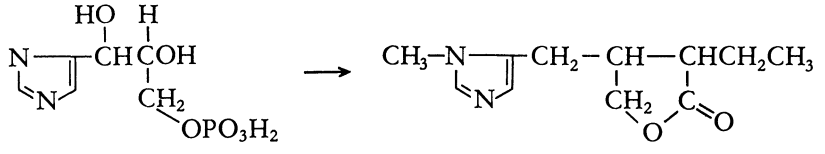
Dihydroxyphenylalanine is also believed to serve as the precursor of the indole unit of betacyanins while other amino acids serve as precursors for the betaxanthins [49,59].



Other feeding experiments have shown tyrosine to be better than DOPA as a precursor of betanidin. Feeding tyrosine doubled the content of amaranthin (a glycoside of betanidine) in *Amaranthus* when it was supplied to 4-day-old seedlings but had no influence on 8-day-old seedlings [23].

Imidazole Alkaloids

Various alkaloids having an imidazole ring are presumably made from the amino acid histidine. The best known of these is pilocarpine, found in various *Pilocarpus* spp. The pathway of its formation has not been studied, and it is not obvious how it might arise from histidine. It might rather come from imidazoleglycerophosphate, a precursor of histidine.

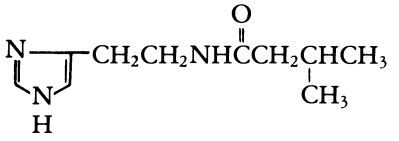


Imidazoleglycerophosphate Pilocarpine

Tracer feeding experiments have found no significant incorporation of histidine, threonine, or acetate into pilocarpine, but methyl groups from methionine went to the methyl group of the alkaloid [9].

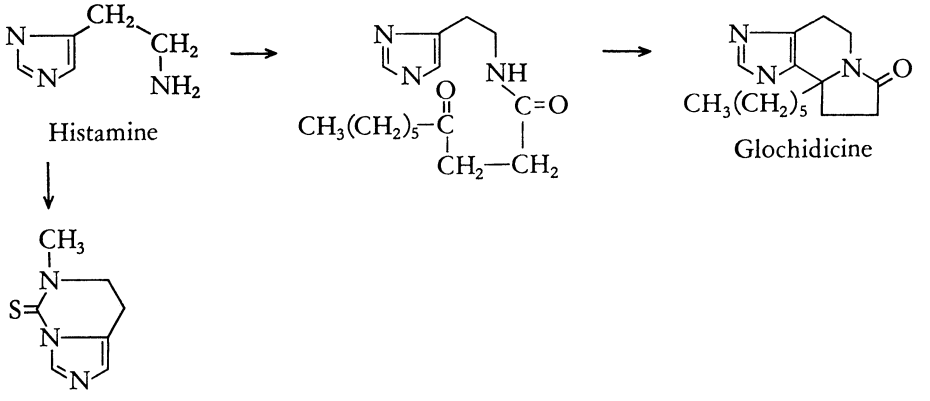
There are experiments showing that histidine and histamine are precursors of dolichotheline in *Dolichothele sphaerica*. The remainder of the alkaloid molecule comes from isovaleric acid, which may itself be derived from

various precursors [24,46]. Experiments have also shown that if substituted analogues of histamine and/or acids other than isovaleric are supplied to the plant, several aberrant alkaloids are produced. If the availability of endogenous histamine was also limited by feeding inhibitors of histidine decarboxylase, the amounts of aberrant products were still further increased [47,48].



Dolichotheline

The imidazole alkaloids of *Glochidion philippicum* seem derived from N-acylation of histamine with a C_{10} keto acid [27]. Zapotidine, the unusual sulfur-containing alkaloid of *Casimiroa edulis*, could be formed by thiomethylation, methylation, and ring closure of histamine [36].



Histamine

Glochidicine

Zapotidine

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Chapter 13

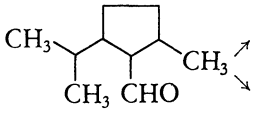
Terpenoid and Steroid Alkaloids

No, no! go not to Lethe, neither twist
Wolf's-bane, tight-rooted, for its poisonous wine;
Nor suffer thy pale forehead to be kiss'd
By nightshade, ruby grape of Proserpine;

Ode on Melancholy, JOHN KEATS

The diverse group of natural products classed as terpenoids and steroids includes some compounds that contain nitrogen and are called alkaloids on that account. Biosynthetically they can be set apart from all other alkaloids because their carbon skeletons are derived from mevalonic acid, while most other alkaloids have their skeletons constructed largely of amino acid residues. This origin from a nonnitrogenous precursor raises a question as to the source of the nitrogen and the stage at which it enters the molecule. For the most part, knowledge about the biosynthesis of these alkaloids is limited to the knowledge that they are indeed derived from mevalonic acid. In other cases even that conclusion is only an inference based on examination of structure. Certain alkaloids, otherwise nonterpenoid, that contain a terpenoid unit are also known and are treated in Chap. 10. Structures of some terpenoid alkaloids are given in Fig. 13-1.

The simplest alkaloid with an apparently terpenoid structure is β-methylpyrroline (1), found in *Piper nigrum*. It could be classed as a hemiterpenoid but might also be derived from 4-methylproline, which is now known to occur naturally [12]. Tracer experiments have shown that skytanthine (2) in *Skytanthus acutus* and actinidine (3) in *Actinidia polygama* are, in fact, made via the terpenoid pathway from mevalonic acid [2,3]. Origin of them from iridial or other iridoids appears plausible [33].



Iriodial

Skytanthine (2)

Actinidine (3)

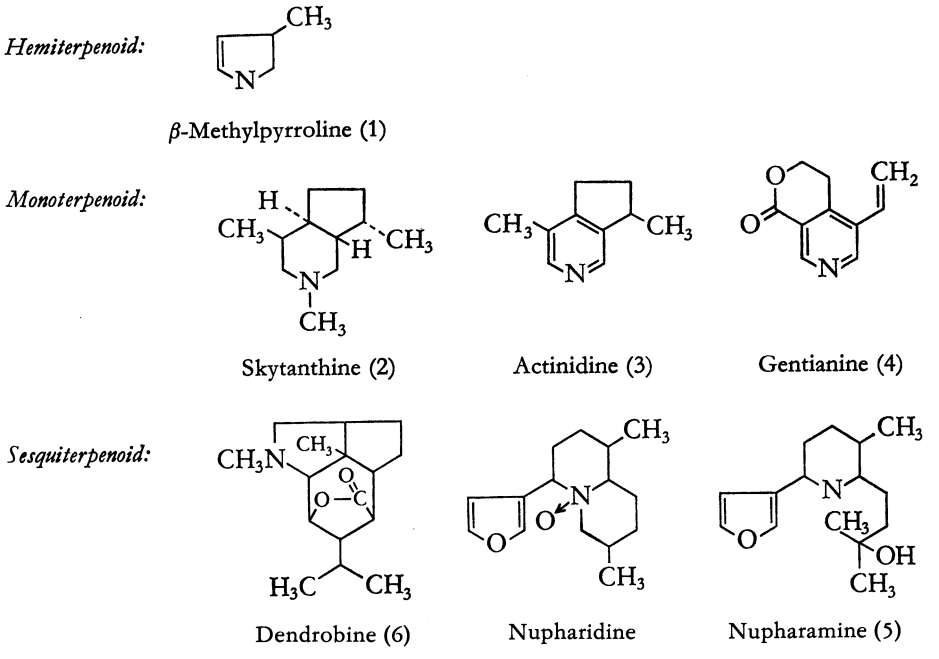


Fig. 13-1. Structures of some terpenoid alkaloids

Gentianine (4) has an irregular arrangement of isoprene units and would require some kind of rearrangement in its formation [41].

Among the sesquiterpenoid alkaloids, the biosynthesis of dendrobine (6) has been studied by feeding mevalonate-2-[^{14}C] to *Dendrobium nobile.* Partial degradations indicated that the alkaloid was made by incorporation of nitrogen into a sesquiterpenoid derived from three molecules of mevalonate [66]. The sulfur-containing alkaloid thiobinupharidine, which occurs along with nupharamine (5) in *Nuphar luteum*, has a structure that appears to be related to that of nupharamine, and tracer feeding experiments have established its specific origin from mevalonic acid [52]. The source of the sulfur atom is unknown, but there are some speculations on a biogenetic scheme for this type of structure [65].

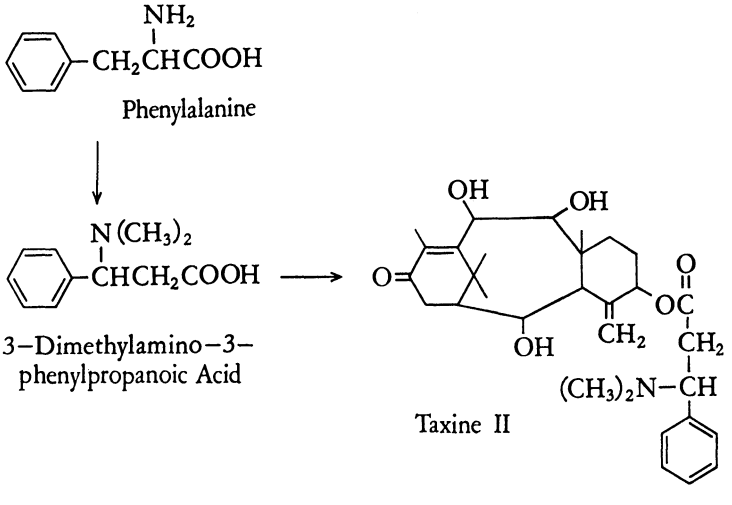
Diterpenoids and Triterpenoids

Diterpenoid alkaloids are conspicuous in the Ranunculaceae genera *Aconitum*, *Consolida*, and *Delphinium* as well as the Cornaceae genus *Garrya* [40]. The diterpenoid alkaloids are subdivided into several groups according to carbon skeleton. The *Garrya* alkaloids are C_{20} compounds with a reduced phenanthrene skeleton. The *Aconitum* and *Delphinium* alkaloids are C_{19}, some with a similar phenanthrene carbon skeleton and some with a modified skeleton having fused cyclohexane, cycloheptane, and cyclopentane rings. The *Aconitum* and *Delphinium* alkaloids also have more oxygen

substitution than the *Garrya* alkaloids and are noted pharmacologically for their much greater toxicity [34]. Among the aconite alkaloids, esterification of hydroxyl groups with anthranilic acid or related compounds is often seen. Figure 13-2 is a hypothetical scheme for formation of some of the *Garrya* alkaloids from the terpenoid alcohol nerolidol according to suggestions of several authors [59,63,64]. Atisine (7), an *Aconitum* alkaloid, also fits appropriately into this scheme. The source of the nitrogen atom and its attached C_2 unit could be ethanolamine derived by decarboxylation of serine. The possible formation of aconitine (8) and related alkaloids from atisine is also suggested in Fig. 13-2 [59]. This whole group of alkaloids is characterized by having an α-oriented substituent methyl group at the A/B ring juncture. This is in contrast to the more usual β-orientation found in abietic acid and suggests a biosynthetic relationship to phyllocladene [10].

Tracer feeding experiments with mevalonic acid-2-[^{14}C] have indicated derivation of brownine and lycoctonine (9) in *Delphinium brownii* by the expected pathway, although incorporation was low [4]. Other feeding experiments, with detached leaves of *D. elatum*, failed to show any incorporation of mevalonic acid-2-[^{14}C] into delpheline (10) [21]. This might be explained by postulating that the roots are the normal site of synthesis [4]. The O- and N-methyl groups of delpheline could be derived from methionine-methyl-[^{14}C] in the leaf-feeding experiment, showing that the leaf is capable of performing the transmethylation reactions although it does not carry out the complete synthesis [21]. Injection of glycine-2-[^{14}C] and mevalonate-2-[^{14}C] into *D. ajacis* has been found to give rise to labeled diterpenoid alkaloids, but degradations have not yet established the location of [^{14}C] in any particular alkaloid [13].

The taxine group of alkaloids from yew (*Taxus* spp.) are esters of various diterpenoids with L-3-dimethylamino-3-phenylpropanoic acid [31]. The



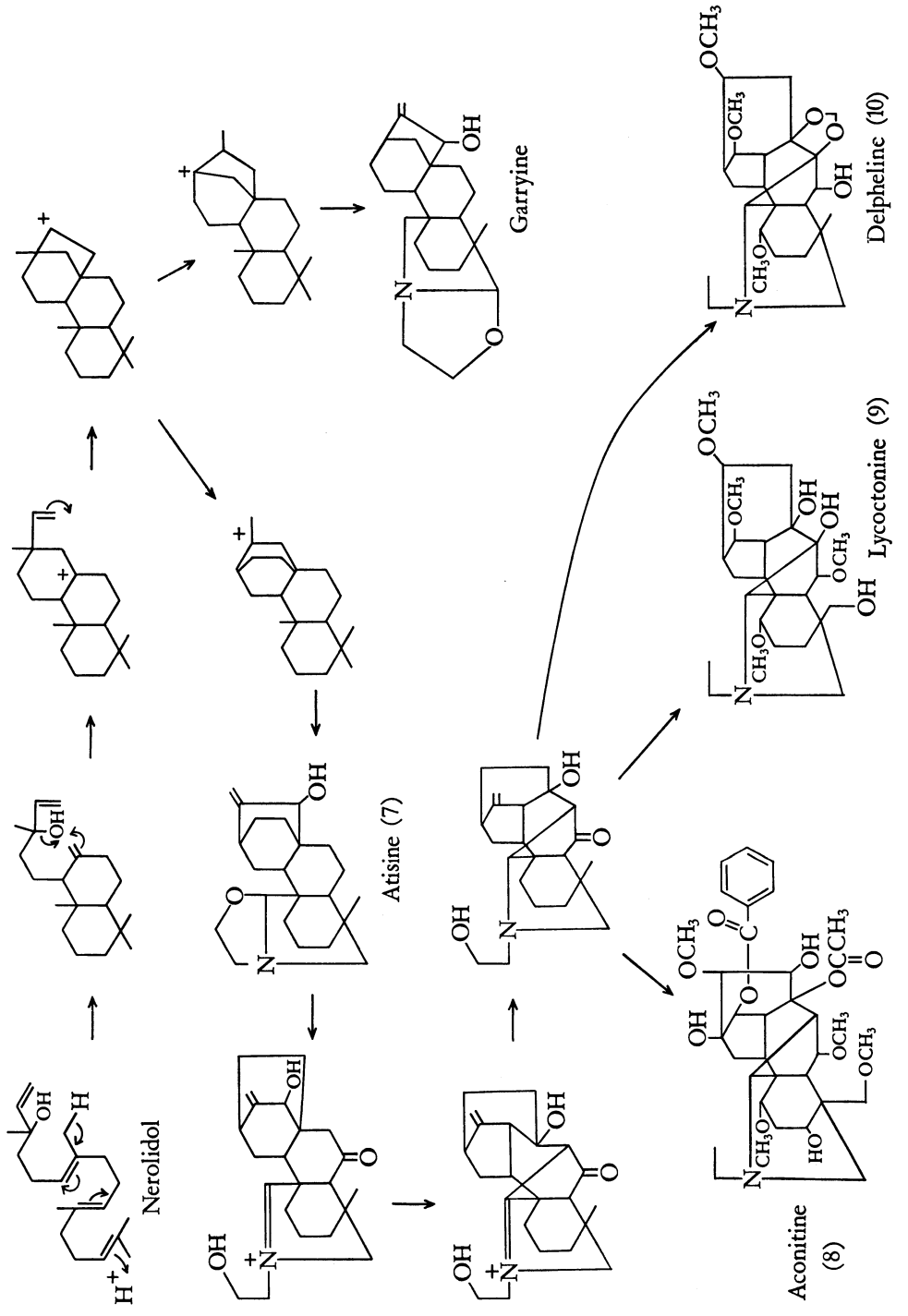


Fig. 13-2. Diterpenoid alkaloid pathways

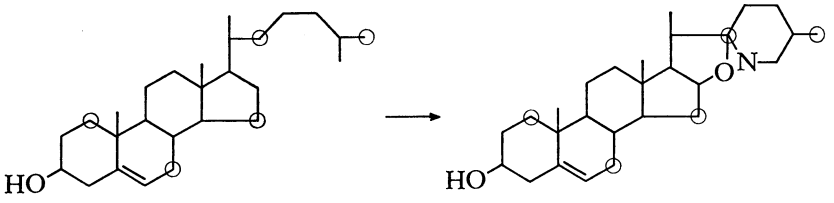
nitrogen atom is thus not directly attached to the terpenoid carbon skeleton, and these compounds could reasonably be classified with the simple amino acid derivatives of Chap. 3. Tracer studies have indicated that the phenylpropane moiety originates from phenylalanine, with an evident α,β-migration of the amino group [31]. Two of the *Delphinium* alkaloids are inhibitors of pea internode growth; and it has been suggested that because of their similarity in structure to the gibberellins, they may act on the regulatory system for synthesis of these hormones [61].

Triterpenoid alkaloids are rare. Two of them are found in *Daphniphyllum teijsmanni*, and tracer experiments have shown their origin from a squalene-like intermediate [38].

Steroids

Steroid and modified steroid alkaloids are divided into three or four groups according to plant source. The carbon skeletons are also characteristic of each plant group. The *Solanum* alkaloids present in *S. tuberosum* and a few related species of the Solanaceae have the C_{27} carbon skeleton of cholesterol changed only by closure of additional rings [45,51]. Modified steroid alkaloids of the genus *Veratrum* and other Liliaceae genera have the same number of carbon atoms in their skeletons as cholesterol, but modification of rings C and D has occurred as well as additional ring closures [37]. Kurchi alkaloids are found in plants of the Apocynaceae genera *Holarrhena* and *Funtumia* and have a C_{21} nucleus similar to pregnane [15]. *Buxus* alkaloids also have a C_{21} pregnane-type nucleus but characteristically have a cyclopropane ring bridging C-9 and C-10, and usually have two methyl groups at C-4 [36]. These various skeletons and their subgroups are illustrated in Fig. 13-3. Many of them exist naturally as esters or glycosides. Some members of both the *Buxus* and *Funtumia* groups have second nitrogen atoms at C-20.

Studies on the biosynthesis of the *Solanum* alkaloids have confirmed their relationship to other steroids derived via the normal mevalonate pathway. Labeled acetate or mevalonate fed to *S. aviculare* or *S. tuberosum* were incorporated into solasodine (11), the acetate in C_2 units following the pattern found for cholesterol, and mevalonate-2-[^{14}C] giving the expected labeling pattern, although complete degradations were not carried out [16,17]. Cultured tomato roots also have the ability to synthesize tomatine from mevalonate [46]. The presumed transformation of cholesterol to the alkaloid is represented below, with carbon atoms presumably derived from C-2 of mevalonic acid *circled*:



Cholesterol Solasodine (11)

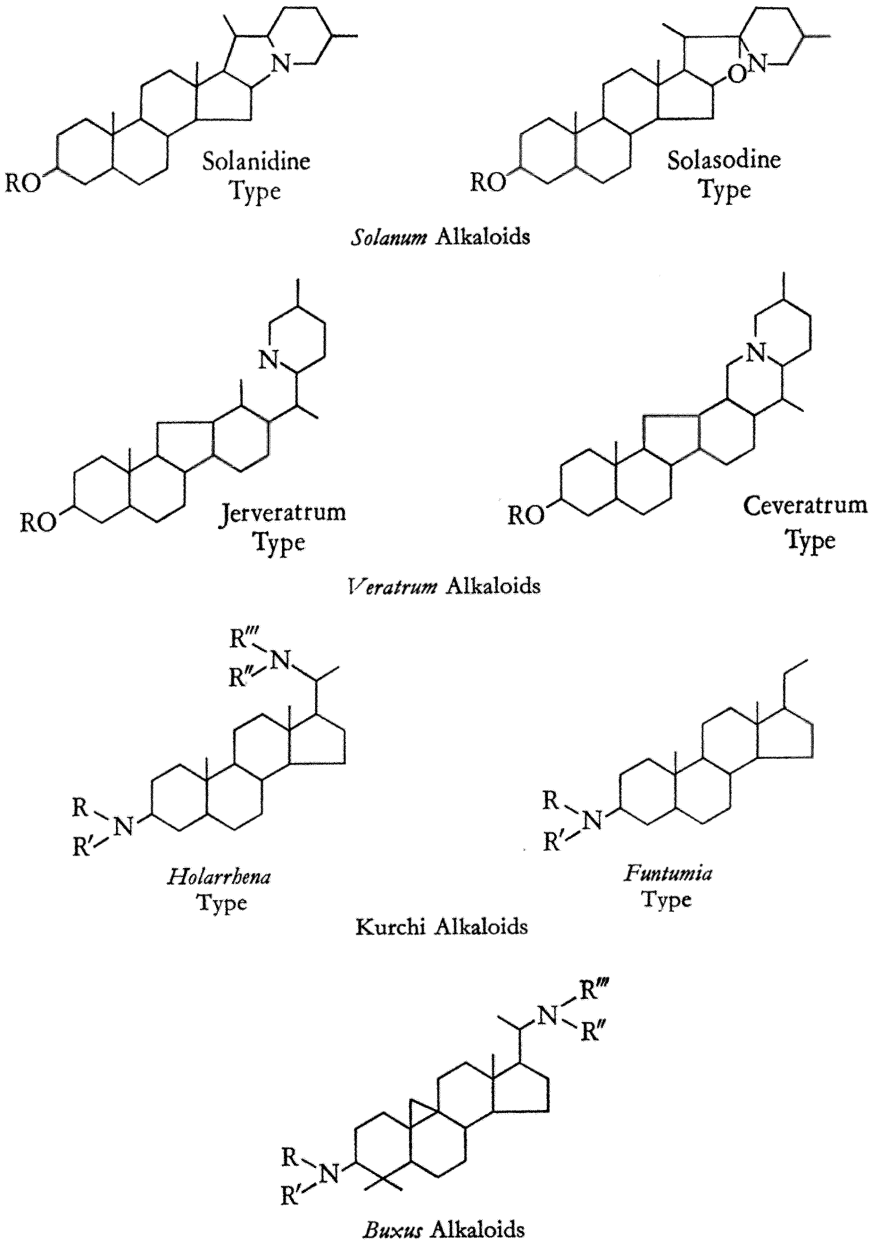
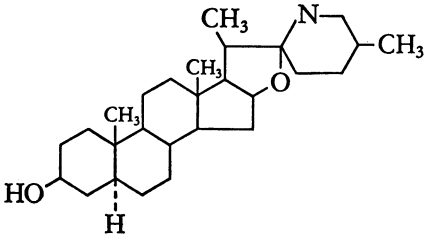


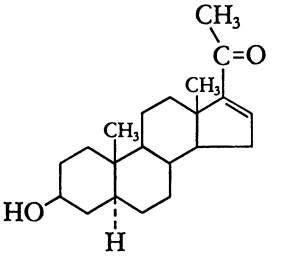
Fig. 13-3. Structural types of steroid alkaloids

The conversion of cholesterol to tomatidine has been found to occur in young plants of *Lycopersicon esculentum* [55] or *L. pimpinellifolium* [19]. The sequence of intermediates between cholesterol and the alkaloids is known at least in broad outline [49,54,57]. Hydroxylation at C-26 is the first step. Then follow replacement of -OH at C-26 by $-NH_2$, hydroxylation at C-22, closure of the piperidine ring, hydroxylation at C-16, and closure of the

spirane ring. Stereospecificity of the first hydroxylation is important; the 25R isomer is a precursor of solasodine, and the 25S isomer of tomatidine. There are indications that the above sequence of reactions is not obligatory but that the order of certain steps may be interchanged—for example, hydroxylation at C-20 before at C-26 [56]. An enzyme from *Solanum laciniatum* catalyzes the glycosylation of solasodine using UDP-glucose as a substrate [32]. When tomatine or its glycoside, tomatidine, is fed to tomato fruits, metabolism to some nitrogen-containing products and some nitrogen-free products occurs [8,20]. Among the latter is 3β-hydroxy-5α-pregn-16-en-20-one [20]:



Tomatidine



3β-Hydroxy-5α-pregn-16-en-20-one

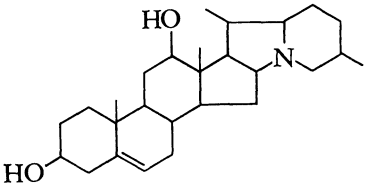
The disappearance of tomatine from tomato fruits as they ripen is of some practical importance because the alkaloid is slightly toxic [60]. When ripening of detached green fruits is accelerated by treatment with Ethrel, tomatine decreases nearly as rapidly as it does in vine-ripened fruits [9].

There are many studies on the genetic and environmental influences on steroid alkaloid content in *Solanum* spp. While normal cultivated potatoes have about 5 mg alkaloids per fresh weight of tuber, some wild varieties have more than 10 times this much [39]. In *Solanum dulcamara* there are three chemical races, differing only in the principal alkaloid of the leaf—soladulcine, solasodine, or tomatidenol. Surprisingly, hybrids produced by crossing the first and third forms contain not only the alkaloids of both parents but also solasodine and tomatidine that are not present in either parent [50]. It is well known that light accelerates the formation of alkaloids in potato tubers. Some of this effect is dependent on the appearance of photosynthesis, but there is probably an additional specific effect of light on alkaloid biosynthesis [22,29,43]. Induced mutants of *Solanum viarum* show variations in alkaloid content of the berries that are related to the total amount of photosynthetic tissue [11]. In *Solanum laciniatum* there is a diurnal variation in alkaloid content, with a maximum concentration present at midday [1]. Treatments of plants with growth regulators have not shown specific effects on alkaloid synthesis, but increased growth correlates with increased alkaloid content [42]. The same correlation is observed in tomato root cultures [48]. Callus tissue cultures of *Solanum* spp. have been used for study of alkaloid synthesis, and cultures that differentiate roots produce more alkaloid [24,67]. Suspension cultures

require light to make alkaloids. Addition of cholesterol, auxin, and cytokinin also favors alkaloid synthesis [23].

The *Solanum* alkaloids have often been claimed to have a protective effect against fungi, insects, or other parasites, and some effects seem indisputable; but there is still doubt about their importance in a normal ecological system [35,44,45,53,58,62]. At the molecular level, they complex with sterols and in this way disrupt membrane structure [47].

Jerveratrum and ceveratrum alkaloids exist simultaneously in the same plant, the former free or as glucosides, the latter as esters. Rubijervine (12),



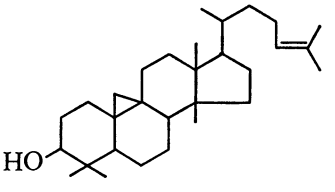
Rubijervine (12)

an alkaloid with a true steroid nucleus, also occurs with them, suggesting a biosynthetic relationship. Several mechanisms have been proposed for the rearrangement of steroid rings C and D to form the modified steroid alkaloids [30,37]. Since modified steroid nucleus has been found to occur naturally only in these alkaloids, it seems likely that the rearrangement occurs after, and is facilitated by, the introduction of nitrogen. In line with this sequence of events is the finding that *Veratrum grandiflorum* plants in the dark accumulate normal steroid alkaloids such as solanidine. On exposure to light rearrangement to modified steroids occurs [25,28]. Mechanisms for the rearrangement are suggested in [26,37]. Tracer experiments have shown that both acetate and cholesterol serve as precursors of the *Veratrum* alkaloids [26]. As the source of nitrogen, arginine is more effective than ammonium ion [27].

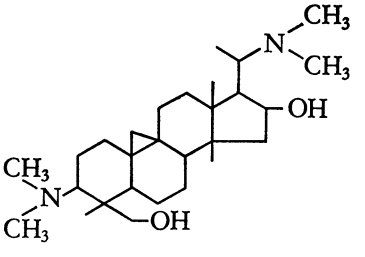
The biosynthesis of *Holarrhena* alkaloids has been investigated by tracer feeding experiments using 4-[^{14}C]-labeled steroids supplied to leaves of *H. floribunda* [5,6]. After feeding of cholesterol 4-[^{14}C], the isolated alkaloid fraction contained 14% of the original radioactivity. Pregnenolone (13) and progesterone (14) also served as alkaloid precursors, although none of the steroids that were effective as precursors could be detected in the plant. Progesterone gave rise to five alkaloids of unknown structure, but cholesterol and pregnenolone were precursors of known compounds holaphyllamine (15), holaphylline (16), and holamine. Comparison of structures and specific activities suggested the pathway shown in Fig. 13-4. Holamine is enantiomorphic with holophyllamine at C-3. Those alkaloids with additional nitrogen at C-20 and with a pyrrolidine ring are probably formed by further reaction of a holaphyllamine-type compound, as suggested in Fig. 13-4, but there is no evidence. Holaphyllamine-4-[^{14}C]

administered to *H. floribunda* has been found to be converted to radioactive pregnenolone. Thus the pathway between these two compounds is reversible [7].

No experimental work has been done with biosynthesis of the *Buxus* alkaloids, but from their structures it appears likely that they are made by a pathway similar to that for the *Holarrhena* alkaloids. Their structural peculiarities, though, make plausible an origin from a compound like cycloartenol [17] rather than cholesterol.



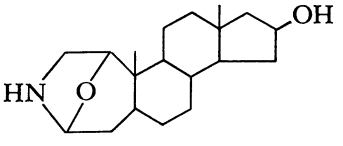
Cycloartenol (17)

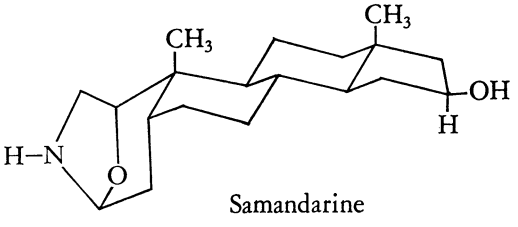


Cyclomicrophyllin A

Cycloartenol is a key intermediate in steroid biosynthesis in higher plants, taking the role that lanosterol fills in animals and fungi [14].

Of the very few animal products that are called alkaloids, the secretions of certain frog and salamander skin glands bear the closest resemblance to typical plant alkaloids. Nine of the salamander alkaloids are now known, and it appears that they are probably derived from steroids by opening of ring A and insertion of nitrogen [18]. The structure of samandarine, for example, is:





Samandarine

Such structures also seem related to the nonnitrogenous toad poisons such as bufotalin. Batrachotoxinin A from the South American frog *Phyllobates aurotaenia* has a normal steroid nucleus to which is fused an additional heterocyclic ring [65]. Nothing is known of its biosynthesis.

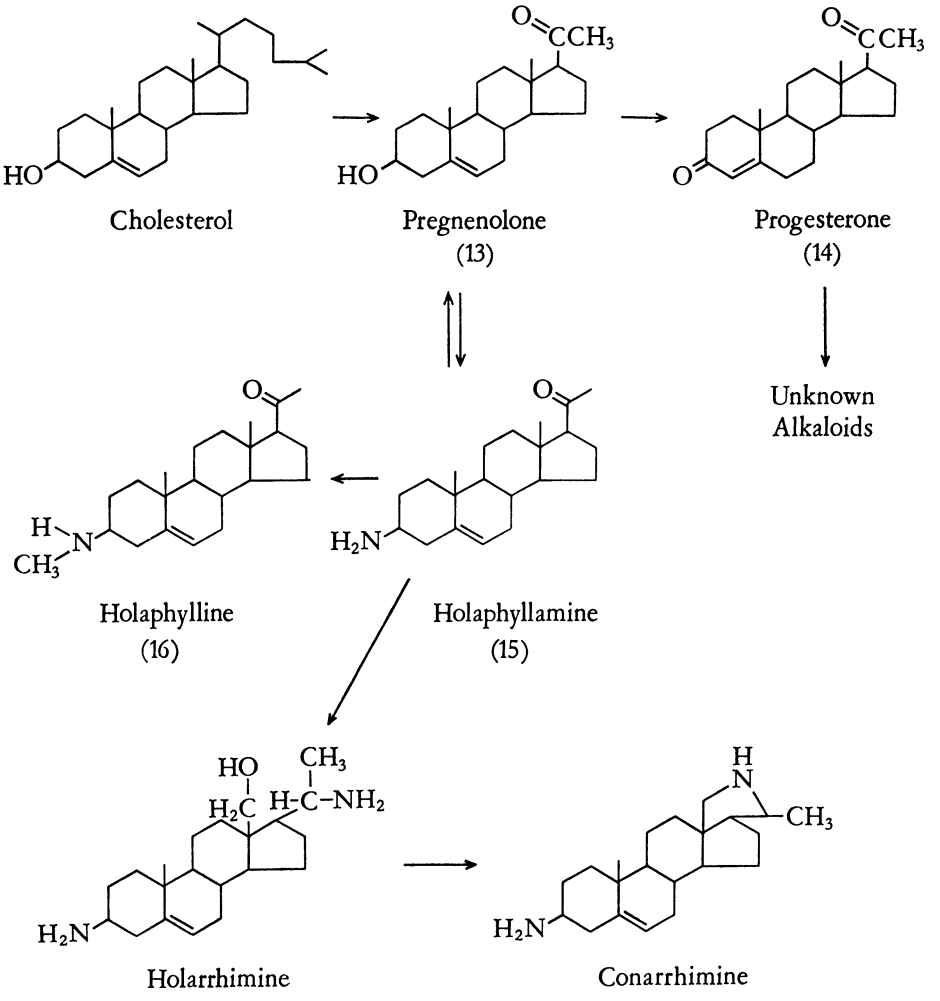


Fig. 13-4. Biosynthesis of *Holarrhena* alkaloids

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Chapter 14

Metabolism of Exogenous Alkaloids

So, naturalists observe, a flea
Hath smaller fleas that on him prey;
And these have smaller still to bite 'em;
And so proceed ad infinitum.

On Poetry, JONATHAN SWIFT

While knowledge about the metabolism of alkaloids in the plants that synthesize them has only recently begun to be developed, pathways of alkaloid degradation by other organisms have been studied for many years, and in a few cases considerable detail is available. The special interest in this area arises, of course, from pharmacological effects of alkaloids, and therefore most studies relate to mammalian metabolism and to alkaloids of widespread use. It is frequently suggested that administered drugs are metabolically converted to the real physiologically active compounds. The metabolism of drugs is a subject of frequent review articles [28,76,134]; and since many drugs are either natural alkaloids or structurally related compounds, these reviews often include information pertinent to the present discussion. There are also two books dealing with the mammalian metabolism of foreign compounds in general [58,116]. Where not specifically annotated, information in this chapter has been derived from these five reviews. The metabolism of alkaloids by microorganisms has received less attention than their mammalian metabolism, but there is practical reason for such studies, since products of microbial transformation of alkaloids may in some cases themselves be pharmacologically valuable or serve as intermediates for chemical syntheses. There is one review in this area [68].

Animals

Oxidation, one of the commonest detoxication processes, is often applicable to alkaloid metabolism. It includes dehydrogenation as well as actual

introduction of oxygen into the molecule by hydroxylation. A phylogenetic correlation has been suggested to the effect that more advanced organisms tend to detoxify foreign compounds by oxidizing them, while primitive organisms have reductive systems instead of the oxidizing systems [2].

A second very general process is conjugation, the conversion of foreign substances to less toxic derivatives by combination with conjugating agents. In mammals, phenols are often converted to β-glucuronides for excretion, acids are converted to substituted amides by combination with amino acids, some nitrogenous compounds are N-methylated, and some are acetylated. Some N-methylated compounds are demethylated. Tertiary amines, including some alkaloids, may be oxidized to N-oxides [101], and preliminary formation of an N-oxide has been suggested as part of the mechanism of demethylation. Demethylations are evidently oxidative since [^{14}C]-labeled N-methyl-pyridinium compounds fed to rats have been shown to give rise to labeled CO_2 [85]. Combinations are also possible, for instance an aromatic compound may first be hydroxylated and then converted to a glucuronide. Insofar as alkaloids contain the appropriate reactive groups, it may be expected that their metabolism in mammals will follow these general pathways. The avoidance of teleology in considering detoxification mechanisms is difficult, but it is ultimately more difficult to justify specialized enzymes waiting for substrates they may never encounter. The physiological roles of the so-called detoxifying enzymes may lie, rather, in normal metabolism; but, lacking absolute specificity, they are able to act on foreign substances that possess appropriate functional groups. In many cases the products of this action are less toxic than the substrates, but in other cases increased toxicity is the result (e.g., the conversion of codeine to morphine).

The site and enzymatic mechanisms of detoxication have been defined to some extent. The light fraction of liver microsomes is active in metabolizing many foreign compounds, and the hydroxylation reactions so characteristic of this metabolism are catalyzed by an enzyme system that incorporates molecular oxygen and requires as cofactors reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$) and a dihydropteridine [75]. Some of the microsomal enzymes involved in these reactions have been solubilized. Drugs that are hydroxylated by liver microsomes have been found to form a spectrally observable complex with a microsomal cytochrome, and this complex may be the first stage of the hydroxylation reaction [117]. An N-methylating enzyme purified from rabbit lung has relatively low specificity for methyl acceptor and can catalyze the formation of morphine, codeine, and nicotine from the corresponding nor-derivatives.

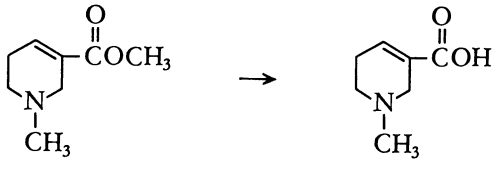
Oxidation of simple amines is brought about by the widely distributed monoamine and diamine oxidases. The first-formed aldehydes undergo further reaction, often being oxidized to the acids and excreted as such—thus, indole-3-acetic acid is the excreted end product of tryptamine metabolism, and 5-hydroxyindoleacetic acid is produced from bufotenine [113]. Mescaline is excreted largely unchanged by humans, but some

oxidation to 3,4,5-trimethoxyphenylacetic acid and 3,4,5-trimethoxybenzoic acid does occur [45,100]. In rats, mice, and rabbits this oxidation goes on to a much greater extent; and these animals are therefore relatively immune to the action of this hallucinogen. There is some question whether the oxidizing enzyme is a microsomal oxygenase or a copper-pyridoxal oxidase [46,107]. In addition to oxidation, mescaline is also converted to N-acetylmescaline and partially demethylated to varying extents by rats, mice, cats, and humans [60,89,122].

Guinea pig liver slices have been shown to split hordenine, probably first to dimethylamine and *p*-hydroxyphenylacetaldehyde, which then go on to yield further products. The metabolism of histamine is complex. In different animals it is variously oxidized, acetylated, methylated, and converted to a riboside. Ephedrine also has various fates. In several animals the initial reaction is a rapid methylation, but hydroxylation also occurs. The imidazole alkaloid pilocarpine is transformed in rat serum to unknown products that lack both imidazole and lactone rings [81].

The metabolism of heterocyclic nitrogen compounds, including several alkaloids, has been found to follow in general the principles outlined above. Hydroxylation of pyridine rings occurs similarly to the oxidation of benzene rings, although it is possible that different enzymatic mechanisms are responsible. Hydroxylation of pyridine compounds at a β-position of the ring seems analogous in its requirements to hydroxylation of a benzene ring. However, oxidation at an α- or γ-position is evidently catalyzed by a system that contains flavine and has no requirement for reduced pyridine nucleotide [55,102].

The betel nut (*Areca catechu*) alkaloid arecoline is hydrolyzed by the action of a liver esterase to arecaidine. Arecaidine appears to account for the physiological properties of betel nut better than arecoline does, and the practice of chewing betel nut with lime probably also favors the ester hydrolysis [96].



Arecoline Arecaidine

In addition to hydrolysis, though, arecoline is also metabolized to its N-oxide, to arecaidine-N-oxide, and to a glucuronide [23]. Furthermore, arecaidine reacts with sulfhydryl compounds to alkylate them, and this reaction is suspected to account for carcinogenicity of the betel nut [91].

The metabolism of the tropane alkaloids cocaine and (±)-hyoscyamine has been investigated in a number of animals. Probably the first stage in metabolism of these compounds is hydrolysis of the esters. The further metabolism of the heterocyclic portion may involve N-demethylation,

hydroxylation, and conjugation with glucuronic acid [86,126,127,140]. Metabolism and excretion of cocaine by mice is slower during pregnancy [124].

The metabolism of nicotine (1) by mammals has probably been as extensively studied as that of any alkaloid. In general, only a small percentage of administered nicotine is excreted unchanged, but the actual figures vary from animal to animal in the range of 1% to 12%. In human smokers, the half-life of nicotine in plasma is less than 30 min [79]. Nonsmokers excrete unchanged a higher percentage of administered nicotine than do smokers [12]. Autoradiography after uptake of labeled nicotine by mice has shown rapid uptake and localization in the brain, somewhat less in the bronchi [135]. Several degradation products have been isolated, and some transformations are shown in Fig. 14-1. In dogs, approximately one-third of the nicotine administered was found to be converted to compounds in which the pyrrolidine ring was oxidized or opened. The other compounds are relatively minor, but their concentrations depend on the animal and the conditions of nicotine administration [133]. In the pathway from nicotine to cotinine, a likely first step is the formation of $\Delta^{1'(5')}$-iminium ion; and this initial reaction is catalyzed by liver microsomes, but the next two reactions are catalyzed by soluble enzymes [24,25,72]. Some other established metabolites that are not shown in Fig. 14-1 are 5′-hydroxycotinine [37], γ-(3-pyridyl-)-γ-oxo-N-methylbutyramide [80], and nicotine-1′-N-oxide. This last product exists in two isomeric forms since the 1′-nitrogen atom has become a chiral center [1,30]. Different animals and different tissues produce different amounts of the two isomers [21]. Human smokers excrete both isomers [20]. Enzyme preparations from rat liver have been found to convert nicotine to nornicotine (2) and formaldehyde, and to oxidize the nornicotine to demethylcotinine (3) [98]. Interestingly, some insects that feed on tobacco handle nicotine in a similar way, excreting the major part of it as cotinine (4) [122]. Army worms have a mixed function oxygenase that brings about N-demethylation of nicotine [26]. Anabasine, which is quite similar in structure to nicotine, is metabolized by mammals in about the same way as nicotine [11].

The metabolism of simple quinoline derivatives has been studied extensively; but the only quinoline alkaloids for which there is any information are the atypical *Cinchona* compounds. An enzyme from rabbit liver has been known for many years to catalyze the oxidation of quinoline derivatives at the 2-position to yield α-pyridones known as carbostyrils (Fig. 14-2). The so-called "quinine oxidase" of rabbit liver has been well-characterized and shown to be a soluble, iron-molybdenum flavoprotein identical to liver aldehyde oxidase [77,103]. The two different types of reaction catalyzed by the same enzyme can be used to support the argument that so-called detoxication reactions are only fortuitous reactions catalyzed by enzymes of low specificity normally having other functions. Although the carbostyrils are the major excretion product of cinchonine (5) and cinchonidine (6), some oxidation of the quinuclidine ring also occurs [137]. In the case of

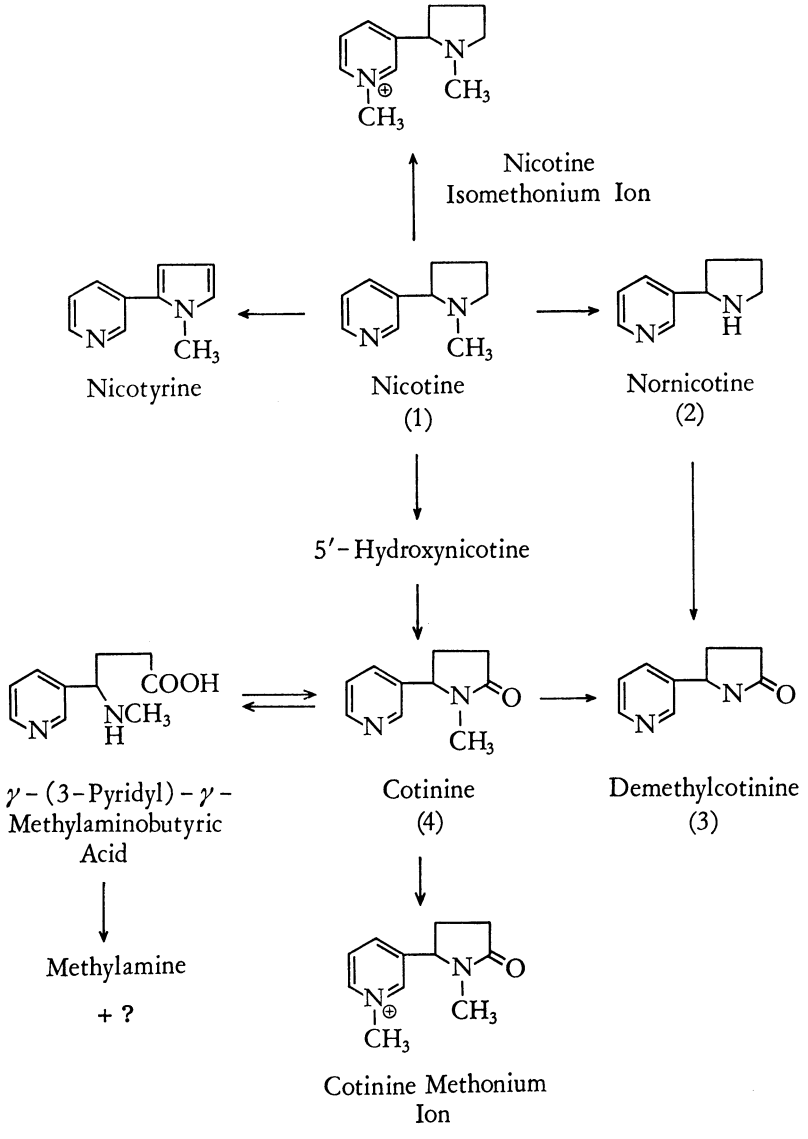


Fig. 14-1. Nicotine metabolism by mammals

quinine (7) and quinidine (8) the same two positions are oxidized. In humans, oxidation of the quinuclidine ring takes precedence over oxidation of the quinoline ring, but both occur [15,30].

Among the isoquinoline alkaloids salsolinol and tetrahydropapaveroline are methylated by rat liver [36], reticuline is converted by rats to berbine and aporphine alkaloids [74], narcotine goes to at lease five different products [54], and papaverine in several animals is both demethylated and conjugated with glucuronic acid [13]. The demethylation of papaverine is brought

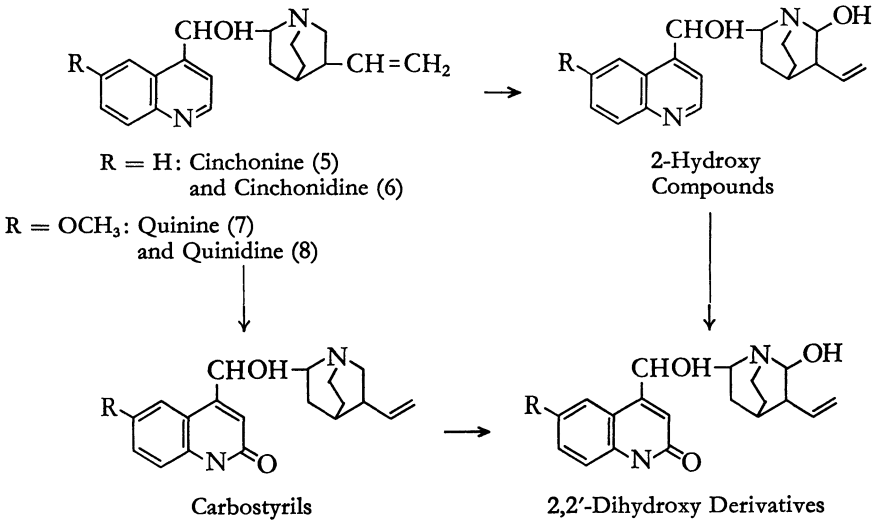


Fig. 14-2. Metabolism of *Cinchona* alkaloids by mammals

about by rat liver microsomes, and pretreatment of the rats with phenobarbital increased this demethylation activity [14]. Much more attention has been given to the metabolism of morphine and related morphinan alkaloids [145]. As with nonalkaloid phenols, conjugation of the hydroxyl groups to glucuronic acid occurs with morphine and codeine. Morphine, which has two hydroxyl groups, conjugates preferentially at C-3. Codeine, which lacks a hydroxyl at C-3, conjugates at C-6. Most animals also make some morphine-3-sulfate, but cats are unusual in having this as the major metabolite [144,146]. While it is called a "detoxication" product, morphine-6-glucuronide is actually more potent than morphine itself [97]. N-demethylation of morphine occurs to a very slight extent (3%–5% in humans). Deuterated morphine is demethylated at a slower rate than ordinary morphine [76]. N-demethylase activity is less in the livers of rats habituated to morphine, but a lower rate is not observed for demethylation in vivo by habituated rats. Codeine, in addition to being conjugated and N-demethylated like morphine, is also partially converted to morphine by an O-demethylation in man and rats, but is not converted in dogs. Several animals convert some codeine to morphine [148,150], and the reverse conversion of morphine to codeine has also been claimed to occur [19] although this claim is disputed [143,146]. The N-demethylation reactions can be brought about in vitro by liver microsomes requiring $NADPH_2$ and O_2. An N-oxide intermediate has been suggested [139]. N-demethylation also occurs in the central nervous system, particularly in regions that are rich in opiate receptors [50]. The conjugation reaction has also been studied in somewhat purified systems. Both intestinal wall and liver contain the conjugating system and use UDP-glucuronic acid as the donor. The

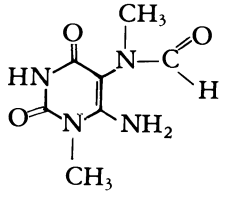
reaction is evidently localized in the microsomal fraction and is inhibited by various narcotics [44,104,112]. In addition to the major metabolites just discussed, several others have been reported—for example, morphine-N-oxide [146], morphine-2,3-quinone [88], and a 2,3-dihydrodiol [87]. Heroin (diacetylmorphine) is metabolized by hydrolysis of one or both acetyl groups and then treated in the same way as morphine [35,147]. Preadaptation of rats to heroin decreased the rate at which they degrade it [35]. Adaptation of rats to morphine may involve some lowering of activity in the enzymes that degrade it, but also an increased ability to excrete unchanged morphine and, consequently, less accumulation of alkaloid in the tissues [38,149].

There are several indole alkaloids whose metabolism in animals has been investigated. Four metabolites of strychnine produced in rabbit liver have been isolated, but only one of these has been identified; 2-hydroxystrychnine, which has only 1% of the toxicity of strychnine [129]. In lysergic acid, hydroxylation at C-2 of the indole nucleus is brought about by the usual liver microsome hydroxylating system, and this is followed by extensive breakdown to unidentified products. It has been shown that reserpine is demethylated and the ester group hydrolyzed, but there are significant species differences in the extent to which these processes occur. It is interesting that the LSD hydroxylating enzyme is inhibited in vitro by reserpine [9]. In vitro experiments have been used to investigate the metabolism of corynantheidine-type alkaloids by liver microsomes. The major reaction with rabbit microsomes was an O-demethylation producing formaldehyde. Ring hydroxylation did not occur [10]. Harmaline and related alkaloids when fed to rats are de-O-methylated, then converted to glucuronides or sulfates [59,125]. Ellipticine in several mammals is hydroxylated in the A ring (primarily at C-9) and then conjugated with glucuronic acid or sulfate [32,78]. Agroclavine is oxidized by a microsomal P-450 system of rat liver to noragroclavine and elymoclavine [142]. Vincristine is hardly metabolized at all by rats; most is excreted unchanged [31].

The metabolism of pyrrolizidine alkaloids is of special interest because it seems that some of their metabolites are more hepatotoxic than the original alkaloids (cf. Chap. 15). For greatest toxicity the allylic ester group remains intact, and the ring it is attached to is dehydrogenated to a pyrrole [84]. Dehydrogenation is accomplished by liver microsomes and can happen with or without preceding hydrolysis [70,141]. Several species of butterflies and moths have an interesting metabolism of pyrrolizidine alkaloids because they eat plants containing them and modify the structures to make their own pheromones [17,119].

There are many publications on metabolism of the methylated xanthines and many different metabolites have been identified. In adult humans, methylated uric acids are the chief products from caffeine and theophylline. These are produced by oxidation at C-8 and usually some loss of N-3 methyl groups. Demethylated products appear in the blood within 3 h of consuming caffeine, but complete clearance of caffeine and its derivatives takes as much

as a week [136]. Theobromine, strangely, is treated differently in that its chief metabolite has undergone opening of the five-membered ring to give a uracil derivative [7]:

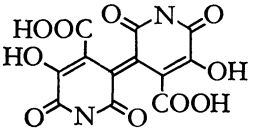


The oxidation and demethylation of these compounds seems to be catalyzed by a microsomal P-450 oxygenase. Smokers metabolize theophylline more rapidly than nonsmokers do, presumably because their P-450 system has been activated by exposure to substances in tobacco smoke [71]. Infants and fetuses, since they have an inactive P-450 system, do not oxidize theophylline but methylate it to produce caffeine. Since theophylline is used to treat neonatal apnea, this finding is more than an irrelevant curiosity [5,22]. In animals other than humans, the metabolism of methylated xanthines runs a similar course with some differences in emphasis [6,105]. For example, rabbits like humans preferentially demethylate caffeine at N-3 but dogs rather at N-1 and N-7. Several sulfur-containing metabolites of caffeine are produced by rabbits, horses, and mice [73]. Pretreatment of rats with 3-methylcholanthrene greatly increased the rate of caffeine elimination. Its half-life in the serum was reduced from 50 min to 15 min. As in the case of smokers vs nonsmokers, it is likely that increased activity of the P-450 system is responsible [4]. Caffeine is peculiarly toxic to squirrel monkeys because they metabolize it very slowly. Its half-life in their plasma is 11 h, but no unusual products are produced [27].

Microorganisms

The metabolism of alkaloids by microorganisms has been studied, but the only case for which a rather complete pathway can be given is the degradation of nicotine (1) by *Arthrobacter* spp. This pathway, developed primarily through the work of Decker, Rittenberg, and their co-workers, interestingly is quite different from the route of nicotine degradation by animals [40,42,43,48,53,61,62,65,108,109]. In *Arthrobacter* spp. the first step in degradation is oxidation to 6-hydroxynicotine (9). The enzyme responsible for this oxidation is induced by growth on nicotine as the sole carbon source and repressed by availability of other carbon-nitrogen sources. Studies with purified enzyme using [^{18}O] have shown that the introduced oxygen is derived from water rather than O_2, so that the reaction probably involves hydroxylation and dehydrogenation [61]. The second step is an oxidative splitting of the pyrrolidine ring. This second step may

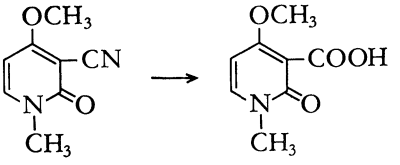
also involve two separate reactions—dehydrogenation and hydrolysis [40, 62]. A third oxidation to the 2,6-dihydroxypyridine ring is followed by a combination of enzymatic and nonenzymatic reactions according to the scheme presented in Fig. 14-3 [48,53,65,108,109]. The structure of the blue pigment produced is not yet known. It probably belongs to the ill-defined group of azaquinones and has no metabolic function [48]. A suggested structure for a similar blue pigment produced by a pseudomonad from isonicotinic acid is [49]:



Although natural nicotine is S-(−), *Arthrobacter oxidans* can also oxidize R-(+)-nicotine to the 6-hydroxy derivative. Two different enzymes are responsible for action on the two enantiomers [41]. Nicotine-N-oxide is also metabolized to a variety of products by *Arthrobacter* [82]. Although microbial fermentation of tobacco is important in the "curing" process, there is very little recent information on changes in alkaloids during this process [132].

For alkaloids other than nicotine there are in the literature some isolated examples of reactions brought about by microorganisms, but no complete metabolic pathways. Some of these transformations have been investigated with a view to provide possible modification of antitumor compounds into more useful drugs.

Ricinine is acted on by a nitrilase of *Pseudomonas* spp., resulting in conversion of the cyano group to carboxyl:



Other (synthetic) 3-cyano-2-pyridones are acted on similarly. It is interesting that although some amide is also produced, it is not an intermediate in formation of the acid [66,110].

Mimosine has been found to inhibit the growth of *Escherichia coli* and to have its inhibition reversed by such indole derivatives as tryptophan. It is split by *E. coli* into serine plus a pyridine derivative of unknown structure [128].

Several bacteria have flavoprotein oxidases that can oxidize pyridine rings, but their activities have been studied with simple pyridine compounds rather than alkaloids [63,138].

A number of microorganisms metabolize atropine, with the initial reactions being demethylation and ester hydrolysis. Induced enzyme

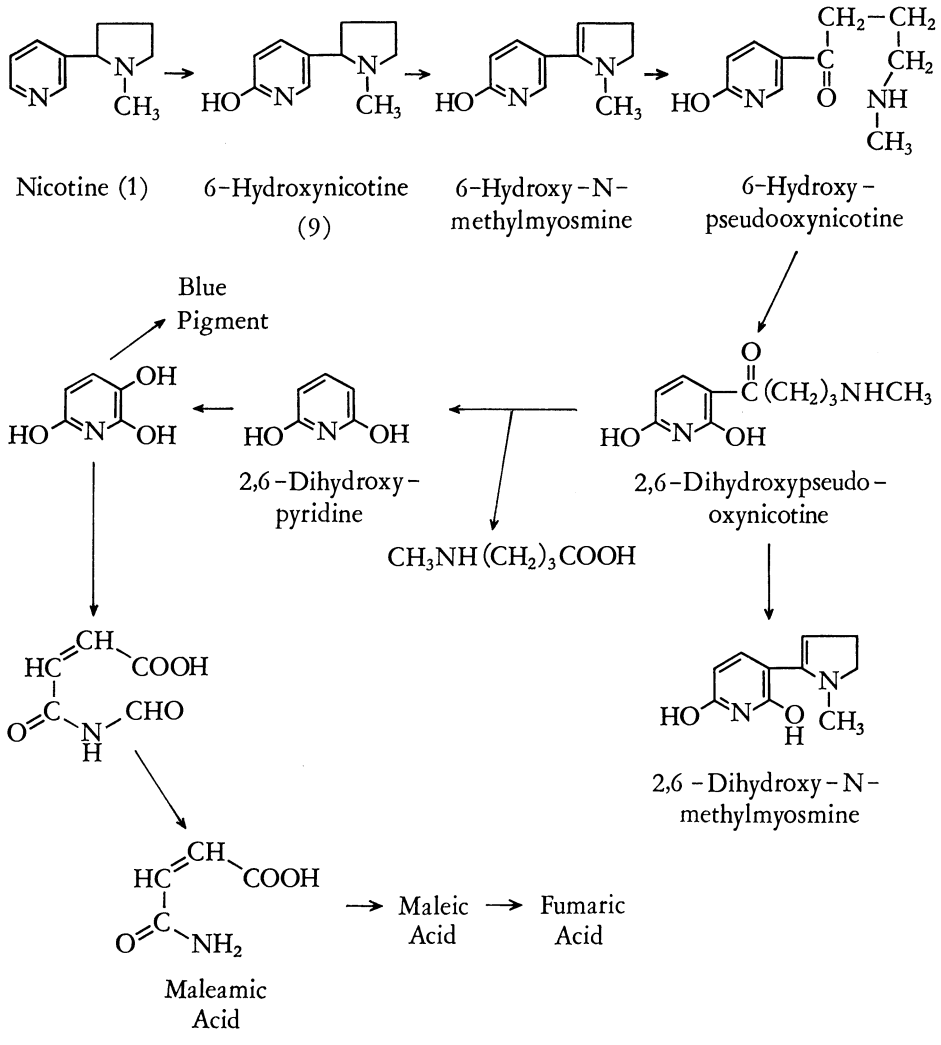
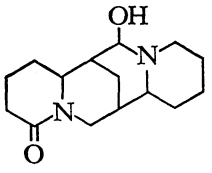


Fig. 14-3. Metabolism of nicotine by *Arthrobacter oxidans*

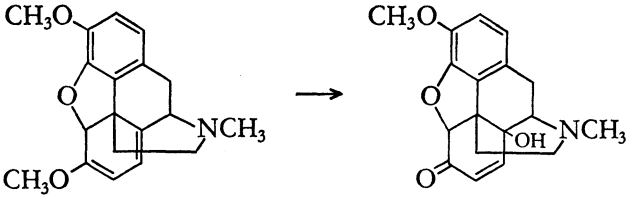
systems are evidently involved in this metabolism since the ability to degrade atropine is not a stable character. Strains of *Aspergillus versicolor* which utilize atropine as their sole carbon source have been isolated [118].

Hydroxylation reactions are a common feature of transformations of alkaloids in microbes just as they are in animals. A strain of *Pseudomonas lupanii* has been found capable of utilizing lupanine as its sole source of both carbon and nitrogen. The first step in lupanine breakdown was hydroxylation at C-17. This is in contrast to the pathway in *Lupinus*, where hydroxylation occurs at C-13 (see Chap. 6). The hydroxylating enzyme is inducible by lupanine [95,131].



17-Hydroxylupanine

Several microorganisms act on benzylisoquinoline and bis-benzylisoquinolines. The most usual action is O-demethylation [39,90,106], but a species of *Nocardia* brings about oxidative splitting of the pyridine ring of papaverine so that two C_6-C_2 fragments are produced [57]. There is a patent [120] claiming that an oxidative enzyme preparation obtainable from several microorganisms catalyzes the conversion of (−)-reticuline to (+)-salutaridine. Conversion of thebaine to 14-hydroxycodeinone can be brought about by the wood-rotting fungus *Trametes sanguinea*, with the introduced oxygen atom derived from O_2 and not from water [3].



Thebaine 14-Hydroxycodeinone

Colchicine is metabolized by *Arthrobacter colchovorum* by deacetylation and then loss of the amino group [151]. *Streptomyces* spp. O-demethylate it [67].

Among the indole alkaloids, vindoline is demethylated by *Streptomyces*, whereas vinblastine is hydroxylated and converted to a bridged ether [93]. A laccase secreted by the fungus *Polyporus anceps* catalyzes the oxidation of vindoline through a series of intermediates to a dimeric product [47]. Several microorganisms oxidize strychnine first to its N-oxide and then to various other products [94]. Yohimbine-type alkaloids are hydroxylated by the fungus *Cunninghamella blakesleeana* [1]. Ellipticine is hydroxylated at the 8- and 9-positions by several microorganisms [33]. 9-Methoxy-ellipticine is O-demethylated [34]. *Streptomyces roseachromogenes* dealkylates or hydroxylates LSD and related compounds [69].

Strains of *Pseudomonas putida* and *Penicillium roquefortii* that can use caffeine as their sole source of carbon and nitrogen have been isolated. In both cases the breakdown pathway begins by oxidative demethylation [18,121]. With *P. putida*, the pathway continues through uric acid to urea and glyoxylic acid [18].

Steroidal alkaloids are frequently glycosides, and a common action of microorganisms on them is hydrolysis of sugar residues. This is a way that some plant pathogens are able to detoxify α-tomatine and solanine, which

are toxic to many other fungi [8,51,64]. Microbial hydroxylation of steroids has been an important source of intermediates for steroid hormone syntheses. Not surprisingly, steroid alkaloids can be hydroxylated in the same way as other steroids [29]. For example, *Aspergillus ochraceus* brings about monohydroxylation at either the 11-α- or 12-β-position of funtumine and funtumidine [56]. Hydroxylations of conessine [83,99], solasodine [144], and tomatidine [115] by other fungi have also been reported. The 7-, 9-, and 11-positions are the ones usually attacked. Hydroxyl groups, either introduced or original, can be dehydrogenated to carbonyl groups [16,64].

Higher Plants

There is very little information on the metabolism of foreign alkaloids by normally alkaloid-free higher plants. However, it has been shown that wheat plants can take up from solution such alkaloids as nicotine, berberine, and codeine and decompose them [52]. In *Salvia officinalis*, there was evidently adaptive formation of a nicotine-decomposing system since plants supplied nicotine over a period of time increased in their ability to decompose it [52]. When alkaloids were fed to various solanaceous plants, those species that did not normally contain the alkaloid tended to degrade it faster than a plant that did [92]. The conversion of thebaine into codeine and morphine by tobacco plants has been mentioned in Chap. 8. In all of these cases, since germ-free plants were not employed, the possibility of alkaloid metabolism by contaminating microorganisms cannot be ruled out.

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Chapter 15

Biochemical Effects of Alkaloids

All o'you cokies is a-gwine to be dead
If you don't stop asniffing that stuff in yo' head.

Cocaine Bill, U.S. FOLK SONG

Ultimately, gross effects of alkaloids and other foreign molecules on the behavior of organisms must have their explanations in terms of specific molecular interactions and chemical reactions. In the course of its development, pharmacology has come from observations of total behavior to observations of gross physiological processes, observations on isolated organs and tissues, and elegant and detailed observations at the cellular level. In a few cases it has started to approach observations at the molecular level. Essential aspects of pharmacology have doubtlessly been overlooked in this chapter, and it should probably be regarded as a smattering of things that have been of interest to one biochemist rather than a balanced review. Where references to literature are not given, the reader is referred to the general sources [51,95,140,165,202,210,270]. Although most of the information in this chapter is from the pharmacological literature—i.e., dealing with animals—there are a few interesting reports dealing with biochemical effects of alkaloids on plants and bacteria. These are introduced at appropriate places rather than having a section to themselves.

The most attractive explanations for the powerful physiological action of small molecules at low concentration concern themselves with the following types of biochemical processes:

1. Mechanisms of DNA replication, RNA transcription, and protein synthesis. Several antibiotics are clearly understood as having their action at this level. Some alkaloids also probably act here (see below).
2. Mechanisms of transport across membranes, both active and passive. The access of substrates to enzymes and the excretion of metabolic products clearly depend on these transport mechanisms. Passive membrane permeability is influenced by

many compounds. Active transport is a more specific and complex process known to be influenced by such drugs as the cardiac glycosides as well as several of the *Veratrum* alkaloids.

3. Mechanisms of enzyme inhibition or activation. These mechanisms are the most convenient to study in vitro but often difficult to relate to gross physiological effects because at a suitable concentration nearly any compound will show some effect on nearly any enzyme. Since active membrane transport is an enzymatic process, it could also be included under these mechanisms (see below).
4. Mechanisms involving structural changes. Changes in macromolecular conformations as the result of adsorption of small molecules or displacement of one small molecule by another may provide the most general kind of explanation for drug effects since all the previous three types of mechanisms can be considered as dependent on particular macromolecular configurations.
5. Blocking of receptor sites for chemical transmitters.

Before any administered alkaloid can have an effect, it must obviously reach its site (or sites) of action. In this simple qualification lurk great difficulties for understanding mechanisms of drug action in complex organisms. Not only are physical barriers and diverse transport systems influential in controlling distribution, but metabolic changes such as those discussed in the previous chapter may intervene. These various and incompletely understood processes cannot be neglected in any complete explanation of the physiological effects of an administered alkaloid, yet they are outside the usual sphere of interest of biochemistry. Biochemistry can begin only with some knowledge of what the active compound is and what its concentration is at the site where it is presumed to act.

Considerations of concentration have been a major stumbling block for explanations of pharmacological mechanisms. In whole organisms as well as in simplified systems a given compound at different concentrations may show effects that appear to be qualitatively and not merely quantitatively different. The literature is rife with examples of drugs which at 10^{-3} M inhibit certain crucial systems in vitro, but at the presumed receptor in vivo, the effective concentration may be found to be lower by several orders of magnitude. At the least, explanations of gross effects based on such findings should be suspect; probably they should often be regarded as entirely irrelevant.

Once knowledge of a known compound is at hand, in a certain concentration range, at a defined site of action, explanations of its action at a molecular level are approachable. Some kind of complementarity between agent and receptor is taken as self-evident. Various types of molecular interaction can be distinguished, and some of these have been shown to have significance in systems of pharmacological interest. The use of gross structural formulae is inadequate for an understanding of such interactions since subtle aspects of shape and electron distribution need to be considered. While quantum biochemistry may have promise of providing deeper understanding in the future [131,209], at its present stage of development it appears to offer very slight advance over cruder theoretical

systems that attempt to relate electronic structures of drugs to their mechanisms of action. Computer modeling of three-dimensional structures is beginning to be useful in drug design [99], and correlation of actual conformations of alkaloids with biological activities has given some interesting generalizations [220].

In some cases, covalent bonding between drug and receptor may occur; but this is probably not as usual as weaker attachments by ionic attractions, hydrogen bonding, metal coordination, and hydrophobic attraction. Considering alkaloids in particular, this statement of Bloom and Laubach [28] seems especially apt: "The amino group knows no peer in the remarkable variety of binding involvement which can result from subtle changes in its functionality." All trivalent nitrogen atoms can act as electron pair donors to suitable acceptors, either metal ions or electrophilic organic molecules. Primary and secondary amines can participate in hydrogen bond formation either as donors or acceptors. Primary amines can form covalent Schiff bases with carbonyl compounds. All amino compounds on the acidic side of their isoelectric points exist as cations which will be attracted to anionic groups. Changes in structure, by modifying the ionization potential of the amino group, can profoundly alter its reactive capabilities where these depend on ionic form. The quaternary amonium compounds found among the alkaloids are, of course, cations at all pH values and are often so strongly adsorbed near their site of entry that they reach internal tissues at lower concentrations than do similar but less highly charged molecules.

The previous paragraph introduces the idea that it is to be taken as a rule rather than an exception for an alkaloid to bind to such macromolecules as proteins and nucleic acids. Many studies have measured the binding of various alkaloids to proteins of blood serum [43,126,193]. Although such binding most often has little obvious relevance to an understanding of the important physiological actions of an alkaloid, two points need to be made:

1. Variations in the amount bound in the serum lead to variations in the amount available at sites of action [247].
2. The binding of an alkaloid to a simple serum protein may provide a useful model for studying binding sites that can be applied to more significant ones [86].

The concept of a receptor on which both endogenous and exogenous small molecules act has become a cornerstone in the understanding of cellular processes at the molecular level. The number of specific receptors in a complex organism is clearly enormous. In the central nervous system alone there are probably at least several dozen neuroregulators, each with its specific receptor [15]. Purification and characterization of receptor molecules is proceeding, but the concentrations of receptors is so low that the work is very difficult. Some general references in this area are in [297]. *Specific* binding to a receptor is established if it is saturable, displaceable by drugs known to act at that receptor, and localized in tissues known to have the receptor [26]. Such standards have not always been

applied to studies on the mode of action of an alkaloid. After isolation and characterization, the other side of understanding the action of receptor molecules is reconstitution, where supposed receptors or parts of them are incorporated into defined membranes and their responses observed. Examples of this approach are studies of the effects of various alkaloids on fluidity of lipid bilayers or transport of molecules and ions through them [44,45,120]. The relevance of these studies to the in vivo system remains to be established.

With the foregoing generalizations and qualifications disposed of, a few examples of alkaloid pharmacology at the molecular level can be considered. These mechanisms have been illuminated as much by studies with synthetic compounds as by studies with naturally occurring alkaloids, and it would therefore be impracticable to limit the discussion rigidly to natural compounds.

Cholinergic and Adrenergic Systems

Drugs acting on the autonomic nervous system have received considerable attention in recent years, and some clearcut biochemical findings have resulted. Anatomically, the peripheral autonomic nervous system can be subdivided into four types of fibers; biochemically, these fibers are divided into "adrenergic" types whose chemical transmitter is noradrenaline, and "cholinergic" types whose chemical transmitter is acetylcholine. Anatomical details can be found in [95]. The chemical and anatomical divisions may be summarized as follows:

	Peripheral autonomic system	
	Thoracolumbar or sympathetic	Craniosacral or parasympathetic
Preganglionic fibers	Cholinergic	Cholinergic
Postganglionic fibers	Mostly adrenergic; a few cholinergic	Cholinergic

The transmitter substances act not only in transmission of the nervous impulse across the synapses between nerve cells but also in the junctions between the nerve cells and the tissues they control. Conduction of the impulse along a nerve fiber is a different kind of process, which will be considered later.

Acetylcholine is the most widespread neurohumoral transmitter, functioning not only in the peripheral autonomic system as shown in the table

above, but also in motor nerves to skeletal muscle and in certain neurons within the central nervous system.

Acetylcholine

Arrival of a nerve action potential at an axonal terminal stimulates release of acetylcholine that has been synthesized and stored in synaptic vesicles. The acetylcholine then diffuses across the synaptic cleft and acts on a receptor of the following neuron, muscle, or gland, which then responds appropriately. Removal of the transmitter from the junction must take place before another impulse can be transmitted. This removal occurs through a combination of diffusion and enzymatic destruction—hydrolysis mediated by acetylcholinesterase. Based on this rough outline of nerve transmission, several types of mechanisms can be distinguished for drugs acting on cholinergic fibers. Some classes of agents are listed below.

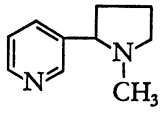
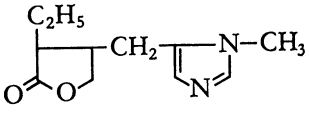
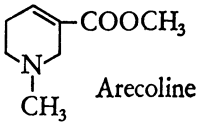
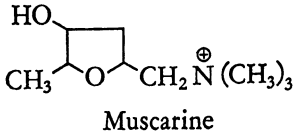
1. Inhibitors of acetylcholine synthesis
2. Substances that affect the release of acetylcholine
3. Substances that act on the receptor as mimics of acetylcholine ("agonists")
4. Substances that occupy the receptor for acetylcholine and thus block its action ("antagonists")
5. Inhibitors of acetylcholine esterase

The mechanisms of these agents cannot in every case be correlated with the kinds of biochemical processes tabulated on p. 182. For instance, inhibitors of acetylcholine synthesis could act either by repressing formation of an enzyme needed for the synthesis or by inhibiting action of an enzyme already present. There do not seem to be any natural alkaloids known at present which owe their effects to inhibition of acetylcholine synthesis. The synthetic drug hemicholinium does act here. The release of acetylcholine is inhibited by morphine [119] and stimulated by several quaternary ammonium compounds [77], and by the alkaloid physostigmine [19]; but this does not seem to be an important explanation for the physiological action of any alkaloid. Several alkaloids are known to mimic the effort of acetylcholine on its receptors. They may be subdivided according to the type of receptor involved: some acting preferentially on the postsynaptic receptor of a neuron, some at the neuromuscular junction, and others at receptors of secretory glands. These various mimetic agents can all be shown to have some kind of structural resemblance to acetylcholine, but precise structural requirements are quite obscure, and prediction of the site of action of a compound from its structure is difficult. Several cholinomimetic alkaloids are shown in Table 15-1 with their presumed sites of action. Nicotine shows first a mimetic effect but then a blocking action on the receptor similar to that of compounds of the fourth type listed above.

With increasing concentrations, the blocking action increasingly predominates over the stimulating action. The active form of nicotine is probably the nicotinium ion, and (−)-nicotine is much more active than (+)-nicotine [1,46]. The action of muscarine can be distinguished from the action of nicotine by the fact that antagonists to nicotine action are not the same as antagonists to muscarine. The terminology that is sometimes used suggests that there are separate "nicotinic" and "muscarinic" receptors, but recent work on receptor purification (see below) suggests that it is more realistic to think of two sites on a single receptor. Other alkaloids that seem to act on the muscarinic site are arecoline, oxotremorine, and pilocarpine [255].

Several other alkaloids act primarily by blocking receptors of acetylcholine. Tubocurarine is the best known and most powerful of the alkaloids that antagonize nicotinic action. Other curare and *Erythrina* alkaloids act similarly. Snake α-toxins also bind at this site [106]. Divalent cations decrease the effectiveness of d-tubocurarine by decreasing the electronegativity of the receptor [276]. Antagonists at the muscarinic site show their greatest action on autonomic effector cells rather than on ganglion cells. There are also differences in the sensitivities of the different types of effector cells, with the general order of some sensitivities being: salivary glands > iris muscles > visceral muscles > gastric secretory cells. The best

Table 15-1. Some cholinomimetic alkaloids

Alkaloid	Site of stimulating effect
 Nicotine	Ganglion cells
 Pilocarpine	Ganglion cells; various autonomic effector cells
 Arecoline	Motor end-plate of skeletal muscle; ganglion cells; various autonomic effector cells
 Muscarine	Various autonomic effector cells

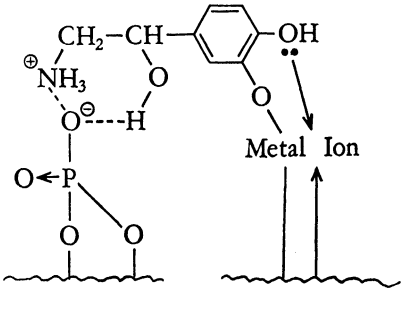
known of these blocking agents is atropine [(±)-hyoscyamine]. Other tropane alkaloids act similarly [255]. Cocaine, which is structurally a tropane alkaloid, shares these properties to only a small extent and is more noted for its effects on adrenergic receptors. Studies of the relation of structure to activity indicate that what is required for antimuscarinic action is an ester of tropic acid with a tertiary amino alcohol [145]. This requirement is met by hyoscyamine and hyoscine but not by cocaine. There is some controversy about whether the molecular configuration responsible for ion conductance across the postsynaptic membrane should be regarded as part of the receptor or a second entity that is acted on by the receptor. In any case, though, it is possible to block ion conductance without blocking the binding of acetylcholine. An alkaloid that has this action is quinacrine [150]. Synthetic local anesthetics such as procaine act similarly [27].

The actions of acetylcholine itself, the cholinomimetic compounds, and the blocking agents all presumably depend on interaction of these compounds with a receptor. It may be inferred that the receptor contains at least an anionic site that is neutralized by ion pair formation with the positively charged nitrogen of acetylcholine. Compounds that mimic acetylcholine, even though not all of them are quaternary, may also attach at this site and activate the receptor. The blocking agents are thought to shield the anionic site of the receptor without activating it. Obviously other sites of the receptor molecule must be involved because of the complex relationships between structure and activity that have been observed. A direct approach to the nature of receptor-transmitter binding has become possible with the availability of purified acetylcholine receptor from *Torpedo californica*. This receptor molecule is a pentameric complex with a molecular weight of 2.55×10^5 daltons. It is a transmembrane protein, and all five subunits are exposed at the outer face [205,277]. Although this purified receptor is a protein, the possible involvement of lipids in receptor action is still a subject of discussion. Gangliosides and inositides do bind such alkaloids as nicotine, d-tubocurarine, and lobeline; and the alkaloids could in this way influence cholinergic transmission at a membrane made of these lipids [44,213]. Any acidic molecule will, of course, bind to basic alkaloids; and the binding of d-tubocurarine to hyaluronic acid should be looked at in this light rather than as implicating acidic polysaccharides as part of the receptor [104]. An unusual and generally discounted explanation of acetylcholine action depends on regulation of triphosphoinositide phosphomonoesterase by the transmitter [269].

Inhibitors of acetylcholinesterase have become prominent in recent years because of their use as insecticides and their potential use as "nerve gases." These agents cause accumulation of acetylcholine and therefore continuous stimulation of cholinergic receptors. Although the vast majority of cholinesterase inhibitors are synthetic, they do include the alkaloid physostigmine (eserine). There has been intensive study of the mechanism of action of cholinesterase, and there is now quite a clear understanding of the details of its catalysis and inhibition. The enzyme has two spatially separated active

sites: an anionic site that binds the quaternary nitrogen of acetylcholine and an esteratic site that binds the carboxyl carbon of the ester. The anionic site has been further subdivided into two or three negatively charged areas, and hydrophobic regions are also associated with them [17,216]. The esteratic site contains the hydroxyl group of a serine residue and the basic imidazole group of histidine. After formation of the enzyme-substrate complex, splitting of the ester by shift of a proton occurs, leaving an acetylated enzyme that is rapidly hydrolyzed to acetate plus the enzyme. Physostigmine, in contrast, forms a carbamylated enzyme whose hydrolysis is very much slower, so that the enzyme is effectually taken out of action. These reactions are shown diagrammatically in Fig. 15-1. Physostigmine also shows other effects, unrelated to its inhibition of cholinesterase [19]. The synthetic cholinesterase inhibitors form phosphorylated enzymes that are hydrolyzed at a still slower rate, so that their action is considered to be practically irreversible. Quite a few alkaloids as well as simple quaternary ammonium salts also inhibit cholinesterase by binding at an anionic site. This binding is generally weak and competitive with that of acetylcholine [6,76]. Out of many alkaloids tested, sempervirine was the most active inhibitor next to physostigmine—but only 1/100 as active. The steroidal *Solanum* alkaloids were slightly less active than that [186,76]. Galanthamine, although it is a reversible inhibitor, shows some properties similar to physostigmine [53, 278].

Alkaloids and other drugs that act on adrenergic transmission show certain analogies with those that act on the cholinergic system, but the parallels are not strict. The most significant difference is that there is no enzyme analogous to acetylcholinesterase for destruction of noradrenaline, and its removal from the junction occurs mostly by reuptake into the nerve terminal. Although the enzymes monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) are two enzymes that act on noradrenaline, they are not thought to be important in terminating its action. Analogous to the cholinomimetic substances discussed above are the sympathomimetic amines, which mimic the action of noradrenaline at adrenergic receptors. The structure of the adrenergic receptor and its binding of noradrenaline has been depicted diagrammatically as follows [28]:



α-Site Excitatory β-Site Inhibitory

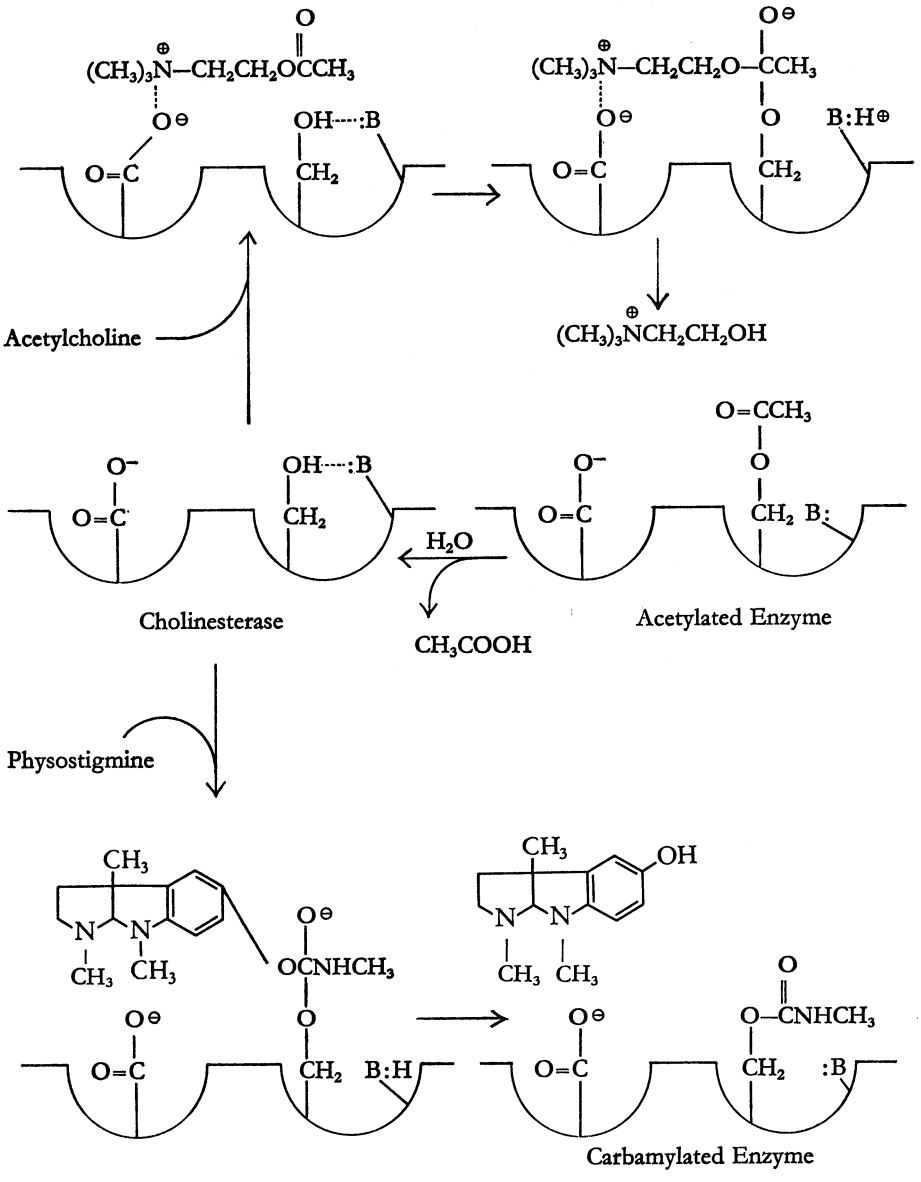


Fig. 15-1. Action and inhibition of cholinesterase

Binding at the α-site is by ionic attraction and hydrogen bonding, whereas a divalent metal ion such as Mg^{2+} serves at the β-site to link noradrenaline to the receptor. Since noradrenaline and related compounds can cause both excitation and inhibition of smooth muscle, the two sites have been proposed. However, as with the cholinergic receptor, it is likely that there is only one adrenergic receptor with its α-ness or β-ness determined by the biological environment [3]. Adrenaline with one N-methyl group shows

greater β-stimulation than noradrenaline. Substitution on the α-carbon of a phenylethylamine prolongs whatever action it has by preventing its destruction by monoamine oxidase.

Some effects of mescaline may be due to its stimulation of α-adrenergic sites [47]. The synthetic alkaloid apomorphine also has this action [23,229,266]. However, most sympathomimetic compounds do not act by directly mimicking the action of noradrenaline on its receptor. Rather, they are either releasers of noradrenaline, inhibitors of its reabsorption, or blockers of either the α- or β-site. Tyramine and nicotine are releasers of noradrenaline, probably from storage vesicles [8]. The protoalkaloid ephedrine acts as a releaser of noradrenaline and is also a true mimetic substance that stimulates both the α- and β-sites, causing both vasoconstriction and bronchial muscle relaxation. Cocaine potentiates the action of noradrenaline by hindering its reabsorption into the nerve ending from the synaptic cleft [139]. Reserpine acts on membranes of the storage vesicles within the nerve terminal, inhibiting their uptake of both noradrenaline and dopamine [88,215]. By this action not only is the store of noradrenaline depleted but the synthesis of new noradrenaline is prevented because β-hydroxylation of dopamine occurs in the vesicles. Noradrenaline outside the vesicles is gradually destroyed by enzymatic action, and all adrenergic responses are diminished. A compound that blocks the α-site allows responses characteristic of the β-site to predominate and thus acts as β-site stimulant. Several alkaloids act as α-adrenergic blocking compounds. These include yohimbine and some of the peptide ergot alkaloids [147,265, 289]. These natural compounds have other complex effects and are largely replaced in medicine by more specific, synthetic α-adrenergic blocking agents. β-Adrenergic blocking agents are less common and include only synthetic compounds. Veratramine increases the rate of catecholamine synthesis in isolated synaptosomes [192], but this may be secondary to its effect on ion transport (see below).

An important group of pharmacological agents are the monoamine oxidase inhibitors [200]. As pointed out above, MAO is not of crucial importance in removal of noradrenaline from junctions but rather acts as a scavenger of other amines that, if present, would stimulate the action of noradrenaline by releasing it from stores or mimicking it at its receptor. Inhibition of MAO therefore has the effect of stimulating adrenergic receptors. The amines that do the actual stimulating may be of endogenous of exogenous origin. For instance, cheese, which contains tyramine, may be quite toxic if consumed concurrently with a MAO inhibitor. Tyramine, dopamine, serotonin, etc., are also normal cell constituents in small amounts. The interpretation of a drug action as due primarily to MAO inhibition is extraordinarily difficult. Most MAO inhibitors show a number of other actions (e.g., inhibition of amine release); and compounds such as cocaine and ephedrine inhibit MAO in vitro, but their familiar pharmacological properties probably have nothing to do with this. The best known MAO inhibitors are synthetic hydrazine derivatives, but nonhydrazine

inhibitors are more selective for MAO. The harmala alkaloids are naturally occurring MAO inhibitors, although they may act in other ways, too [96]. Under certain conditions harmaline counteracts the amine-releasing effect of reserpine. Several tetrahydroisoquinolines and tetrahydroberberines are also powerful inhibitors of MAO [172]. The structural requirements for MAO inhibition are not clearly defined.

The Opiate Receptor

An enormous amount of information has accumulated on the biochemistry of the so-called opiate receptor. Although this receptor has not been purified in the way that the acetylcholine receptor has, much has been learned about it by indirect means. By using radioactively labeled alkaloids it was found that certain neurons of the brain bound morphine and related compounds stereoselectively and with high affinity. These neurons are concentrated in the limbic system of vertebrates [141,199]. Similar opiate binding does not occur in the nervous system of invertebrates [198] or in simpler organisms [82], even though morphine has effects on them [82,125]. There are also morphine receptors in other organs of vertebrates, but they show significant differences from the brain receptors [242]. Since morphine-like compounds were not believed to be endogenous to brain, a search for the natural ligand of the receptors began in the early 1970s and resulted in the discovery of the peptide transmitters enkephalins and endorphins. The former are pentapeptides, while the latter are larger molecules that incorporate the enkephalins as part of them [93,115,243]. The amino acid sequences of the two enkephalins are:

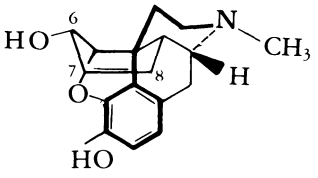
met-enkephalin: tyr-gly-gly-phe-met
leu-enkephalin: tyr-gly-gly-phe-leu

Pig brain has more of the first and beef brain more of the second [115,243]. The foregoing account glosses over the probability that there are probably several types of opiate receptors in the brain. Some may be less stereoselective than others, and it is even possible that besides the known peptides other endogenous transmitters may be discovered [41,118,250]. A non-peptide, morphine-like compound has been found in human spinal fluid [238]. Reviews on the opiate receptor appear regularly [156,246,254].

In spite of the reservation that there may be more than one type of opiate receptor, much can be said about the stereospecific receptor of brain synaptosomes. It evidently has both protein and lipid moieties since digestion with either proteolytic or lipolytic enzymes destroys it [190]. A partially purified proteolipid shows stereospecific binding of opiates with saturation at one molecule of opiate per 6.0×10^4 daltons of proteolipid [157]. A third group of the receptor is essential for opiate binding [168,245], and various inorganic ions have considerable influence on the binding—

presumably by controlling conformation of the receptor protein [29,191, 245,254]. The lipid part of the receptor is most likely a cerebroside. Treatment of synaptic membranes with cerebroside sulfatase destroys binding sites [151], antibodies to cerebroside sulfate inhibit morphine effects [54], and cerebroside sulfate by itself is physically affected by the presence of morphine [45,120,254]. Opiates also bind to phosphoglycerides, and this may account for some of the nonspecific binding to lipid membranes.

Many analogues of morphine and the enkephalins have been tested for their binding activity in order to define the nature of the binding site. A quaternary carbon atom to which is attached a phenyl group and a β-aminoethyl group is a requirement for activity. This grouping is emphasized in the following conformational structure of morphine:



The phenolic ring and nitrogen atom of morphine correspond to the tyrosine residue of the enkephalins. Although the phenolic hydroxyl group of morphine is at its optimal position for activity, its complete loss does not destroy activity [111]. C-7 and C-8 of morphine correspond to the leucyl or methionyl side chains of the enkephalins, and the 6-OH group to the carboxyl terminus [251]. This alignment of functional groups is dependent on the enkephalins having a β-bend in the second glycine molecule [31,225]. Some potent synthetic derivatives of morphine have hydrophobic substituent groups analogous to the phenylalanine side chain of enkephalins, but no natural alkaloids have this. Conformationally, mobile compounds show more divergent modes of action because they can interact with receptors in more than one way [204]. Some receptor models assume that there are different preferred conformations for reaction with agonists or antagonists [191], but other models assume that a single conformation is sufficient [138].

The sequence of molecular events that follows binding of morphine (or other opiate) to the receptor is still a subject of vigorous research and speculation. Clearly, complex interactions with other regulatory molecules remain to be sorted out. One line of investigation relates morphine action to cyclic AMP. Although morphine has no effect on basal levels of cAMP in brain cells, it counteracts the increase in cAMP that can be caused by such substances as noradrenaline [271], dopamine [288], or prostaglandins [50,105]. In turn, this effect of morphine can be reversed by naloxone, adenosine, or potassium ion [50,105]. It appears from these results that the presence of receptor-opiate complex somehow interferes with the reception of other transmitters that stimulate adenylate cyclase [271]. Cultured

neural cells respond somewhat differently than slices or homogenates of brain. Their basal adenylate cyclase is inhibited by morphine and after a time activity is restored as a result of a more active form of the enzyme becoming predominant [117,234,235]. Besides antagonizing the effects of prostaglandins and catecholamines on adenylate cyclase, morphine also stimulates the synthesis of prostaglandins in homogenates [18,49] and the synthesis of dopamine in whole animals [38,48,81]. Some longer-term effects of morphine on the metabolism of whole animals are doubtless secondary effects that result from changed levels in several hormones—not only catecholamines and prostaglandins but also serotonin [137], pituitary hormones [171], and steroid hormones [146]. These later metabolic effects show up as alterations in carbohydrate metabolism [67,294], lipid metabolism [64, 180,263], and glycoprotein synthesis [64]. It would not be surprising to find some involvement of calmodulin with the effects of morphine on brain cells. This important regulatory protein is present at high concentrations in brain, it regulates certain adenylate cyclases, and morphine inhibits calcium transport across membranes [222]. As yet, though there is no information on this possibility [134].

Several attempts have been made to explain addiction to opiates in molecular terms. There is no difference in morphine binding or rate of breakdown when morphine-dependent rats are compared with controls [135]. A generalized biochemical theory proposes that addicting drugs inhibit the action of some enzyme (or enzymes) whose synthesis is repressed by a product (or products) of its action [12,94]. When the enzyme's action is inhibited, less product is made; and as a result more enzyme is synthesized in order to maintain the normal metabolic equilibrium. In the presence of an inhibitor, normal metabolism can be maintained only by abnormally high concentrations of enzyme—a situation corresponding to drug "tolerance." When the inhibiting agent is removed, drastic metabolic imbalance occurs—a situation corresponding to the "withdrawal reaction." It has been shown that development of tolerance to morphine can be prevented by administering cycloheximide to mice. This is evidence that some new protein must be made for tolerance to be established [283]. This could be an enzyme in the catecholamine biosynthetic pathway since the rate of conversion of tyrosine to dopamine is increased in morphine-treated rats [48,81]. Catecholamines also appear responsible for some withdrawal symptoms [108]. A different enzyme that could be implicated is a peptidase of brain that is highly specific for leu-enkephalin. In mice treated chronically with morphine, the activity of this peptidase increases greatly while other peptidases are unaffected. Upon removal of morphine, withdrawal symptoms could result from lack of both the alkaloid and the enkephalin at the receptor [162]. Still other explanations suggest that antibodies to morphine [22] or the α-aminobutyric acid system [109] are involved in morphine tolerance.

Serotonin Receptors

Although relatively little is known about the properties of isolated serotonin receptors, they are of great interest as the presumptive sites for action of several hallucinogenic compounds. The most powerful of the hallucinogenic drugs is the partially synthetic derivative of ergot alkaloids, lysergic acid diethylamide (LSD). Natural ergot alkaloids, psilocin, bufotenine, and harmine derivatives are believed to have a similar mechanism of action [2,154,228,253]. The binding activity of these compounds to brain membranes and their ability to displace serotonin correlates with their pharmacological activity [21,241]. When the two isomers of LSD are compared, the natural D-isomer binds with much greater affinity than the L-form [21]. Several observations have suggested that a ganglioside is an essential part of the serotonin receptor. A ganglioside with high affinity for serotonin has been prepared, and reserpine releases serotonin quickly and completely from the complex [89–91]. Other aspects of the serotonin system are that mescaline can act to stimulate the synthesis of serotonin from tryptophan [236], and that quinidine or reserpine depress the ability of blood platelets to take up and hold serotonin [32]. A peculiar effect of LSD on higher plants is that it appears to cause mutations in genes required for chlorophyll synthesis [249].

Miscellaneous Membrane Receptors

Besides the much-studied systems discussed above, several other types of cell membrane receptors are known to be affected by particular alkaloids at concentrations low enough to have physiological relevance. γ-Aminobutyric acid (GABA) and glycine are inhibitory transmitters in certain brain neurons. The benzylisoquinoline alkaloid (+)-bicuculline is a specific antagonist of GABA at the receptor [163,176,244] as are the pyridine alkaloids guvacine and arecaidine [122]. Strychnine is a specific antagonist of glycine at its receptors [256,299]. Although the major effect of d-tubocurarine is on the cholinergic system, it is also an antagonist of L-glutamate at neuroreceptors for this amino acid [296]. Receptors for adenosine are widespread but comparatively little studied. They are probably involved with the cyclic-AMP system (see below) and are antagonized by methylated xanthines such as caffeine [24].

Ion Transport and Membrane Permeability

Several alkaloids evidently owe their effects to their activity in affecting the transport of metal ions across biological membranes. Since these ions are

important in regulating enzymatic activity, structural components, and electrical properties, important consequences follow from changes in ionic equilibria. One of the best-known ion transport systems is the sodium-potassium ATPase that is present in many cell membranes and is responsible for uptake of potassium ions and extrusion of sodium ions. The most familiar inhibitors of this system are cardiac glycosides such as ouabain and digitoxin, but several alkaloids also inhibit this enzyme. Harmaline and related compounds interact with both the sodium and potassium sites. Transport of amino acids is also inhibited by these alkaloids, but probably as a secondary effect of the sodium ion imbalance [232,248,257]. Sanguinarine is another alkaloid that inhibits the same enzyme, but unlike harmaline it is not competitive with sodium or potassium ions [230,261]. It may also have additional effects on sodium transport that are not connected with the ATPase [181]. Some cases of dropsy and glaucoma may be caused by consumption of plant products that contain sanguinarine [71]. Cassaine, a diterpenoid alkaloid, and the modified steroid alkaloids of *Veratrum* spp. also have been shown to inhibit Na-K ATPase; but the latter may have additional effects on the lipid bilayer [80,87,203,268].

In addition to the Na-K ATPase, there are other systems involved in sodium and potassium transport. In nerve axons, for example, it is believed that ion-specific pores open and close in response to changes in membrane potential. Ion movements then propagate the change in potential along the axon. A few compounds appear to affect the ion channels fairly directly. Batrachotoxin increases permeability to sodium ion [5] while tetrodotoxin specifically blocks the sodium channels [5]. Yohimbine also acts to close sodium channels, but perhaps not directly [13]. Several peptide antibiotics act as ionophores, inserting themselves into membranes and increasing the conductance of specific ions. The cyclopeptide alkaloid frangulanine appears to act this way with potassium or rubidium ions [132]. The presence of calcium in the axonal membrane is essential for proper functioning of the sodium and potassium exchanges. Cocaine and related synthetic compounds such as procaine inhibit axonal conduction by competing with Ca^{2+} for sites on the membrane. Displacement of calcium by such substances increases the permeability and therefore decreases the ease with which an action potential can be initiated. Most of the calcium in the membrane appears to be bound to phosphatidylserine [27]. Plants may have a similar system since the inhibition of lettuce seed germination by procaine is relieved by Ca^{2+} [178]. The *Veratrum* alkaloids cause increased sodium permeability that can be prevented by either Ca^{2+} or cocaine [87,201]. Theophylline increases potassium conductance, perhaps by increasing the availability of calcium [252].

Calcium is also important for the functioning of muscle, and several alkaloids by controlling Ca^{2+} availability affect a muscle's response to stimulation. Caffeine causes a release of accumulated Ca^{2+} and potentiation of the twitch [185,221]. Quinine has a similar end result, but the detailed

mechanism may be different [88]. Papaverine decreases the availability of Ca^{2+} and acts spasmolytically [78].

Several alkaloids seem to affect the permeability of membranes to organic molecules. The effect of harmaline on amino acids has already been mentioned [257]. Veratridine not only increases Na^{+} permeability but also stimulates release of ATP from cholinergic synaptosomes [201]. α-Tomatine increases the permeability of plant cell membranes to many different solutes. Its effect can be overcome by increased Ca^{2+} concentrations [211,212]. Glucose uptake into ascites cells is inhibited by berberine [56].

Metabolic Effects

So-called metabolic effects of several alkaloids have been noted from time to time. These are effects on degradative pathways of metabolism such as glycolysis, the citric acid cycle, or fat oxidation. A few of these effects can now be explained as resulting from more or less specific enzyme inhibitions. The best examples of such effects are connected with the role of cyclic 3′,5′-adenosine monophosphate (3′,5′-AMP). This nucleotide activates phosphorylase a, thus stimulating glycolysis, and also activates lipase, thus stimulating fat breakdown. Its formation from ATP is stimulated by adrenaline or noradrenaline (although all adenylate cyclases are not responsive to catecholamines [153]); its destruction by phosphodiesterase is inhibited competitively by theophylline. Thus methylxanthines and catecholamines can act together, but at different sites, to increase blood sugar levels and oxygen consumption, or to stimulate other processes dependent on 3′,5′-AMP. Papaverine is another alkaloid that inhibits some phosphodiesterases. It must be remembered, though, that theophylline and papaverine both have other effects, so that their effect on a process cannot be taken as firm evidence that cyclic AMP is involved [88,183,279,286]. Theophylline and caffeine inhibit many enzymes of purine metabolism [183] as well as some alkaline phosphatases [58]. Caffeine also has effects on glycogen synthesis and utilization that are unrelated to cyclic AMP [130]. It would be interesting to know whether methylated xanthines or papaverine might function in the plants that contain them as regulators of cyclic-AMP levels. However, the whole question of cAMP function in plants remains unsettled. It has been shown that some phosphodiesterases from higher plants are inhibited by these alkaloids, but others are not [34,75,129].

Several miscellaneous inhibiting effects of alkaloids on enzymes do not lend themselves to an organized discussion but are presented in Table 15-2.

A number of alkaloids can be grouped together as inhibitors of the kind of electron transport processes that occur in mitochondria, microsomes, and chloroplasts. Papaverine [173], tubocurarine, lasiocarpine, and heliotrine are inhibitors [83] of mitochondrial respiration, but the sites of action are not known. Reserpine is an uncoupling agent that may act by increasing

Table 15-2. Enzyme inhibition by alkaloids

Alkaloid	Enzyme inhibited	Reference
Ajmaline	ATPase of chloroplasts	275
Berberine	Alcohol dehydrogenase	194
Cinchona alkaloids	Diamine oxidase	196
Cocaine	Particulate tryptophan hydroxylase	137
Colchiceine	Phosphatases	240
Harmaline	Sucrase	160
Mimosine	Pyridoxal-dependent enzymes	136
Quinidine	ATPase, pyruvate kinase, lactic dehydrogenase	110
Salsolinol	Tyrosine hydroxylase	285
d-Tubocurarine	Dehydrogenases	264

permeability to hydrogen ion [161]. Spegazzine inhibits oxidative phosphorylation at a site on the ATP side of the oligomycin-sensitive site [217]. Several quinoline alkaloids inhibit electron transport between the B and C cytochromes [4]. The microsomal P-450 system is inhibited by ellipticine, 9-hydroxyellipticine [155], emetine [121], nicotine [284], pilocarpine [272], and pyrrolizidines [73]. Photophosphorylation in chloroplasts is inhibited by several quinolines at several different sites [287]; by benzophenanthridines, which appear to act as uncouplers [274]; and by many peptide alkaloids, some acting as uncouplers and some stimulating H^+ uptake [206].

Two biosynthetic processes that are specifically inhibited by alkaloids although the exact sites are not yet identified are carotenoid biosynthesis and ascorbic acid biosynthesis. In both microorganisms and higher plants, nicotine inhibits normal carotenoid synthesis. Many observations indicate that it interferes with cyclization so that only open-chain compounds are made [98,114,169]. In halophilic bacteria, nicotine inhibits the hydroxylation of C_{50} carotenoid-like pigments that are characteristic of these organisms [143, 144]. Lycorine shows various inhibitory effects on the growth of higher plants, and its primary effect is likely to be as an inhibitor of ascorbic acid biosynthesis. Secondary effects appear as anomalies of cell division, elongation, and synthetic processes [9,65]. In *Clinia miniata*, which contains lycorine, the alkaloid does not inhibit synthesis of ascorbic acid [10].

Effects on Nucleic Acids and Protein Synthesis

Amid the enormous amount of information now available regarding nucleic acid structure and the processes of replication, transcription, and translation, there are several observations that show specific effects of alkaloids on these systems. While some of these effects may have no pharmacological

relevance, others go a long way toward explaining the behavioral or morphological effects of certain alkaloids.

It is not surprising that many alkaloids bind to nucleic acid molecules simply by electrostatic attraction between anionic phosphate groups and cationic nitrogen atoms. Besides this, though, there are more specific interactions between alkaloids and nucleic acid molecules. The best-defined of these specific interactions is described as intercalation, where the alkaloid molecules are inserted and occupy sites at the interior of the DNA double helix. Intercalation can be recognized as a high-affinity process in contrast to low-affinity binding by electrostatic attraction at the periphery. Physical evidence of intercalation into the DNA molecule has been obtained for berberine [142], colchicine [36], ellipticine [258], harman derivatives [69,149], irediamine A [223], quinacrine [179], and quinine [72]. Other alkaloids, which bind reversibly but in an unknown way, are caffeine [148,174], LSD [35,281], mimosine [208], quaternary *trans*-quinolizidines [59], and steroidal diamines [159]. None of the above form covalent bonds with the DNA molecule, but certain carcinogenic alkaloids are believed to owe their toxicity to being alkylating agents that permanently modify DNA or other essential macromolecules. A general theory of alkylating drugs is presented in [177]. Among alkaloids the most likely candidates for acting by this mechanism are certain pyrrolizidines [116] and arecaidine [11].

The immediate molecular consequences of an alkaloid's binding to DNA are certainly not uniform or predictable. Sometimes the presence of an alkaloid may affect the binding of a normal regulator. For example, caffeine enhances binding of the glucocorticoid receptor protein from rat liver to DNA [37]. Caffeine also inhibits binding of the carcinogen dimethylbenzanthracene [239] and protects DNA in whole cells against irradiation damage—perhaps partly by an effect on the repair mechanism [227,237].

The inhibition of DNA replication by alkaloids could be the result of binding to the template, but it could just as well be the result of binding to the DNA polymerase. Therefore, while it is known that in several cases alkaloids inhibit replication, there are few cases where it is known exactly how they do so. The presence of caffeine or camptothecin in the medium causes cultured tumor cells to make DNA of abnormally low molecular weight [112,152], suggesting interference with the template rather than the enzyme. Mimosine [208,282], reserpine [100], and vinleurosine [55] all inhibit incorporation of nucleotides into DNA. One case of inhibition of DNA polymerase is shown by a mixture of alkaloids from *Narcissus tazetta* [189].

Alkaloids may also affect the transcription process either by binding to the DNA template or to RNA polymerases. Although several are known to influence the overall process, little detail is known about their mechanisms of action. There are conflicting reports about the effect of caffeine on RNA synthesis—that it has no effect [175], that it is inhibitory [301], or that it is stimulatory [174,182]. The last cases are most interesting because they show

caffeine not as an overall stimulant of transcription but as a selective activator of specific messenger-RNA molecules for microsomal enzymes [174] and for adenylosuccinate lyase [182]. Quinine applied to pea pods induces phenylalanine-ammonia lyase by stimulating formation of the mRNA for it [101]. Quite a few other alkaloids inhibit transcription as measured by incorporation of nucleotides into RNA. Among these are berberine [56], colchicine [57], emetine [92], lasiocarpine [207], and several *Vinca* alkaloids [55]. Reserpine and ergocornine specifically inhibit synthesis of a steroid-induced mRNA in bacteria [280], and colchicine similarly inhibits synthesis of mRNA for S-100 protein in rat glial cell cultures [167].

Reverse transcription of DNA from RNA templates is an important process in the infection of mammalian cells with certain tumor-inducing viruses. The enzyme known as "reverse transcriptase" (RNA-dependent DNA polymerase) is responsible for this process. Methylxanthines such as caffeine [259], or more effectively several benzophenanthridines [233], are inhibitors of this reverse transcription process, and they evidently interact with the primer rather than with the enzyme.

Ribosomal protein synthesis can be interfered with by alkaloids at several different stages. Mescaline treatment of isolated ribosomes makes them less stable so that various components are released into the medium [62], and ribonuclease or trypsin catalyze faster breakdown [61]. Replacement of endogenous spermidine by mescaline may be a partial explanation for the lowered stability [61]. Affected ribosomes are less efficient in synthesizing protein [60,63]. The site of action of narciclasine in inhibiting protein synthesis is well-defined. It acts only on eukaryotic ribosomes, binding to the 60S subunit at the peptidyl transferase center so that access of the 3'-OH of the donor substrate is blocked and no peptide bond can be formed [14,39]. At concentrations in the range of 10^{-5}M, narciclasine inhibits growth of onion roots and causes mitotic aberrations [231]. The related alkaloid, lycorine, also inhibits ribosomal protein synthesis, perhaps by the same mechanism [66]. LSD modifies the immunoglobulin produced by rabbit lymphoid cells in culture. It evidently interferes specifically with tryptophan incorporation, and a derivative of LSD replaces a tryptophan residue [293]. Several alkaloids act similarly to the well-known antibiotic, cycloheximide, in that they inhibit eukaryotic ribosomal protein synthesis by inhibiting translocation of the growing peptide chain from the A site to the P site of the ribosome. Several ipecachuana, phenanthroquinolizidine, and phenanthroindolizidine alkaloids act in this way [37,68,123]. Surprisingly, pretreatment of whole animals with these alkaloids *increases* the activity of protein synthesis in ribosomes prepared for them [123,124].

Mitosis Inhibitors

As mentioned earlier, some alkaloids may secondarily cause mitotic aberrations, but there are a few alkaloids that show a very powerful and primary effect on this process. Colchicine has been known for many years to allow

chromosome duplication while blocking nuclear division, so that polyploid cells are produced. More recently, vinblastine and related alkaloids have been found to act similarly, causing dissolution of the mitotic spindle that is necessary for nuclear division [30,70]. The mechanism of this effect has been studied extensively; and there is no doubt that these alkaloids bind to tubulin, which is the structural protein of microtubules that, in turn, are essential to the spindle apparatus [70]. Tubulin from many different sources—both from different organs in the same species and between species—appears to be essentially the same protein, a polymer based on two quite similar subunits [218,262]. These α and β monomers join to form a dimer of molecular weight about 1.2×10^5 daltons. Binding of one molecule of colchicine or vinblastine per dimer prevents microtubule formation [166], but some further polymerization can occur after binding of colchicine [197]. Neither alkaloids binds to completed microtubules or destroys them [291,292]. The interrelationships between vinblastine and colchicine are complex. Although vinblastine can displace colchicine from tubulin, it does so noncompetitively, showing that they have different sites [33,302]. Vinblastine even stabilizes colchicine binding [290]. A number of observations can be interpreted as showing that the tubulin dimer first binds colchicine at a low-affinity site and that this binding changes the conformation of the protein to expose a high-affinity site. As a result, colchicine binding is completed over a period of hours [85]. In contrast, two sites for vinblastine are both available for immediate reaction. Moreover, binding to vinblastine may cause the conformational change that exposes the high-affinity site for colchicine [292]. Other compounds such as podophyllotoxin react at the low-affinity colchicine site but not at the high-affinity site [52,302]. The two vinblastine sites have affinity constants differing by about a factor of 100. There is a range of values reported by various authors, but order of magnitude values appear to be:

Colchicine, high affinity	$3 \times 10^7\ M^{-1}$
Vinblastine, high affinity	$6 \times 10^6\ M^{-1}$
Vinblastine, low affinity	$8 \times 10^4\ M^{-1}$
Colchicine, low affinity	$6 \times 10^3\ M^{-1}$

Binding of vinblastine to the high-affinity site stabilizes colchicine binding, prevents assembly of microtubules, and causes crystal formation in cells [25,291]. Binding at the low-affinity site results in precipitation of tubulin [25,291]. Additional proteins, guanosine triphosphate, and magnesium ion are all involved in the process of microtubule formation. There are some competitive effects between colchicine and the proteins but not between colchicine and GTP or colchicine and magnesium [103,158,184,197].

The structural requirements for binding of colchicine and vinblastine to tubulin have been investigated by measuring binding constants and competitions for many analogous structures. For colchicine it is clear that two aromatic rings and the methoxyl groups are necessary. The tropolone ring C can be replaced by a benzene ring, but a polar group corresponding to the carbonyl must be present. The N-acetyl group has only a slight importance

[79,133]. The low-affinity binding site may interact with the methoxyl groups of ring A, and the high-affinity site with ring C. For vinblastine the dimeric structure is very important. The vindoline moiety alone has no activity and the catharanthine moiety only about 1/1000 the activity of the dimeric alkaloid [291]. The methoxycarbonyl group of the catharanthine moiety is important. Although the vindoline portion by itself is inactive, it cannot be replaced in the dimeric alkaloid with a simpler indole [300]. Loss of the acetyl group increases activity [188].

Although colchicine was discovered by its effect on mitosis, it has other effects on cells. Some of these may relate to its binding to tubulin, since microtubules participate in processes besides mitosis. Intracellular transport of proteins is a process dependent on microtubules and inhibited by colchicine [40,170]. Microtubules may be involved in the response of adipose tissue to insulin; and colchicine inhibits the insulin response [226], although it does not bind to the presumed insulin receptor of muscle cells [74]. Related effects may be the inhibition by colchicine of cholesterol synthesis [187], or ornithine decarboxylation [42], of the release of norepinephrine from hypogastric nerve [26]; and its stimulation of triglyceride synthesis [187], and of histamine sensitivity [219]. The inhibition of normal or gibberellin-induced elongation might be a result of colchicine's effect on cortical microtubules that control orientation of cellulose microfibrils [107,195,224]. Other effects of colchicine may have nothing to do with tubulin and result from its lower-affinity binding to many other proteins, perhaps sufficiently to affect membrane-bound processes [7,260,295]. Examples of this kind of activity could be the inhibition of cell aggregation or agglutination [273,298] and an inhibition of gibberellin-induced α-amylase synthesis [195].

Some effects of colchicine on whole cells may even be effects of its metabolites. For instance, colchiceine, which has a free hydroxyl group on ring C, binds to membrane gangliosides [214]. A last explanation for some colchicine effects depends only on properties of the tropolone ring. Tropolones are powerful metal-ion chelators and could inhibit processes dependent on such ions. The resemblance of tropolones to phenols may account for the fact that colchicine is a powerful inhibitor of catechol-O-methyltransferase [20].

In some of the miscellaneous processes discussed in the preceding paragraph vinblastine acts similarly to colchicine [42,219,298]. Unlike colchicine, however, it inhibits binding of insulin to muscle cells [74].

Several less well investigated alkaloids act similarly to colchicine and vinblastine as inhibitors of tubulin polymerization. Mescaline binds to tubulin and from its structure may be presumed to act at the low-affinity colchicine site [102]. Caffeine and other xanthines inhibit cytokinesis, possibly but not necessarily by an effect on microtubules [16,107,127]. Maytansine resembles vinblastine more than colchicine [164]. It competes with vinblastine at both the high- and low-affinity sites. It does not cause aggregation as vinblastine does and even prevents the aggregation caused by

the latter [25]. Finally, even morphine, which has enough other ramifications, can affect mitosis in onion root tips [218].

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