6

ALKALOIDS

The alkaloids are low molecular weight nitrogen-containing compounds found mainly in plants, but also to a lesser extent in microorganisms and animals; over 27 000 different alkaloid structures have been characterized, with 21 000 from plants. They contain one or more nitrogen atoms, typically as primary, secondary, or tertiary amines, and this usually confers basicity on the alkaloid, facilitating isolation and purification, since water-soluble salts can be formed in the presence of mineral acids. The name alkaloid is in fact derived from alkali. However, the degree of basicity varies greatly, depending on the structure of the alkaloid molecule and on the presence and location of other functional groups. Indeed, some alkaloids, e.g. where the nitrogen is part of an amide function, are essentially neutral. Alkaloids containing quaternary amines are also found in nature. The biological activity of many alkaloids is often dependent on the amine function being transformed into a quaternary system by protonation at physiological pH values.

Alkaloids are often classified according to the nature of the nitrogen-containing structure (e.g. pyrrolidine, piperidine, quinoline, isoquinoline, indole), though the structural complexity of some examples rapidly expands the number of subdivisions. The nitrogen atoms in alkaloids originate from an amino acid, and, in general, the carbon skeleton of the particular amino acid precursor is also largely retained intact in the alkaloid structure, though the carboxylic acid carbon is often lost through decarboxylation. Accordingly, subdivision of alkaloids into groups based on amino acid precursors forms a rational and often illuminating approach to classification. Relatively few amino acid precursors are actually involved in alkaloid biosynthesis, the principal ones being ornithine, lysine, nicotinic acid, tyrosine, tryptophan, anthranilic acid, and histidine. Building blocks from the acetate, shikimate, or methylerythritol phosphate pathways are also frequently incorporated into the alkaloid structures. However, a large group of alkaloids are found to acquire their nitrogen atoms via transamination reactions, incorporating only the nitrogen from an amino acid, whilst the rest of the molecule may be derived from acetate or shikimate; others may be terpenoid or steroid in origin. The term 'pseudoalkaloid' is sometimes used to distinguish this group.

ALKALOIDS DERIVED FROM ORNITHINE

L-**Ornithine** (Figure 6.1) is a non-protein amino acid forming part of the urea cycle in animals, where it is produced from L-arginine in a reaction catalysed by the enzyme arginase. In plants it is formed mainly from L-glutamic acid (Figure 6.2). Ornithine contains both δ- and α-amino groups, and it is the nitrogen from the former group which is incorporated into alkaloid structures along with the carbon chain, except for the carboxyl group. Thus, ornithine supplies a C_4N building block to the alkaloid, principally as a pyrrolidine ring system, but also as part of the tropane alkaloids (Figure 6.1). Most of the other amino acid alkaloid precursors typically supply nitrogen from their solitary α-amino group. However, the reactions of ornithine are almost exactly paralleled by those of L-lysine, which incorporates a C_5N unit containing its ε-amino group (see page 326).

Polyamines

Simple polyamines were first isolated from human semen over 300 years ago, though another 250 years

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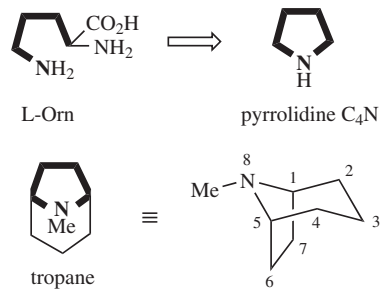


Figure 6.1

passed before their chemical characterization as **spermidine** and **spermine** (Figure 6.2). The simpler compound **putrescine** (1,4-diaminobutane) was first isolated from *Vibrio cholerae*, but its common name relates to its presence in decomposing animal flesh.

We now know polyamines are found in virtually all living species and are critical regulators of cell growth, differentiation, and cell death. In eukaryotic cells, the three polyamines are synthesized from L-ornithine and L-methionine. In animals, PLP-dependent decarboxylation (see page 22) of ornithine gives putrescine. In plants and microorganisms, an alternative sequence to putrescine starting from arginine also operates concurrently as indicated in Figure 6.2. The arginine pathway also involves decarboxylation, but requires additional hydrolysis reactions to cleave the guanidine portion. Aminopropyl groups are then transferred from a decarboxylated SAM (dcSAM) to putrescine. The alkylation reaction is mechanistically analogous to SAM-mediated methylation, though an alternative alkyl group is transferred. These reactions give firstly spermidine and then spermine. The polyamine pathway is an important target for chemotherapy, since depletion of polyamines can lead to the disruption of a variety of cellular functions. Spermidine synthase is currently considered a promising drug target in the malaria parasite *Plasmodium falciparum* (see page 218).

Pyrrolidine and Tropane Alkaloids

Simple pyrrolidine-containing alkaloid structures are exemplified by hygrine and cuscohygrine found in those plants of the Solanaceae which accumulate medicinally valuable tropane alkaloids such as hyoscyamine or cocaine (see Figure 6.4). The pyrrolidine ring system is formed initially as a Δ^1-pyrrolinium cation (Figure 6.3). **Putrescine** is methylated to *N*-methylputrescine, then oxidative deamination of *N*-methylputrescine by the action of a diamine oxidase (see page 27) gives the aldehyde. The *N*-methyl-Δ^1-pyrrolinium cation is then generated via imine formation. Indeed, the aminoaldehyde in aqueous solution is known to exist as an equilibrium mixture with the imine.

The extra carbon atoms required for hygrine formation are derived from acetate via acetyl-CoA, and the sequence appears to involve stepwise addition of two acetyl-CoA units (Figure 6.4). In the first step, the enolate anion from acetyl-CoA acts as nucleophile towards the pyrrolinium ion in a Mannich-like reaction, which could yield products with either *R* or *S* stereochemistry. The second addition is then a Claisen condensation extending the side-chain, and the product is the 2-substituted pyrrolidine, retaining the thioester group of the second acetyl-CoA. **Hygrine** and most of the natural tropane alkaloids lack this particular carbon atom, which can be lost by suitable hydrolysis/decarboxylation reactions.

The bicyclic structure of the tropane skeleton in **hyoscyamine** and **cocaine** is achieved by a repeat of the Mannich-like reaction just observed. This requires an oxidation step to generate a new Δ^1-pyrrolinium cation, and removal of a proton α to the carbonyl. The *intramolecular* Mannich reaction on the *R* enantiomer accompanied by decarboxylation generates **tropinone**, and stereospecific reduction of the carbonyl yields **tropine** with a 3α-hydroxyl, or the isomeric **ψ-tropine**, the precursor of the calystegines (see page 321). Hyoscyamine is the ester of tropine with (*S*)-tropic acid, which is derived from L-phenylalanine via phenyl-lactic acid (see Figure 6.5).

Should the carboxyl carbon from the acetoacetyl side-chain not be lost as it was in the formation of tropine, then the subsequent intramolecular Mannich reaction will generate a tropane skeleton with an additional carboxyl substituent (Figure 6.4). However, this event is rare, and is only exemplified by the formation of ecgonine derivatives such as **cocaine** in *Erythroxylum coca* (Erythroxylaceae). The pathway is in most aspects analogous to that already described for hyoscyamine, but must proceed through the *S*-enantiomer of the *N*-methylpyrrolidineacetoacetyl-CoA. The ester function is then modified from a coenzyme A thioester to a simple methyl oxygen ester, and **methylecgonine** is subsequently obtained from the methoxycarbonyltropinone by stereospecific reduction of the carbonyl. Note that, in this case, reduction of the carbonyl occurs from the opposite face to that noted with the tropinone → tropine conversion and, thus, yields the 3β configuration in ecgonine. **Cocaine** is a diester of ecgonine, the benzoyl moiety arising from phenylalanine via cinnamic acid and benzoyl-CoA (see page 157).

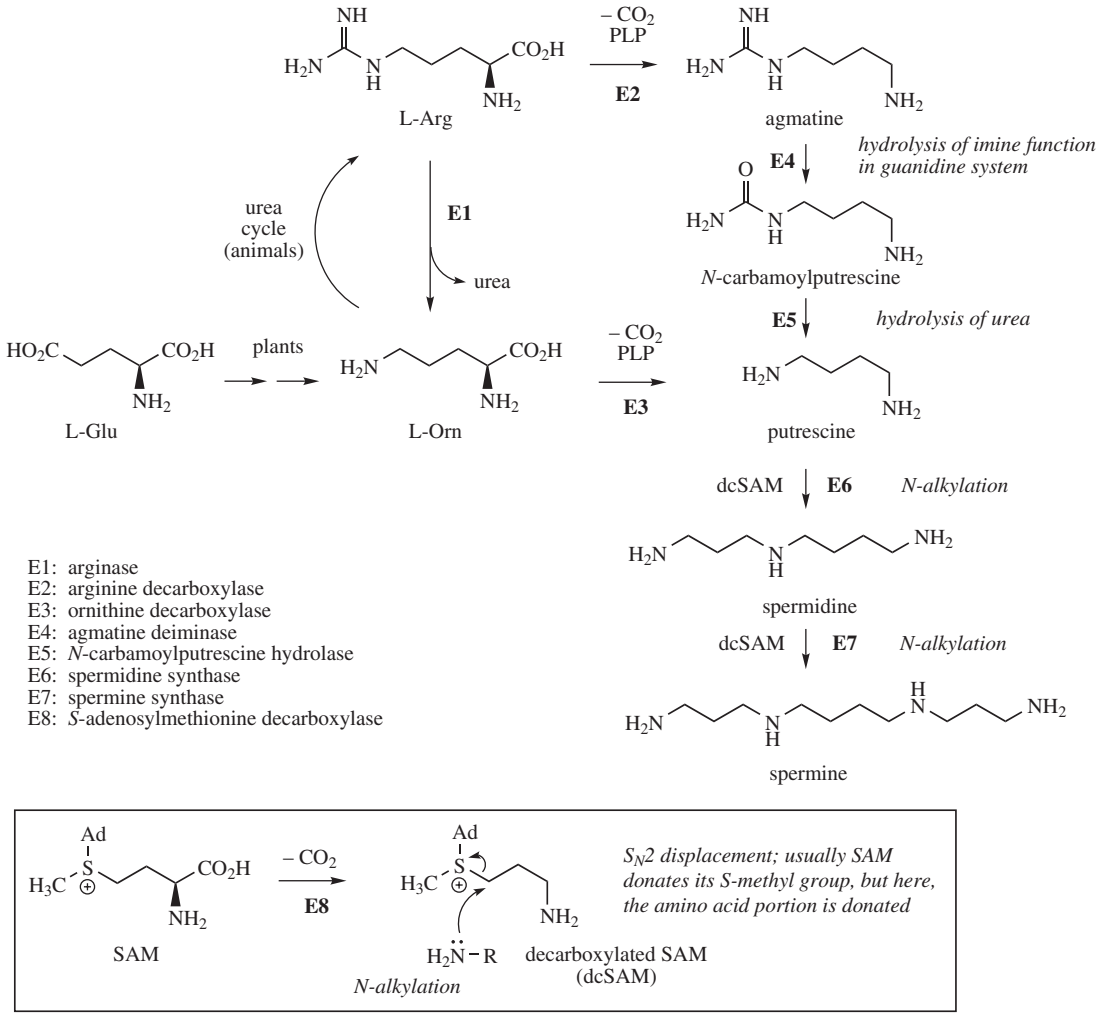


Figure 6.2

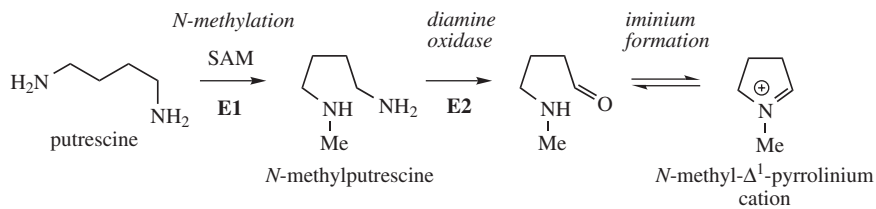


Figure 6.3

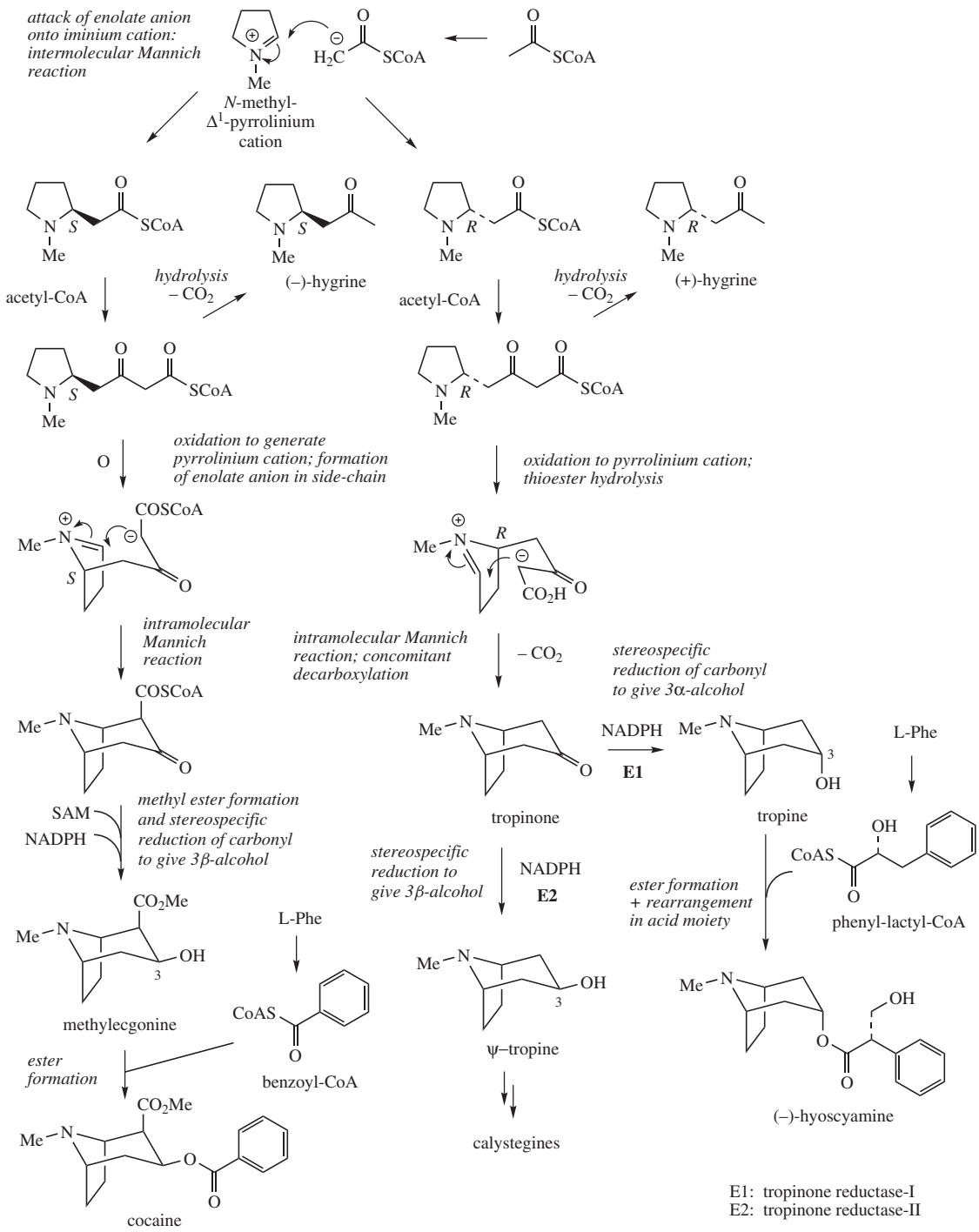

attack of enolate anion onto iminium cation: intermolecular Mannich reaction
N-methyl-Δ1-pyrrolinium cation
hydrolysis – CO2
(–)-hygrine
(+)-hygrine
acetyl-CoA
oxidation to generate pyrrolinium cation; formation of enolate anion in side-chain
oxidation to pyrrolinium cation; thioester hydrolysis
intramolecular Mannich reaction
intramolecular Mannich reaction; concomitant decarboxylation
– CO2
stereospecific reduction of carbonyl to give 3α-alcohol
NADPH
E1
tropinone
tropine
L-Phe
phenyl-lactyl-CoA
ester formation + rearrangement in acid moiety
methyl ester formation and stereospecific reduction of carbonyl to give 3β-alcohol
SAM
NADPH
methylecgonine
benzoyl-CoA
ester formation
cocaine
stereospecific reduction to give 3β-alcohol
NADPH
E2
ψ–tropine
calystegines
(–)-hyoscyamine
E1: tropinone reductase-I
E2: tropinone reductase-II

Figure 6.4

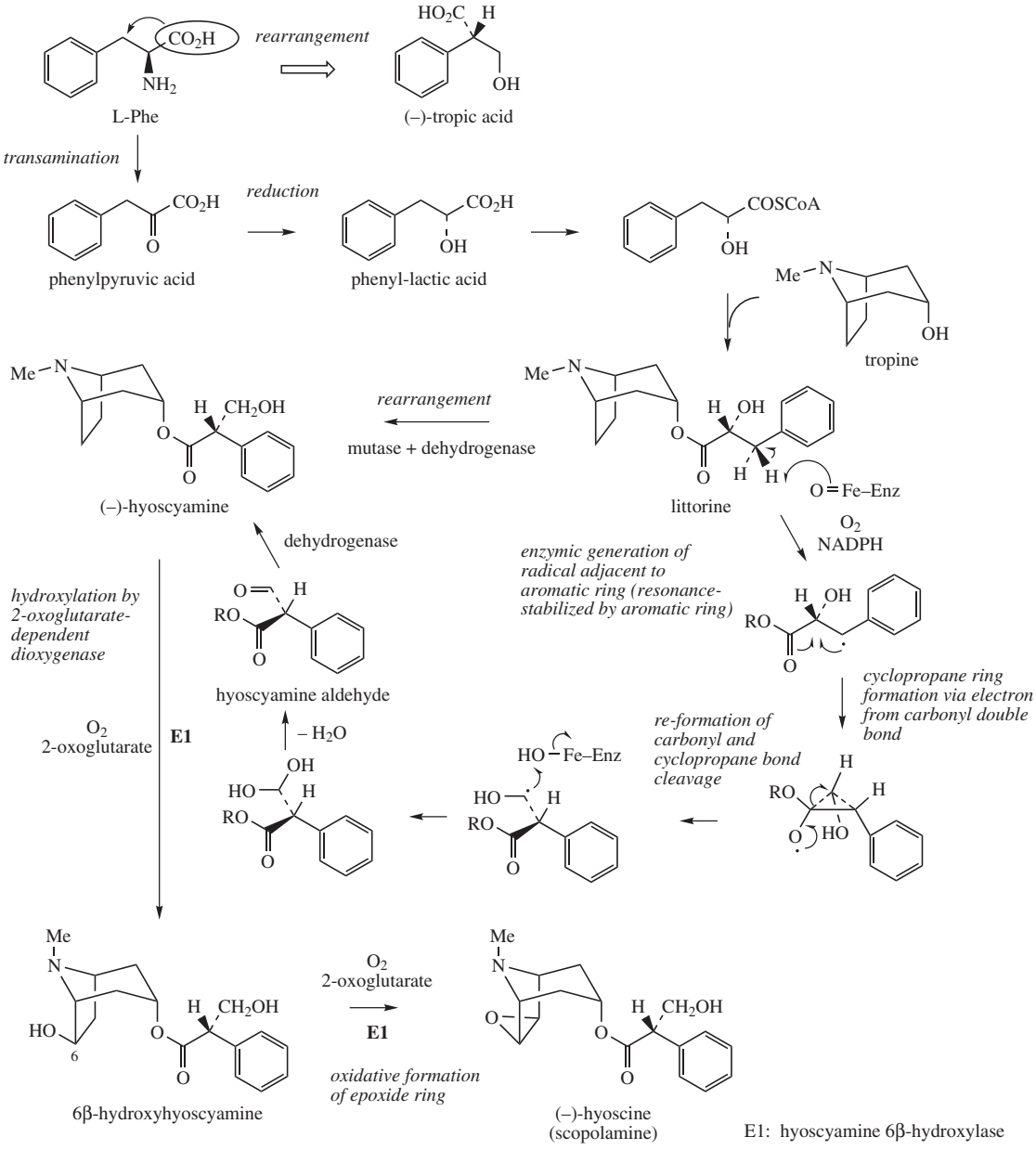


Figure 6.5

A novel rearrangement process occurs in the phenylalanine → tropic acid transformation in which the carboxyl group apparently migrates to the adjacent carbon (Figure 6.5). Phenylpyruvic acid and phenyl-lactic acid have been shown to be involved, and tropine becomes esterified with phenyl-lactic acid (most likely via the coenzyme-A ester) to form **littorine** before the rearrangement occurs. A cytochrome P-450-dependent enzyme transforms littorine to hyoscyamine aldehyde, and a radical process (Figure 6.5) with an intermediate cyclopropane-containing radical would accommodate the available data. Further modifications to the tropane

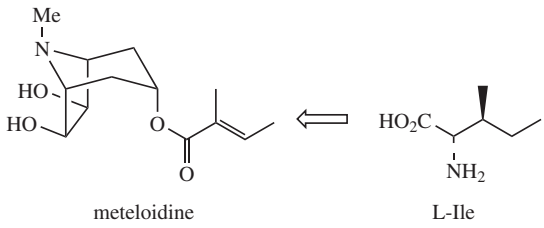


Figure 6.6

skeleton then occur on the ester, not on the free alcohol. These include hydroxylation to 6β-hydroxyhyoscyamine and additional oxidation, allowing formation of an epoxide grouping as in **hyoscine** (**scopolamine**). Both of these reactions are catalysed by a single 2-oxoglutarate-dependent dioxygenase (see page 27). Other esterifying acids may be encountered in tropane alkaloid structures, e.g. tiglic acid in **meteloidine** (Figure 6.6) from *Datura meteloides* and phenyl-lactic acid in littorine above, which is a major alkaloid in *Anthocercis littorea*. Tiglic acid is known to be derived from the amino acid L-isoleucine (see page 216).

The structure of **cuscohygrine** arises by an *intermolecular* Mannich reaction involving a second *N*-methyl-Δ^1-pyrrolinium cation (Figure 6.7). Cuscohygrine (and also hygrine) racemize rapidly in solution via a self-catalysed (i.e. base-catalysed) Michael-type retroconjugate addition process.

The tropane alkaloids (−)-hyoscyamine and (−)-hyoscine are among the most important of the natural alkaloids used in medicine. They are found in a variety of solanaceous plants, including *Atropa belladonna* (deadly nightshade), *Datura stramonium* (thornapple) and other *Datura* species, *Hyoscyamus niger* (henbane), and *Duboisia* species [Box 6.1]. These alkaloids are also responsible for the pronounced toxic properties of these plants.

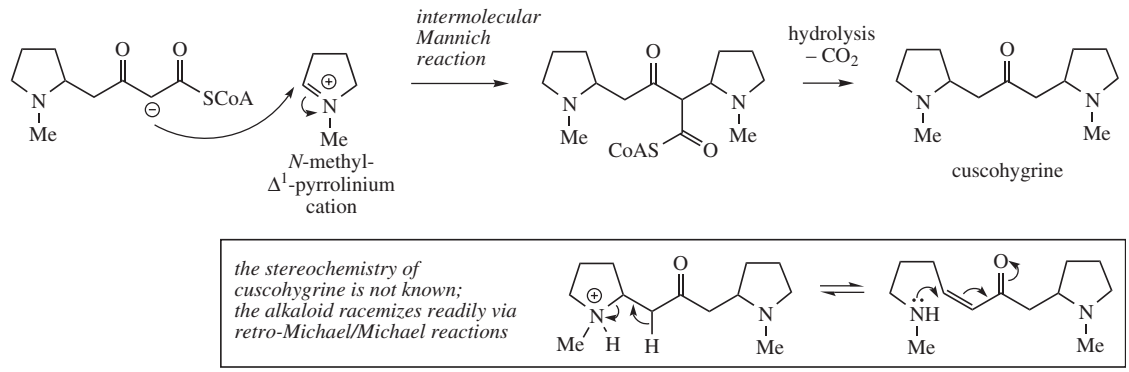


Figure 6.7

Box 6.1

Belladonna

The deadly nightshade *Atropa belladonna* (Solanaceae) has a long history as a highly poisonous plant. The generic name is derived from *Atropos*, in Greek mythology the Fate who cut the thread of life. The berries are particularly dangerous, but all parts of the plant contain toxic alkaloids, and even handling of the plant can lead to toxic effects, since the alkaloids are readily absorbed through the skin. Although humans are sensitive to the toxins, some animals, including sheep, pigs, goats, and rabbits, are less susceptible. Cases are known where the consumption of rabbits or birds that have ingested belladonna has led to human poisoning. The plant is a tall perennial herb producing dull-purple bell-shaped flowers followed by conspicuous shiny black fruits, the size of a small cherry. *Atropa belladonna* is indigenous to central and southern Europe, though it is not especially common. It is cultivated for drug use in Europe and the United States. The tops of the plant are harvested two or three times per year and dried to give **belladonna** herb. Roots from plants some 3–4 years old are less commonly employed as a source of alkaloids.

Box 6.1 (continued)

Belladonna herb typically contains 0.3–0.6% of alkaloids, mainly (−)-hyoscyamine (Figure 6.4). Belladonna root has only slightly higher alkaloid content at 0.4–0.8%, again mainly (−)-hyoscyamine. Minor alkaloids, including (−)-hyoscine (Figure 6.5) and cuscohygrine (Figure 6.7), are also found in the root, though these are not usually significant in the leaf. The mixed alkaloid extract from belladonna herb is still used as a gastrointestinal sedative, usually in combination with antacids. Root preparations can be used for external pain relief, e.g. in belladonna plasters.

Stramonium

Datura stramonium (Solanaceae) is commonly referred to as thornapple on account of its spikey fruit. It is a tall bushy annual plant widely distributed in Europe and North America, and because of its alkaloid content is potentially very toxic. Indeed, a further common name, Jimson or Jamestown weed, originates from the poisoning of early settlers near Jamestown, Virginia. At subtoxic levels, the alkaloids can provide mild sedative action and a feeling of well-being. In the Middle Ages, stramonium was employed to drug victims prior to robbing them. During this event, the victim appeared normal and was cooperative, though afterwards could usually not remember what had happened. For drug use, the plant is cultivated in Europe and South America. The leaves and tops are harvested when the plant is in flower. **Stramonium** leaf usually contains 0.2–0.45% of alkaloids, principally (−)-hyosycamine and (−)-hyoscine in a ratio of about 2:1. In young plants, (−)-hyoscine can predominate.

The generic name *Datura* is derived from dhat, an Indian poison used by the Thugs. The narcotic properties of *Datura* species, especially *Datura metel*, have been known and valued in India for centuries. The plant material was usually absorbed by smoking. Most species of *Datura* contain similar tropane alkaloids and are potential sources of medicinal alkaloids. In particular, *Datura sanguinea*, a perennial of tree-like stature with blood-red flowers, is cultivated in Ecuador and yields leaf material with a high (0.8%) alkaloid content in which the principal component is (−)-hyoscine. The plants can be harvested several times a year. *Datura sanguinea* and several other species of the tree-daturas (now classified as a separate genus *Brugmansia*) are widely cultivated as ornamentals, especially for conservatories, because of their attractive large tubular flowers. The toxic potential of these plants is not always recognized.

Hyoscyamus

Hyoscyamus niger (Solanaceae), or henbane, is a European native with a long history as a medicinal plant. Its inclusion in mediaeval concoctions and its power to induce hallucinations with visions of flight may well have contributed to our imaginary view of witches on broomsticks. The plant has both annual and biennial forms, and is cultivated in Europe and North America for drug use, the tops being collected when the plant is in flower and then dried rapidly. The alkaloid content of **hyoscyamus** is relatively low at 0.045–0.14%, but this can be composed of similar proportions of (−)-hyoscine and (−)-hyosycamine. Egyptian henbane, *Hyoscyamus muticus*, has a much higher alkaloid content than *Hyoscyamus niger*, and although it has mainly been collected from the wild, especially from Egypt, it functions as a major commercial source for alkaloid production. Some commercial cultivation occurs in California. The alkaloid content of the leaf is from 0.35 to 1.4%, of which about 90% is (−)-hyoscyamine.

Duboisia

Duboisia is a small genus of Australian trees, containing only three species, again from the family Solanaceae. Two of these, *Duboisia myoporoides* and *Duboisia leichhardtii* are grown commercially in Australia for tropane alkaloid production. The small trees are kept as bushes to allow frequent harvesting, with up to 70–80% of the leaves being removed every 7–8 months. The alkaloid content of the leaf is high (up to 3% has been recorded), and it includes (−)-hyoscyamine, (−)-hyoscine, and a number of related structures. The proportion of hyoscyamine to hyoscine varies according to the species used and the area in which the trees are grown. The hyoscine content is frequently much higher than that of hyoscyamine. Indeed, interest in *Duboisia* was very much stimulated by the demand for hyoscine as a treatment for motion sickness in military personnel during the Second World War. Even higher levels of alkaloids, and higher proportions of hyoscine, can be obtained from selected *Duboisia myoporoides* × *Duboisia leichhardtii* hybrids, which are currently cultivated. The hybrid is superior to either parent and can yield 1–2.5% hyoscine and 0–1% hyoscyamine. *Duboisia* leaf is an important commercial source of medicinal tropane alkaloids.

Duboisia hopwoodi, the third species of *Duboisia*, contains little tropane alkaloid content, but produces mainly nicotine and related alkaloids, e.g. nornicotine (see page 331). Leaves of this plant were chewed by aborigines for their stimulating effects.

Box 6.1 (continued)

Allied Drugs

Tropane alkaloids, principally hyoscyamine and hyoscine, are also found in two other medicinal plants, namely scopolia and mandrake, but these plants find little current use. Scopolia (*Scopolia carniolica*; Solanaceae) resembles belladonna in appearance, though it is considerably smaller. Both root and leaf materials have been employed medicinally. The European mandrake (*Mandragora officinarum*; Solanaceae) has a complex history as a hypnotic, a general panacea, and an aphrodisiac. Its collection has been surrounded by much folklore and superstition, in that pulling it from the ground was said to drive its collector mad due to the unearthly shrieks emitted. The roots are frequently forked and are loosely likened to a man or woman. Despite the Doctrine of Signatures, which teaches that the appearance of an object indicates its special properties, from a pharmacological point of view this plant would be much more efficient as a pain reliever than as an aphrodisiac.

Hyoscyamine, Hyoscine, and Atropine

All the above solanaceous plants contain as main alkaloidal constituents the tropane esters (−)-**hyoscyamine** and (−)-**hyoscine**, together with other minor tropane alkaloids. The piperidine ring in the bicyclic tropane system has a chair-like conformation, and there is a ready inversion of configuration at the nitrogen atom so that the *N*-methyl group can equilibrate between equatorial and axial positions (Figure 6.8). An equatorial methyl is strongly favoured provided there are no substituents on the two-carbon bridge, in which case the axial form may predominate. (−)-Hyoscyamine is the ester of tropine (Figure 6.4) with (−)-(*S*)-tropic acid, whilst (−)-hyoscine contains scopine (see Figure 6.10) esterified with (−)-(*S*)-tropic acid. The optical activity of both hyoscyamine and hyoscine stems from the chiral centre in the acid portion, (*S*)-tropic acid. Tropine itself, although containing chiral centres, is a symmetrical molecule and is optically inactive; it can be regarded as a *meso* structure. The chiral centre in the tropic acid portion is adjacent to a carbonyl and the aromatic ring, and racemization can be achieved under mild conditions by heating or treating with base. This will involve an intermediate enol (or enolate) which is additionally favoured by conjugation with the aromatic ring (Figure 6.9). Indeed, normal base-assisted fractionation of plant extracts to isolate the alkaloids can sometimes result in production of significant amounts of racemic alkaloids. The plant material itself generally contains only the enantiomerically pure alkaloids. Hyoscyamine appears to be much more easily racemized than hyoscine. Hydrolysis of the esters using acid or base usually gives racemic tropic acid. Note that littorine (Figure 6.5), in which the chiral centre is not adjacent to the phenyl ring, is not readily racemized, and base hydrolysis gives optically pure phenyl-lactic acid. The racemic form of hyoscyamine is

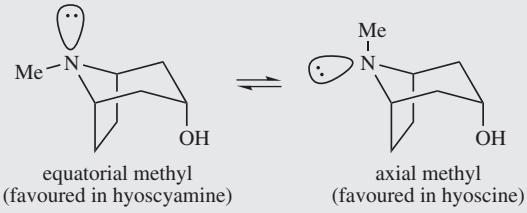


Figure 6.8

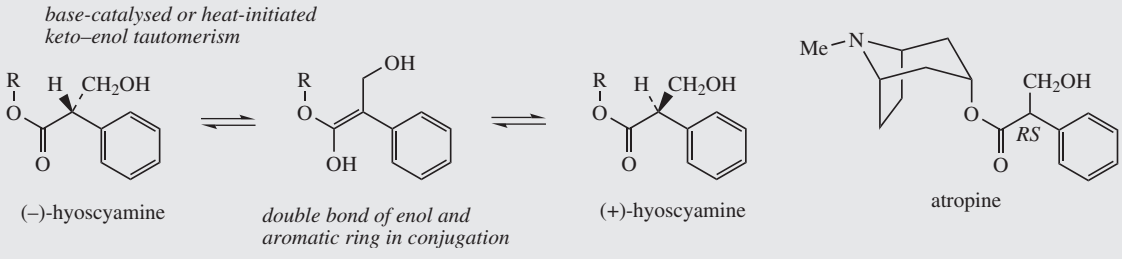


Figure 6.9

Box 6.1 (continued)

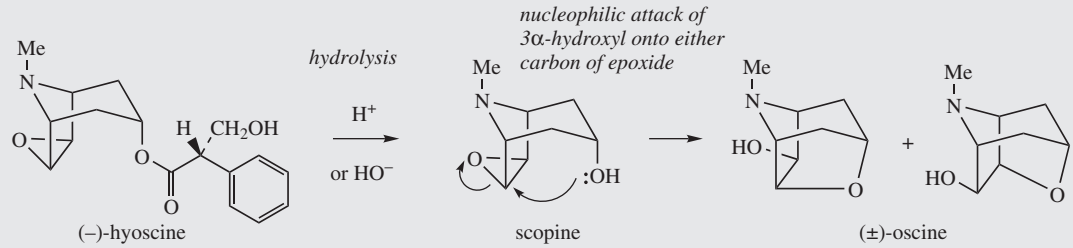


Figure 6.10

called **atropine** (Figure 6.9), whilst that of hyoscine is called atroscine. In each case, the biological activity of the (+)-enantiomer is some 20–30 times less than that of the natural (−)-form. Chemical hydrolysis of hyoscine in an attempt to obtain the alcohol scopine is not feasible. Instead, the alcohol oscine is generated because of the proximity of the 3α-hydroxyl group to the reactive epoxide function (Figure 6.10).

Probably for traditional reasons, salts of both (−)-hyoscyamine and (±)-hyoscyamine (atropine) are used medicinally, whereas usage of hyoscine is restricted to the natural laevorotatory form. These alkaloids compete with acetylcholine for the muscarinic site of the parasympathetic nervous system, thus preventing the passage of nerve impulses, and are classified as anticholinergics. Acetylcholine binds to two types of receptor site, described as muscarinic or nicotinic. These are triggered specifically by the alkaloid muscarine from the fly agaric fungus *Amanita muscaria* or by the tobacco alkaloid nicotine (see page 334) respectively. The structural similarity between acetylcholine and muscarine (Figure 6.11) can readily be appreciated, and hyoscyamine is able to occupy the same receptor site by virtue of the spatial relationship between the nitrogen atom and the ester linkage (Figure 6.11). The side-chain also plays a role in the binding, explaining the marked difference in activities between the two enantiomeric forms. The agonist properties of hyoscyamine and hyoscine give rise to a number of useful effects, including antispasmodic action on the gastrointestinal tract, antisecretory effect controlling salivary secretions during surgical operations, and as mydriatics to dilate the pupil of the eye. Hyoscine has a depressant action on the central nervous system and finds particular use as a sedative to control motion sickness. One of the side-effects from oral administration of tropane alkaloids is dry mouth (the antisecretory effect), but this can be much reduced by transdermal administration. In motion sickness treatment, hyoscine can be supplied via an impregnated patch worn behind the ear. Hyoscine under its synonym **scopolamine** is also well known, especially in fiction, as a 'truth drug'. This combination of sedation, lack of will, and amnesia was first employed in childbirth to give what was termed 'twilight sleep', and may be compared with the mediaeval use of stramonium. The mydriatic use also has a very long history. Indeed, the specific name *belladonna* for deadly nightshade means 'beautiful lady' and refers to the practice of ladies at court who applied the juice of the fruit to the eyes, giving widely dilated pupils and a striking appearance, though at the expense of blurred vision through an inability to focus. Atropine also has useful antidote action in cases of poisoning caused by acetylcholinesterase inhibitors e.g. physostigmine and neostigmine (see page 386) and organophosphate insecticides.

Hyoscine is commercially more valuable than hyoscyamine, but most of the plant sources described produce considerably more hyoscyamine. Researchers are thus trying to establish plants or plant systems that accumulate predominantly hyoscine. Outside of conventional plant breeding and selection, genetic manipulation offers considerable scope. For example, it has been demonstrated that introducing the hyoscyamine 6β-hydroxylase gene from *Hyoscyamus niger* into *Atropa belladonna* increases hyoscine content, whilst overexpressing the *Hyoscyamus niger* gene in *Hyoscyamus muticus* root cultures can increase hyoscine production up to 100-fold. An alternative approach is to express the hyoscyamine 6β-hydroxylase gene in an organism that does

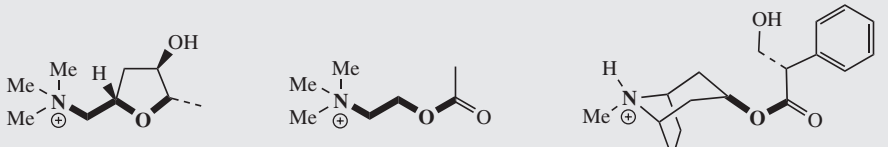


Figure 6.11

Box 6.1 (continued)

not produce tropane alkaloids, supply the cultured cells with hyoscyamine, and allow the cells to carry out a biotransformation. This has been successful in *Escherichia coli* and tobacco cell cultures.

It is valuable to reiterate here that the tropane alkaloid-producing plants are all regarded as very toxic, and that since the alkaloids are rapidly absorbed into the bloodstream, even via the skin, first aid must be very prompt. Initial toxicity symptoms include skin flushing with raised body temperature, mouth dryness, dilated pupils, and blurred vision.

Homatropine (Figure 6.12) is a semi-synthetic ester of tropine with racemic mandelic (2-hydroxyphenylacetic) acid and is used as a mydriatic, as are **tropicamide** and **cyclopentolate** (Figure 6.12). Tropicamide is an amide of tropic acid, though a pyridine nitrogen is used to mimic that of the tropane. Cyclopentolate is an ester of a tropic acid-like system, but uses a non-quaternized amino alcohol resembling choline. **Glycopyrronium** (Figure 6.12) has a quaternized nitrogen in a pyrrolidine ring, with an acid moiety similar to that of cyclopentolate. This drug is an antimuscarinic used as a premedicant to dry bronchial and salivary secretions. **Hyoscine butylbromide** (Figure 6.13) is a gastrointestinal antispasmodic synthesized from (−)-hyoscine by quaternization of the amine function with butyl bromide. The quaternization of tropane alkaloids by *N*-alkylation proceeds such that the incoming alkyl group always approaches from the equatorial position. The potent bronchodilator **ipratropium bromide** (Figure 6.13) is thus synthesized from noratropine by successive isopropyl and methyl alkylations. This drug is used in inhalers for the treatment of chronic bronchitis. **Tiotropium bromide** (Figure 6.13) is a newer, longer-acting agent.

Benzatropine (**benztropine**; Figure 6.12) is an ether of tropine used as an antimuscarinic drug in the treatment of Parkinson's disease. It is able to inhibit dopamine reuptake, helping to correct the deficiency which is characteristic of Parkinsonism.

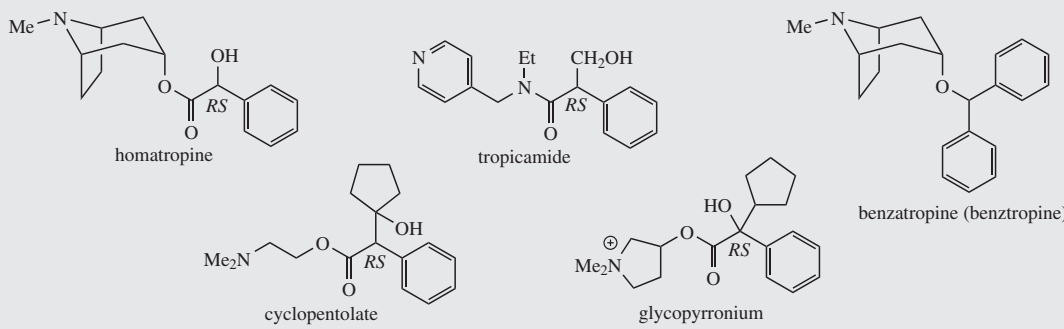


Figure 6.12

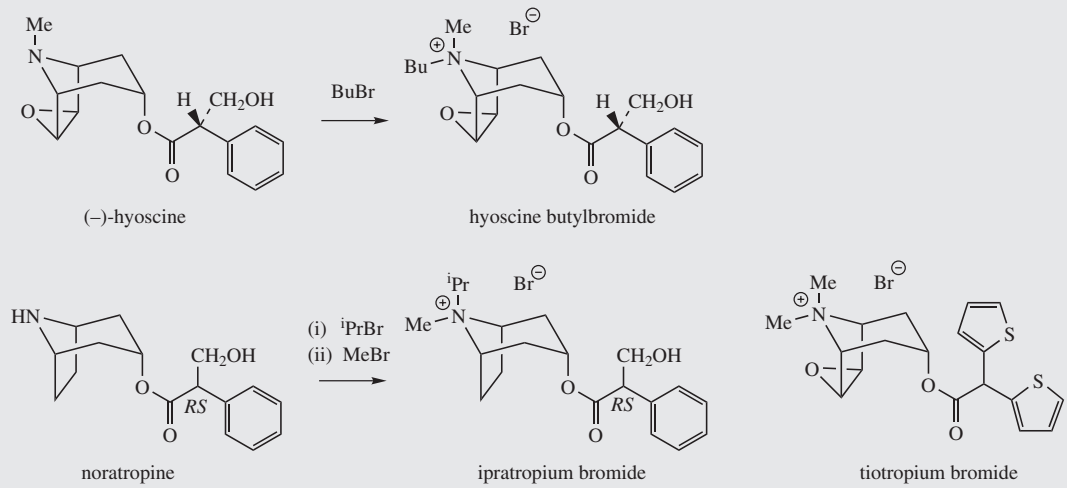
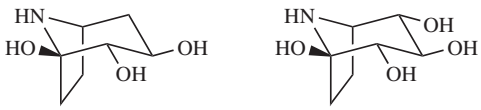


Figure 6.13



calystegine A_3 calystegine B_2

Figure 6.14

The **calystegines** are a group of recently discovered, water-soluble, polyhydroxy nortropane derivatives that are found in the leaves and roots of many of the solanaceous plants, including *Atropa, Datura, Duboisia, Hyoscyamus, Mandragora, Scopolia* and *Solanum*. They were first isolated from *Calystegia sepium* (Convolvulaceae). These compounds, e.g. calystegin A_3 and calystegin B_2 (Figure 6.14), are currently of great interest as glycosidase inhibitors. They have similar potential for the development of drugs with activity against the AIDS virus HIV as the polyhydroxyindolizidines such as castanospermine (see page 330), and the aminosugars such as deoxynojirimycin (see page 498). Examples of tri-, tetra-, and penta-hydroxy calystegines are currently known. These alkaloids appear to be produced from the 3β-alcohol ψ-tropine (Figure 6.4) by a sequence of *N*-demethylation, followed by further hydroxylation steps. *N*-Demethylation is also a feature in the formation of nornicotine from nicotine (see page 333).

Cocaine (Figure 6.4) is a rare alkaloid restricted to some species of *Erythroxylum* (Erythroxylaceae). *Erythroxylum coca* (coca) is the most prominent as a source of cocaine, used medicinally as a local anaesthetic, and as an illicit drug for its euphoric properties [Box 6.2]. Coca also contains significant amounts of **cinnamoylcocaine** (**cinnamylcocaine**; Figure 6.15), where cinnamic acid rather than benzoic acid is the esterifying acid, together with some typical tropine derivatives without

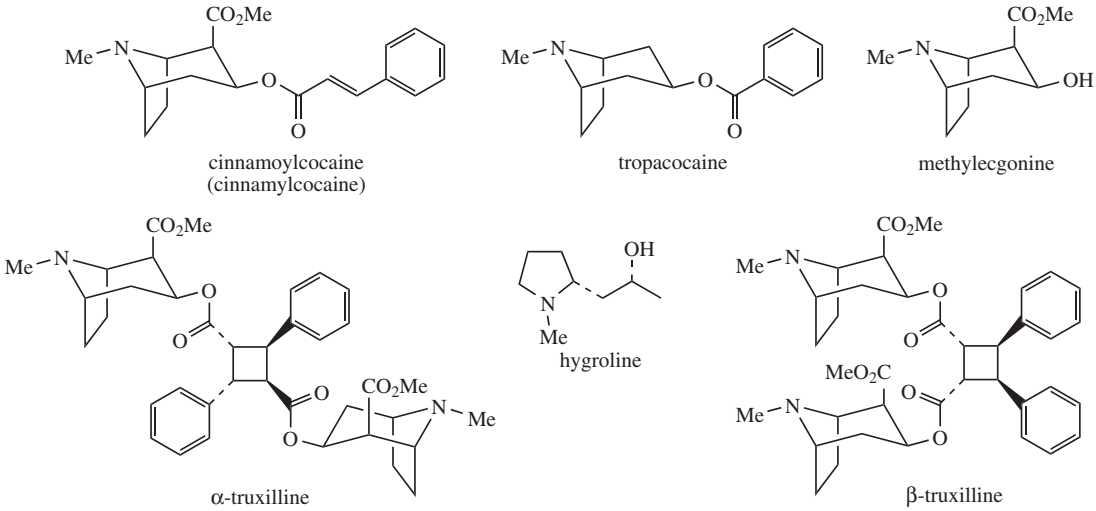


Figure 6.15

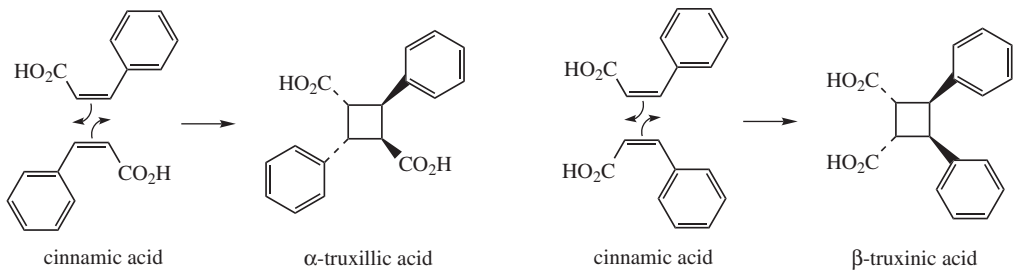


Figure 6.16

the extra carboxyl, e.g. **tropacocaine** (Figure 6.15). Tropacocaine still retains the 3β-configuration, showing that the stereospecific carbonyl reduction is the same as with the cocaine route, and not as with the hyoscyamine pathway. The **truxillines** contain dibasic acid moieties, α-truxillic and β-truxinic acids, which are cycloaddition products from two cinnamic acid units (Figure 6.16).

Box 6.2

Coca

Coca leaves are obtained from species of *Erythroxylum* (Erythroxylaceae), small shrubs native to the Andes region of South America, namely Colombia, Ecuador, Peru, and Bolivia. Peru is the only producer of medicinal coca; illicit supplies originate from Colombia, Peru, and Bolivia. Two main species provide drug materials, *Erythroxylum coca* and *Erythroxylum novogranatense*, though each species exists in two distinguishable varieties. *Erythroxylum coca* var. *coca* provides Peruvian or Huanaco coca, *Erythroxylum coca* var. *ipadu* Amazonian coca, *Erythroxylum novogranatense* var. *novogranatense* Colombian coca, and *Erythroxylum novogranatense* var. *truxillense* gives Trujillo coca. Cultivated plants are kept small by pruning, and a quantity of leaves is harvested from each plant three or more times per year.

Coca-leaf chewing has been practised by South American Indians for many years and has been an integral part of the native culture pattern. Leaf is mixed with lime, thus liberating the principal alkaloid cocaine as the free base, and the combination is then chewed. Cocaine acts as a potent antifatigue agent, and allows labourers to ignore hunger, fatigue, and cold, enhancing physical activity and endurance. Originally, the practice was limited to the Inca high priests and favoured individuals, but it became widespread after the Spanish conquest of South America. It is estimated that 25% of the harvest is consumed in this way by the local workers, who may each use about 50 g of leaf per day (≡ 350 mg cocaine). Only a tiny amount (1–2%) of the coca produced is exported for drug manufacture. The rest contributes to illicit trade and the world's drug problems. Efforts to stem the supply of illicit coca and cocaine have been relatively unsuccessful.

Coca leaf contains 0.7–2.5% of alkaloids, the chief component (typically 40–50%) of which is (−)-cocaine (Figure 6.4), a diester of (−)-ecgonine. Note that although tropine is an optically inactive *meso* structure, ecgonine contains four chiral centres, is no longer symmetrical, and is, therefore, optically active. Cinnamoylcocaine (cinnamylcocaine), α-truxilline, β-truxilline, and methylecgonine (Figure 6.15) are minor constituents also based on ecgonine. Other alkaloids present include structures based on φ-tropine (the 3β-isomer of tropine), such as tropacocaine (Figure 6.15), and on hygrine, e.g. hygrine, hygroline (Figure 6.15), and cuscohygrine (Figure 6.7). Cuscohygrine typically accounts for 20–30% of the alkaloid content. *Erythroxylum novogranatense* varieties have a higher cocaine content than *Erythroxylum coca* varieties, but also a higher cinnamoylcocaine content and are less desirable for illicit cocaine production, in that the latter alkaloid hinders crystallization of cocaine.

Illegal production of cocaine is fairly unsophisticated, but can result in material of high quality. The alkaloids are extracted from crushed leaf using alkali (lime) and petrol. The petrol extract is then re-extracted with aqueous acid, and this alkaloid fraction is basified and allowed to stand, yielding the free alkaloid as a paste. Alternatively, the hydrochloride or sulfate salts may be prepared. The coca alkaloids are often diluted with carrier to give a preparation with 10–12% of cocaine. The illicit use of cocaine and cocaine hydrochloride is a major problem worldwide. The powder is usually sniffed into the nostrils, where it is rapidly absorbed by the mucosa, giving stimulation and short-lived euphoria through inhibiting reuptake of neurotransmitters dopamine, noradrenaline, and serotonin, so prolonging and augmenting their effects. Regular usage induces depression, dependence, and damage to the nasal membranes. The drug may also be injected intravenously or the vapour inhaled. For inhalation, the free base or 'crack' is employed to increase volatility. The vaporized cocaine is absorbed extremely rapidly and carried to the brain within seconds, speeding up and enhancing the euphoric lift. Taken in this form, cocaine has proved highly addictive and dangerous. Cocaine abuse is currently regarded as a greater problem than heroin addiction, and despite intensive efforts, there is no useful antagonist drug available to treat cocaine craving and addiction. Considerable research is being directed towards an alternative strategy, namely to enhance enzymic hydrolysis of cocaine and to accelerate its clearance from the body. The major cocaine-metabolizing enzymes in humans are a non-specific serum cholinesterase (termed butyrylcholinesterase), which hydrolyses the benzoyl ester, and two liver carboxylesterases, one of which catalyses hydrolysis of the methyl ester, the other the benzoyl ester. Enhancement of benzoyl ester hydrolysis is the more desirable, since the alternative product benzoylecgonine is itself psychoactive. Further, when users consume cocaine and alcohol concurrently, transesterification of benzoylecgonine may occur, giving the corresponding ethyl ester cocaethylene, which has increased toxicity and half-life.

In the 1800s, coca drinks were fashionable, and one in particular, Coca-Cola®, became very popular. This was originally based on extracts of coca (providing cocaine) and cola (supplying caffeine) (see page 415), but although the coca content was omitted from 1906 onwards, the name and popularity continue.

Box 6.2 (continued)

Medicinally, **cocaine** is of value as a local anaesthetic for topical application. It is rapidly absorbed by mucous membranes and paralyses peripheral ends of sensory nerves. This is achieved by blocking ion channels in neural membranes. It was widely used in dentistry, but has been replaced by safer drugs, though it still has applications in ophthalmic and in ear, nose and throat surgery.

The essential functionalities of cocaine required for activity were eventually assessed to be the aromatic carboxylic acid ester and the basic amino group, separated by a lipophilic hydrocarbon chain. Synthetic drugs developed from the cocaine structure have been introduced to provide safer, less toxic local anaesthetics (Figure 6.17). **Procaine**, though little used now, was the first major analogue employed. **Benzocaine** is used topically, but has a short duration of action. **Tetracaine** (**amethocaine**), **oxybuprocaine**, and **proxymetacaine** are valuable local anaesthetics employed principally in ophthalmic work. The ester function can be replaced by an amide, and this gives better stability towards hydrolysis in aqueous solution or by esterases. **Lidocaine** (**lignocaine**) is an example of an amino amide analogue and is perhaps the most widely used local anaesthetic, having rapid action, effective absorption, good stability, and may be used by injection or topically. Other amino amide local anaesthetic structures include **prilocaine**, with similar properties to lidocaine and very low toxicity, and **bupivacaine**, which has a long duration of action and is currently the most widely used local anaesthetic agent in both surgery and obstetrics. The (*S*)-enantiomer **levobupivacaine** has considerably fewer side-effects than the (*R*)-isomer, and is thus preferred over the racemic drug. **Ropivacaine, mepivacaine**, and **articaine** (**carticaine**) are some recently inroduced amide-type local anaesthetics, the latter two being used predominantly in dentistry. **Cinchocaine** is often incorporated into preparations to soothe haemorrhoids.

Lidocaine, although introduced as a local anaesthetic, was subsequently found to be a potent antiarrhythmic agent, and it now finds further use as an antiarrhythmic drug, for treatment of ventricular arrhythmias especially after myocardial infarction. Other cocaine-related structures also find application in the same way, including **procainamide** and **flecainide** (Figure 6.17). Procainamide is an amide analogue of procaine, whilst in **mexiletene**, a congener of lidocaine, the amide group has been replaced by a simple ether linkage.

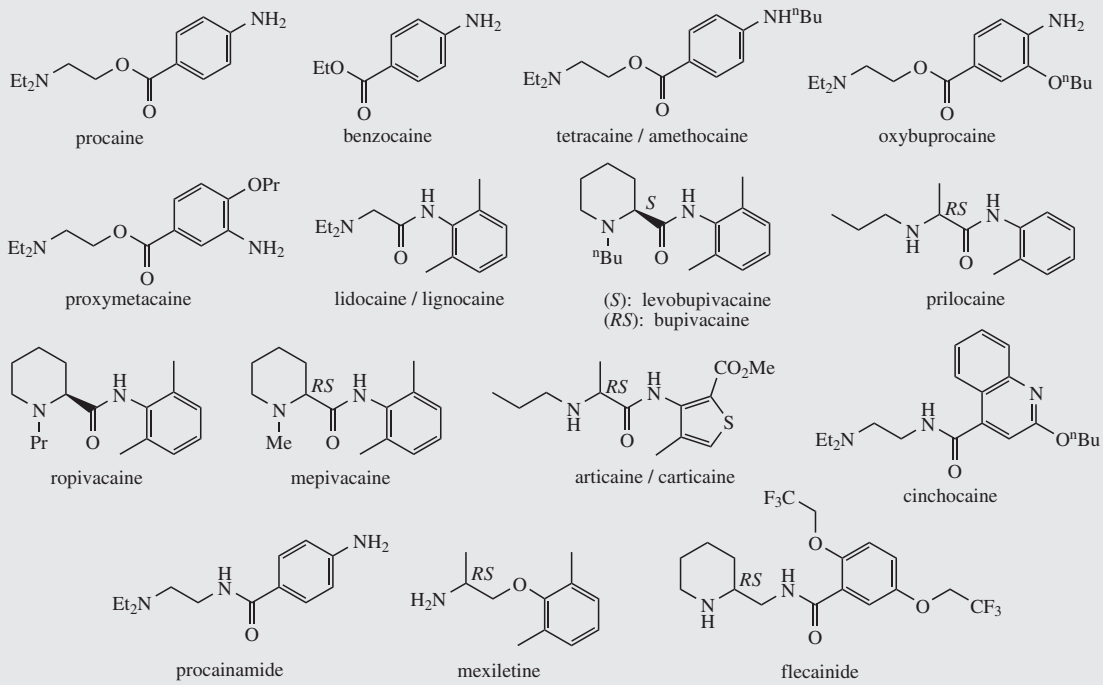


Figure 6.17

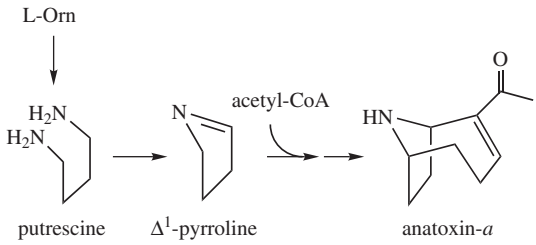


Figure 6.18

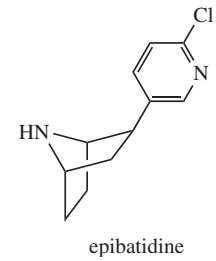


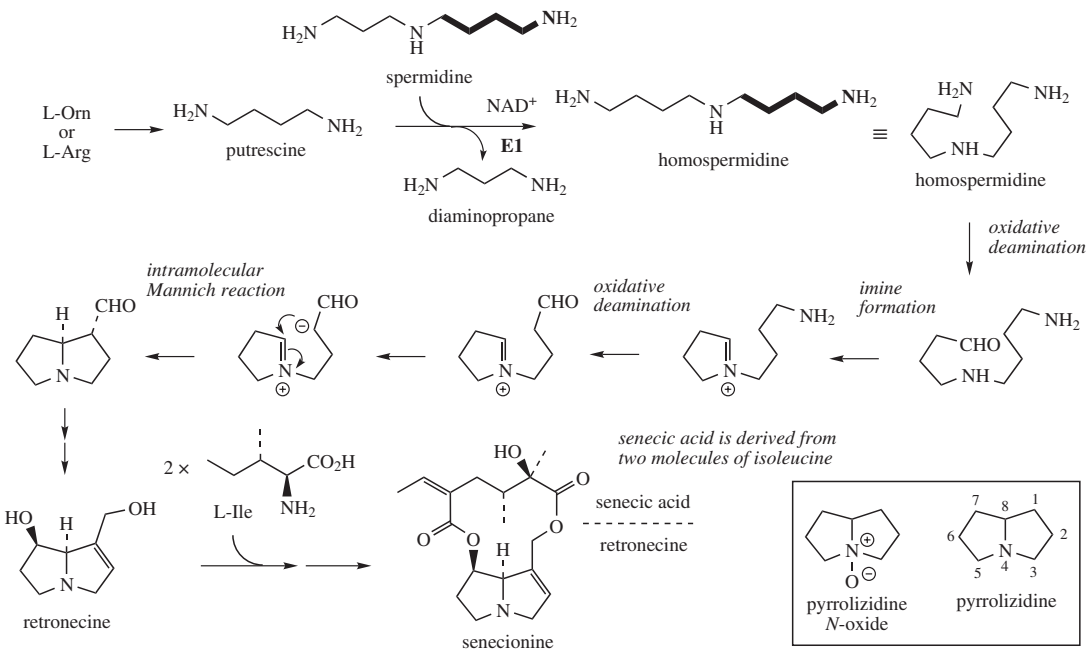
Figure 6.19

Anatoxin-*a* (Figure 6.18) is a toxic tropane-related alkaloid produced by a number of cyanobacteria, e.g. *Anabaena flos-aquae* and *Aphanizomenon flos-aquae*, species which proliferate in lakes and reservoirs during periods of hot, calm weather. A number of animal deaths have been traced back to consumption of water containing the cyanobacteria and ingestion of the highly potent neurotoxin anatoxin-*a*, which has been termed Very Fast Death Factor. Anatoxin-*a* is one of the most powerful nicotinic acetylcholine receptor agonists known, and has become a useful pharmacological probe for elucidating the mechanism of acetylcholine-mediated neurotransmission and disease states associated with this process. The ring system may be regarded as a homotropane, and it has been suggested that the pyrrolidine ring originates from ornithine via putrescine and Δ^1-pyrroline, in a way similar to the tropane alkaloids (Figure 6.18). The remaining carbon atoms may originate from acetate precursors. A remarkable compound with a nortropane ring system has been isolated from the highly coloured skin of the Ecuadorian poison frog *Epipedobates tricolor* in tiny amounts (750 frogs would yield only 1 mg). This compound, called **epibatidine** (Figure 6.19), is exciting considerable interest as a lead compound for analgesic drugs. It is 200–500 times more potent than morphine (see page 349) and does not act on normal opioid receptors, but is a specific agonist at nicotinic acetylcholine receptors. Unfortunately, it is also highly toxic, so most research is now focused on the synthesis of structural analogues. Whether or not the nortropane ring system is ornithine derived remains to be established.

Pyrrolizidine Alkaloids

The bicyclic pyrrolizidine skeleton is elaborated from **putrescine** derived from arginine. Whilst ornithine may act as precursor, it is actually incorporated by way of arginine, because plants synthesizing pyrrolizidine alkaloids appear to lack the decarboxylase enzyme transforming ornithine into putrescine (Figure 6.2). Putrescine is converted into the polyamine **homospermidine** by a process that transfers an aminopropyl group from spermidine (Figure 6.2) in an NAD^+-dependent reaction (Figure 6.20). The cofactor requirement suggests this may involve imine intermediates. The pyrrolizidine skeleton is built up from homospermidine by a sequence of oxidative deamination, imine formation, and an intramolecular Mannich reaction which exploits the enolate anion generated from the aldehyde. This latter reaction is analogous to that proposed in formation of the tropane ring system (see page 314). A typical simple natural pyrrolizidine structure is that of **retronecine** (Figure 6.20), which can be derived from the pyrrolizidine aldehyde by modest oxidative and reductive steps. The pyrrolizidine skeleton thus incorporates a C_4N unit from ornithine, plus a further four carbon atoms actually from the same amino acid precursor, but via the polyamine spermidine.

Pyrrolizidine alkaloids have a somewhat restricted distribution, but are characteristic of many genera of the Boraginaceae (e.g. *Heliotropium, Cynoglossum*, and *Symphytum*), the Compositae/Asteraceae (e.g. *Senecio* and *Eupatorium*), and certain genera of the Leguminosae/Fabaceae (e.g. *Crotalaria*), and Orchidaceae. The pyrrolizidine bases rarely occur in the free form, and are generally found as esters with rare mono- or di-basic acids, the necic acids. Thus, **senecionine** (Figure 6.20) from *Senecio* species is a diester of retronecine with senecic acid. Inspection of the 10-carbon skeleton of senecic acid suggests it is potentially derivable from two isoprene units, but experimental evidence has demonstrated that it is in fact obtained by incorporation of two molecules of the amino acid L-isoleucine. Loss of the carboxyl from isoleucine supplies a carbon fragment analogous to isoprene units (compare tiglic acid in the tropane alkaloid meteloidine, Figure 6.6). Other necic acid structures may incorporate fragments from valine, threonine, leucine, or acetate. It is also worthy of note that, in general, the pyrrolizidine alkaloids accumulate in the plant as polar, salt-like *N*-oxides, facilitating their transport and,



E1: homospermidine synthase

Figure 6.20

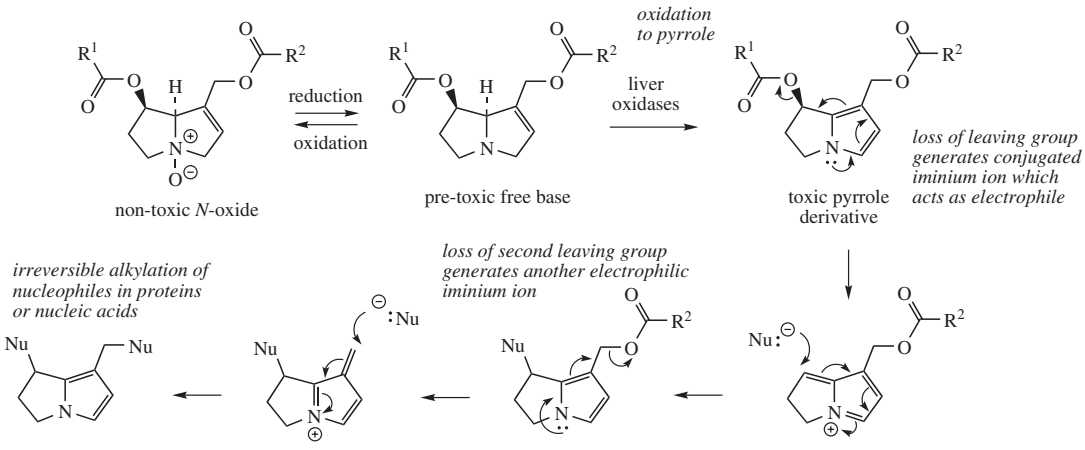
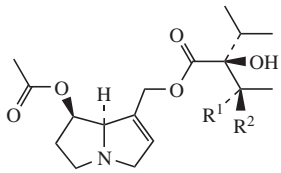


Figure 6.21

above all, maintaining them in a non-toxic form. The *N*-oxides are easily changed back to the tertiary amines by mild reduction, as will occur in the gut of a herbivore (Figure 6.21).

Many pyrrolizidine alkaloids are known to produce pronounced hepatic toxicity, and there are many recorded cases of livestock poisoning, particularly from ingestion of *Senecio* species (ragworts). Potentially toxic structures have 1,2-unsaturation in the pyrrolizidine ring and an ester function on the side-chain. Although themselves non-toxic, these alkaloids are transformed by mammalian liver oxidases into reactive pyrrole



R^1 = H, R^2 = OH, acetyl-intermedine
R^1 = OH, R^2 = H, acetyl-lycopsamine

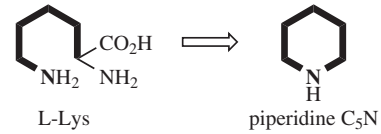
Figure 6.22

structures, which are potent alkylating agents and react with suitable cell nucleophiles, e.g. nucleic acids and proteins (Figure 6.21). *N*-Oxides are not transformed by these oxidases, only the free bases. The presence of pyrrolizidine alkaloids, e.g. **acetyl-intermedine** and **acetyl-lycopsamine** (Figure 6.22), in medicinal comfrey (*Symphytum officinale*; Boraginaceae) has emphasized potential dangers of using this traditional herbal drug as a remedy for inflammatory, rheumatic, and gastrointestinal disorders. Prolonged usage may lead to liver damage. Caterpillars of the cinnabar moth *Tyria jacobaeae* feed on species of *Senecio* (e.g. ragwort, *Senecio jacobaea*, and groundsel, *Senecio vulgaris*) with impunity, building up levels of pyrrolizidine alkaloids in their bodies (in the form of non-toxic *N*-oxides). This makes them distasteful to predators and also potentially toxic should the predator convert the alkaloids into the free bases.

Some of the tobacco alkaloids, e.g. nicotine, contain a pyrrolidine ring system derived from ornithine as a portion of their structure. These are described under nicotinic acid derivatives (see page 331).

ALKALOIDS DERIVED FROM LYSINE

L-**Lysine** is the homologue of L-ornithine, and it, too, functions as an alkaloid precursor, using pathways analogous to those noted for ornithine. The extra methylene group in lysine means this amino acid participates in forming six-membered piperidine rings, just as ornithine provided five-membered pyrrolidine rings. As with ornithine, the carboxyl group is lost, the ε-amino nitrogen rather than the α-amino nitrogen is retained, and lysine thus supplies a C_5N building block (Figure 6.23).



L-Lys piperidine C_5N

Figure 6.23

Piperidine Alkaloids

***N*-Methylpelletierine** (Figure 6.24) is an alkaloidal constituent of the bark of pomegranate (*Punica granatum*; Punicacae), where it cooccurs with **pelletierine** and **pseudopelletierine** (Figure 6.24), the mixture of alkaloids having activity against intestinal tapeworms. *N*-Methylpelletierine and pseudopelletierine are homologues of hygrine and tropinone respectively, and a pathway similar to Figure 6.4 using the diamine **cadaverine** (Figure 6.24) may be proposed. As with putrescine, the rather distinctive name cadaverine also reflects its isolation from decomposing animal flesh. In this pathway, the Mannich reaction involving the Δ^1-piperidinium salt utilizes the more nucleophilic acetoacetyl-CoA rather than acetyl-CoA, and the carboxyl carbon from acetoacetate appears to be lost during the reaction by suitable hydrolysis/decarboxylation reactions (Figure 6.24). **Anaferine** from *Withania somnifera* (Solanaceae; Figure 6.24) is an analogue of cuscohygrine in which a further piperidine ring is added via an intermolecular Mannich reaction.

The alkaloids found in the antiasthmatic plant *Lobelia inflata* (Campanulaceae) contain piperidine rings with C_6C_2 side-chains derived from phenylalanine via cinnamic acid. These alkaloids are produced as in Figure 6.25, in which benzoylacetyl-CoA, an intermediate in the β-oxidation of cinnamic acid (see page 160), provides the nucleophile for the Mannich reaction. Oxidation in the piperidine ring gives a new iminium species, and this can react further with a second molecule of benzoylacetyl-CoA, again via a Mannich reaction. Naturally, because of the nature of the side-chain, the second intramolecular Mannich reaction as involved in pseudopelletierine biosynthesis is not feasible. Alkaloids such as **lobeline** and **lobelanine** from *Lobelia inflata*, or **sedamine** from *Sedum acre* (Crassulaceae), are products from further *N*-methylation and/or carbonyl reduction reactions (Figure 6.25). The North American Indians smoked lobelia rather like tobacco (*Nicotiana tabacum*; Solanaceae). **Lobeline** stimulates nicotinic acetylcholine receptor sites in a similar way to nicotine, but with a weaker effect. It has been employed in smoking cessation preparations, and more recently to treat methamphetamine abuse (see page 404). Ketopiperidines such as lobeline, *N*-methylpelletierine, and related structures epimerize readily at the centre adjacent to the nitrogen via retro-Michael/Michael conjugate addition reactions (compare cuscohygrine, page 316).

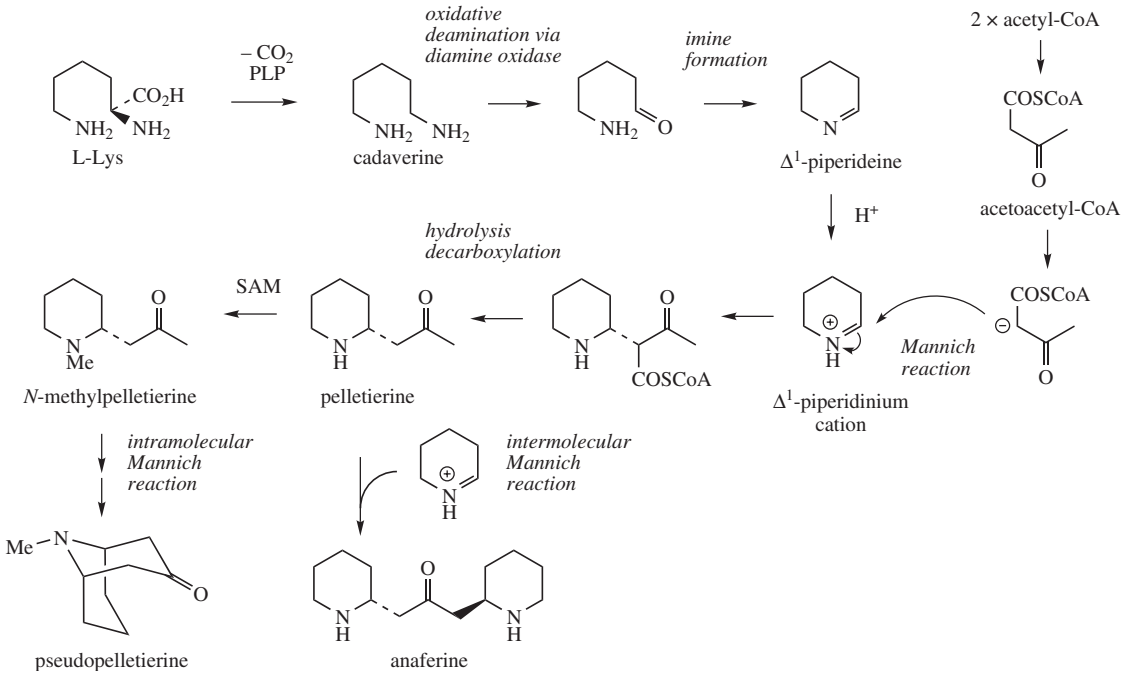


Figure 6.24

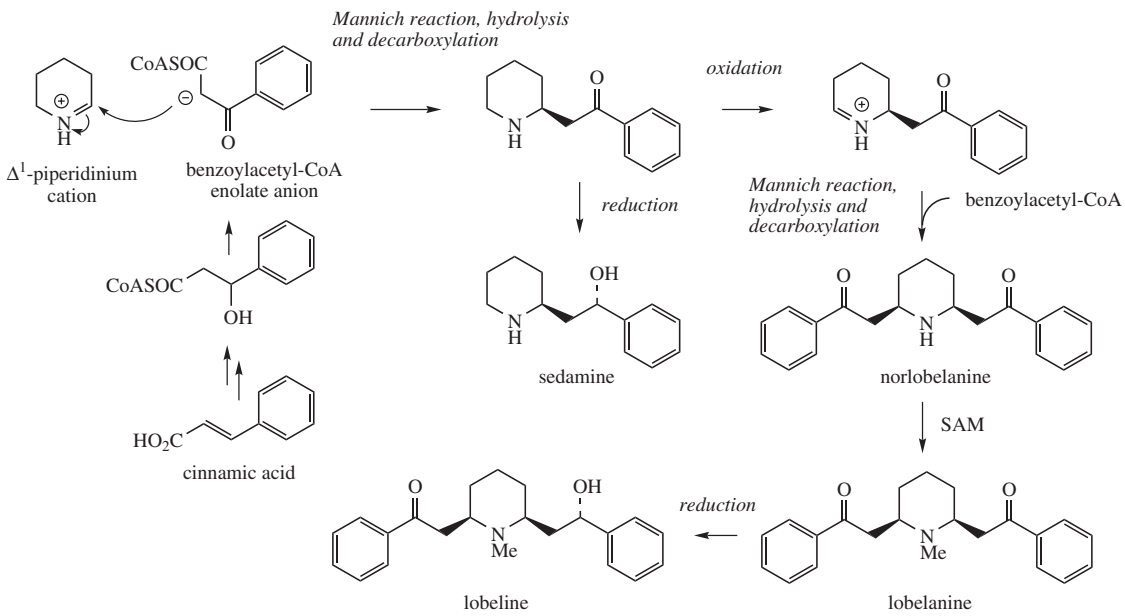


Figure 6.25

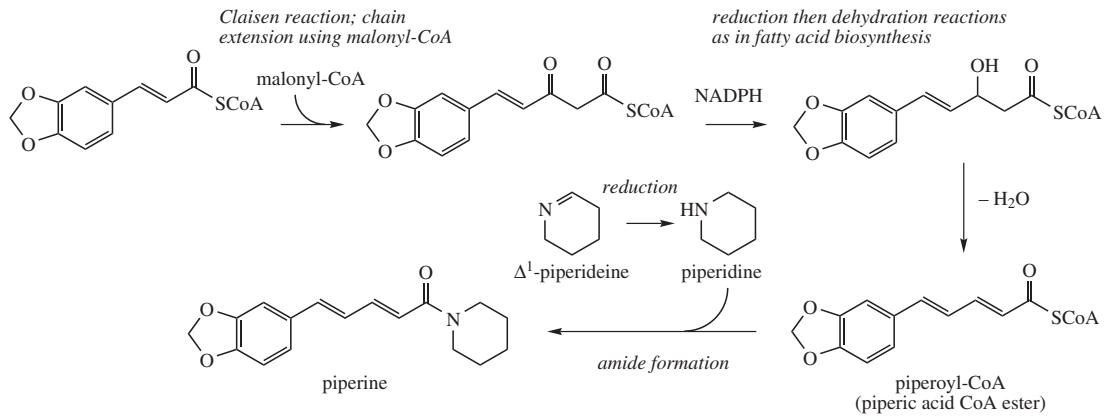


Figure 6.26

The simple piperidine alkaloid coniine from poison hemlock is not derived from lysine, but originates by an amination process and is discussed on page 401.

The pungency of the fruits of black pepper (*Piper nigrum*; Piperaceae), a widely used condiment, is mainly due to the piperidine alkaloid **piperine** (Figure 6.26). In this structure, the piperidine ring forms part of a tertiary amide structure and is incorporated via piperidine itself, the reduction product of Δ^1-piperideine (Figure 6.24). The piperic acid portion is derived from a cinnamoyl-CoA precursor, with chain extension using acetate/malonate (see page 168), and combines as its CoA ester with piperidine.

Quinolizidine Alkaloids

The lupin alkaloids, found in species of *Lupinus* (Leguminosae/Fabaceae) and responsible for the toxic properties associated with lupins, are characterized by a quinolizidine skeleton (Figure 6.27). This bicyclic ring system is closely related to the ornithine-derived pyrrolizidine system, but is formed from two molecules of lysine. **Lupinine** from *Lupinus luteus* is a relatively simple structure, very comparable to the basic ring system of the pyrrolizidine alkaloid retronecine (see page 325), but other lupin alkaloids, e.g. **lupanine** and **sparteine** (Figure 6.27) contain a tetracyclic bis-quinolizidine ring system and are formed by incorporation of a third lysine molecule. (−)-Sparteine is also the major alkaloid in Scotch broom (*Cytisus scoparius*; Leguminosae/Fabaceae); both enantiomeric forms of sparteine are found in nature. The alkaloid (−)-**cytisine**, a toxic component of *Laburnum* species (Leguminosae/Fabaceae), contains a modified tricyclic ring system, and comparison with the structures of lupanine or sparteine shows its likely relationship by loss of four carbon atoms from the tetracyclic system of (+)-sparteine (Figure 6.27). The structural similarity of lupinine and retronecine is not fully reflected in the biosynthetic pathways. Experimental evidence shows lysine to be incorporated into lupinine via **cadaverine**, but no intermediate corresponding to homospermidine is implicated. Δ^1-Piperideine seems to be an important intermediate after cadaverine, and the pathway proposed (Figure 6.27) invokes coupling of two such molecules. The two tautomers of Δ^1-piperideine, nitrogen analogues of keto–enol systems, are able to couple by an aldol-type mechanism (see page 20). Indeed, this coupling occurs in solution at physiological pH values, though stereospecific coupling to the product shown in Figure 6.27 would require appropriate enzyme participation. Following the coupling, it is suggested that the imine system is hydrolysed, the primary amine group then oxidized, and formation of the quinolizidine ring is achieved by imine formation. **Lupinine** is then synthesized by a reductive step.

The pathway to **sparteine** and **lupanine** undoubtedly requires participation of another molecule of cadaverine or Δ^1-piperideine. Experimental data are not clear cut, and Figure 6.27 merely indicates how incorporation of a further piperidine ring might be envisaged. Loss of the fourth ring and oxidation to a pyridone system offers a potential route to **cytisine**. Note that either of the outermost rings could be lost to yield the same product.

Quinolizidine alkaloids are mainly found in plants of the Leguminosae/Fabaceae family. They deter or repel the feeding of herbivores and are toxic to them by a variety of mechanisms. A number of plants (*Laburnum, Cytisus, Lupinus*) containing significant quantities of these

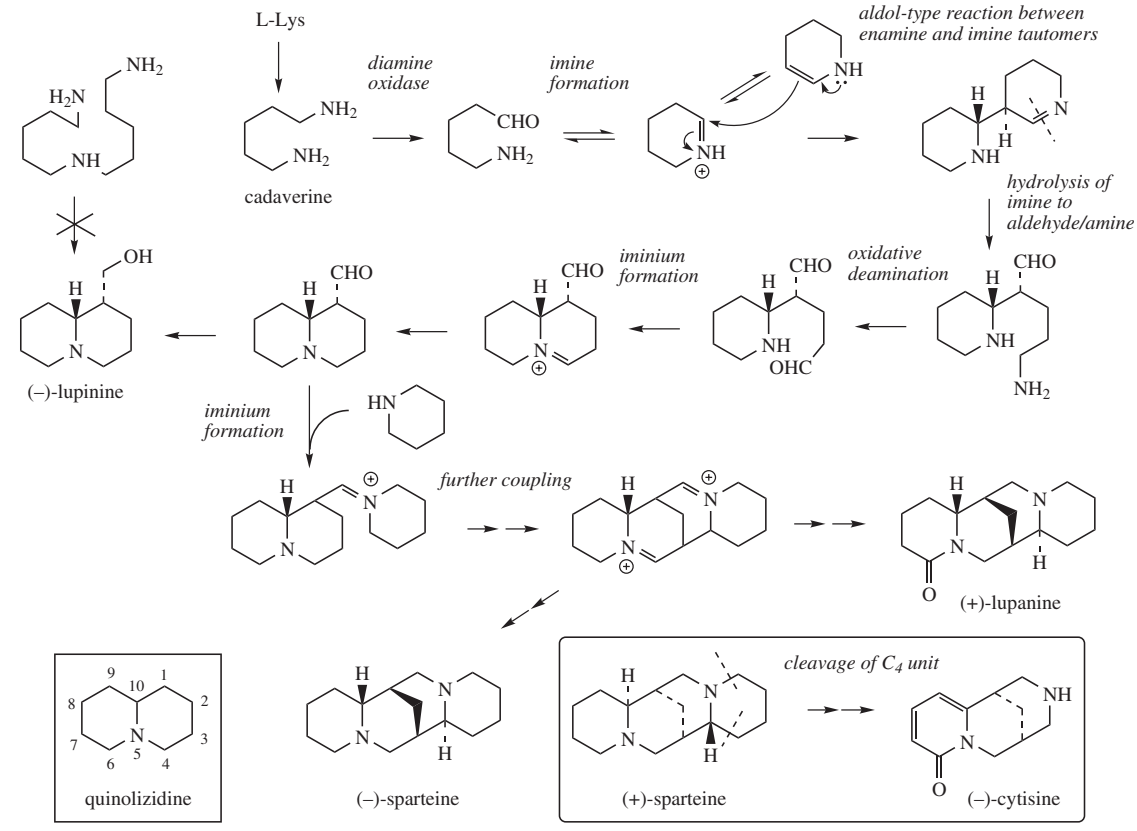


Figure 6.27

alkaloids must be regarded as potentially toxic to humans, and are known to be responsible for human poisoning. The widely planted and ornamental laburnum trees offer a particular risk, since all parts, including the pea-like seeds, contain dangerously high amounts of alkaloids. So-called 'sweet lupins' are selected strains with an acceptably low alkaloid content (typically about a quarter of the total alkaloids of 'bitter' strains), and are cultivated as a high-protein crop. Natural (−)-sparteine, a chiral diamine, is a useful chiral ligand in asymmetric synthesis; it can be extracted readily from branches of *Cytisus scoparius*. (−)-Cytisine can be obtained in large amounts from the seeds of *Laburnum anagyroides* and is a potent agonist for nicotinic acetylcholine receptors, more so than nicotine itself. It has been used in eastern Europe for many years as a smoking cessation aid. The synthetic **varenicline** (Figure 6.28) is loosely based on the cytisine structure and has also been introduced to help stop smoking; it is

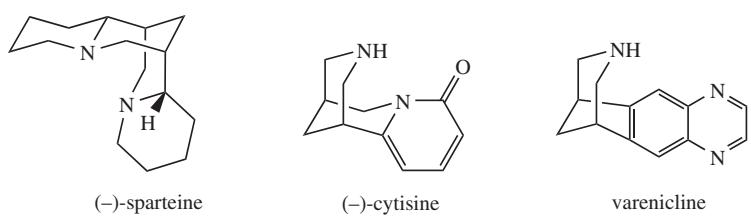


Figure 6.28

a partial agonist at nicotinic acetylcholine receptors, and also seems to deter ethanol consumption in patients.

Indolizidine Alkaloids

Indolizidine alkaloids (Figure 6.29) are characterized by fused six- and five-membered rings, with a nitrogen atom at the ring fusion, e.g. **swainsonine** from *Swainsona canescens* (Leguminosae/Fabaceae) and **castanospermine** from the Moreton Bay chestnut *Castanospermum australe* (Leguminosae/Fabaceae). In this respect, they appear to be a hybrid between the pyrrolizidine and quinolizidine alkaloids described above.

Although they are derived from lysine, their origin deviates from the more common lysine-derived structures in that L-**pipecolic acid** is an intermediate in the pathway. Two routes to pipecolic acid are known in nature, as indicated in Figure 6.29, and these differ with respect to whether the nitrogen atom originates from the α- or the ε-amino group of lysine. For indolizidine alkaloid biosynthesis, pipecolic acid is formed via the aldehyde and imine with retention of the α-amino group nitrogen. This can then act as starter for malonate chain extension, incorporating an extra C_2 unit. The indolizidinone may then be produced by simple reactions, though no details are known. This compound leads to castanospermine by a sequence of hydroxylations, but is also a branch-point compound to alkaloids such as swainsonine which have the opposite configuration at the ring fusion. Involvement of a planar iminium ion would account for the change in stereochemistry. Polyhydroxyindolizidines such as swainsonine and castanospermine displayed activity against the AIDS virus HIV, by their ability to inhibit glycosidase enzymes involved in glycoprotein biosynthesis. The glycoprotein coating is essential for the proliferation of AIDS and some other viruses. This has stimulated considerable research on related structures and their mode of action. The ester 6-*O*-butanoyl-castanospermine (**celgosivir**; Figure 6.30) was unsuccessful in clinical trials as an anti-AIDS agent,

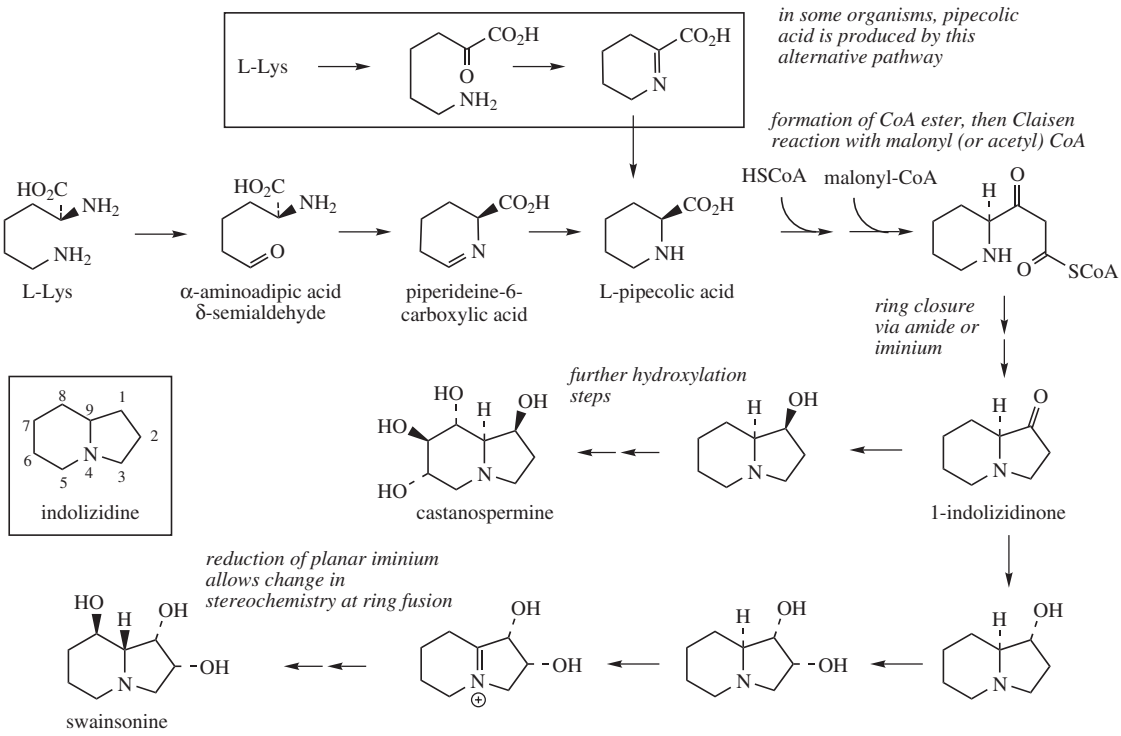


Figure 6.29

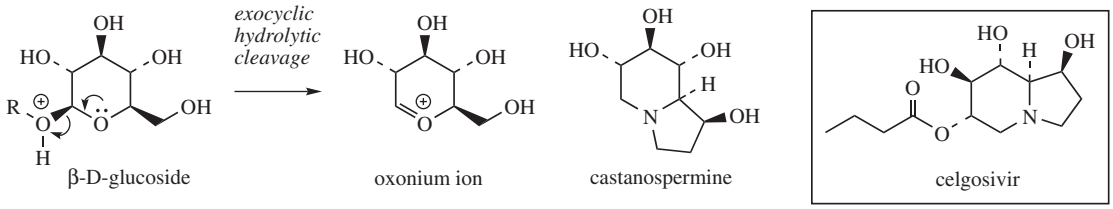


Figure 6.30

but is still being evaluated against hepatitis C. There is a strong similarity between castanospermine and the oxonium ion formed by hydrolytic cleavage of a glucoside (Figure 6.30) (see page 30), but there appears to be little stereochemical relationship with some other sugars, whose hydrolytic enzymes are also strongly inhibited. These alkaloids are also toxic to animals, causing severe gastrointestinal upset and malnutrition by severely affecting intestinal hydrolases. Indolizidine alkaloids are found in many plants in the Leguminosae/Fabaceae (e.g. *Swainsona, Astragalus, Oxytropis*) and also in some fungi (e.g. *Rhizoctonia leguminicola*, which produces swainsonine).

ALKALOIDS DERIVED FROM NICOTINIC ACID

Pyridine Alkaloids

The alkaloids found in tobacco (*Nicotiana tabacum*; Solanaceae) [Box 6.3] include **nicotine** and **anabasine** (Figure 6.31). The structures contain a pyridine system together with a pyrrolidine ring (in nicotine) or a piperidine ring (in anabasine), the latter rings arising from ornithine and lysine respectively. The pyridine unit has its origins in **nicotinic acid** (**vitamin B_3**; Figure 6.31), the vitamin sometimes called **niacin** (see page 31). The amide nicotinamide forms an essential component of coenzymes such as NAD^+ and $NADP^+$ (see page 25). The nicotinic acid component of nicotinamide is synthesized in animals by degradation of L-tryptophan through the **kynurenine** pathway and **3-hydroxyanthranilic acid** (Figure 6.32) (see also dactinomycin, page 452), the pyridine ring being formed by oxidative cleavage of the benzene ring and subsequent inclusion of the amine nitrogen (Figure 6.32). However, plants such as *Nicotiana* use a different pathway employing dihydroxyacetone phosphate and L-aspartic acid precursors (Figure 6.33). The dibasic acid **quinolinic acid** features in both pathways. Decarboxylation to nicotinic acid involves assimilation of the quinolinic acid into the pyridine nucleotide cycle, and the subsequent side-chain modifications take place whilst the pyridine system is part of a nucleotide complex. Nicotinamide and nicotinic acid are subsequently released from nucleotide carriers. The pyridine nucleotide cycle is responsible for the biosynthesis of NAD^+, the degradation of NAD^+ to nicotinic acid, and the recycling of nicotinic acid to NAD^+. **Picolinic acid** (Figure 6.32) is an isomer of nicotinic acid also originating from the kynurenine pathway.

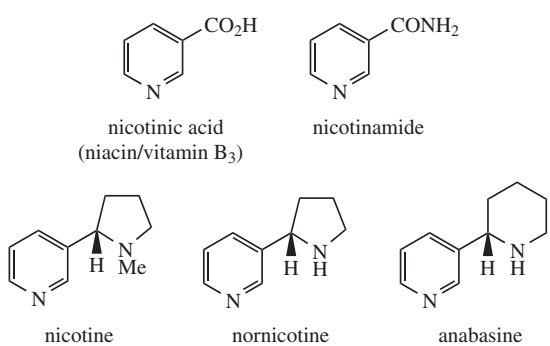
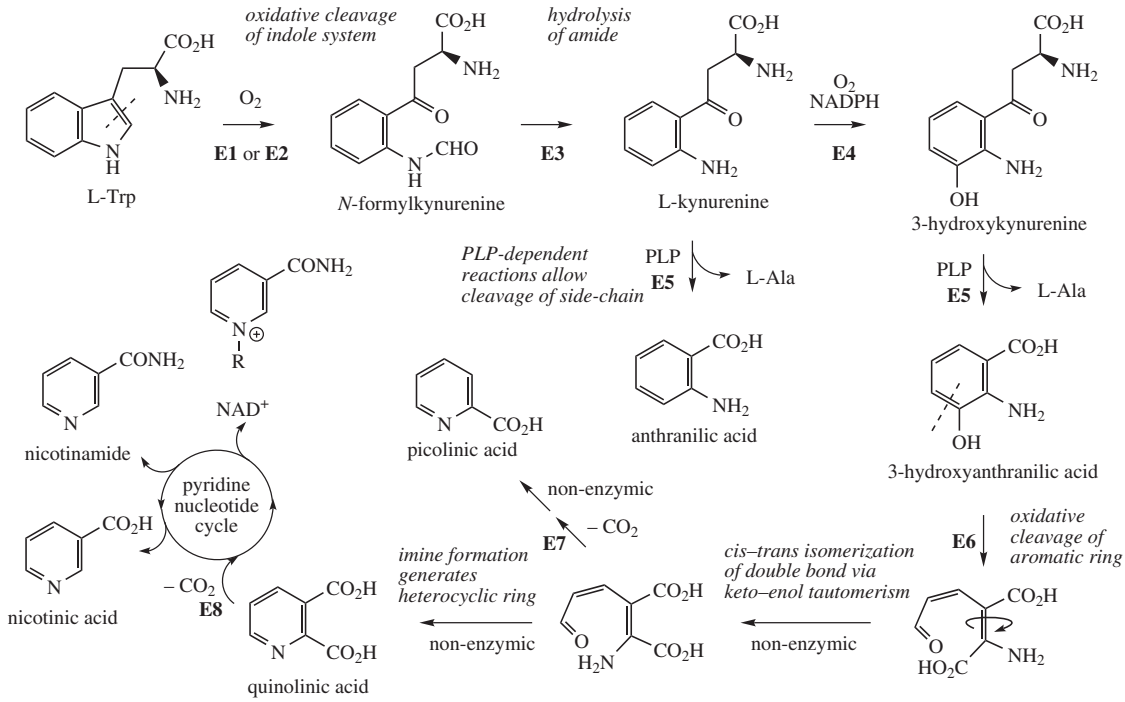


Figure 6.31

In the formation of **nicotine**, a pyrrolidine ring derived from ornithine, most likely as the *N*-methyl-Δ^1-pyrrolinium cation (see Figure 6.3), is attached to the pyridine ring of nicotinic acid, displacing the carboxyl during the sequence (Figure 6.34). A dihydronicotinic acid intermediate is likely to be involved allowing decarboxylation to the enamine 1,2-dihydropyridine. This allows an aldol-type reaction with the *N*-methylpyrrolinium cation, and finally dehydrogenation of the dihydropyridine ring back to a pyridine gives nicotine. **Nornicotine** is derived by oxidative demethylation of nicotine involving a cytochrome P-450-dependent enzyme. **Anabasine**



E1: L-tryptophan 2,3-dioxygenase (L-Trp-specific)
E2: indoleamine 2,3-dioxygenase (broad specificity)
E3: kynurenine formamidase
E4: kynurenine 3-monooxygenase
E5: kynureninase
E6: 3-hydroxyanthranilate 3,4-dioxygenase
E7: aminocarboxymuconate-semialdehyde decarboxylase
E8: quinolinic acid phosphoribosyltransferase

Figure 6.32

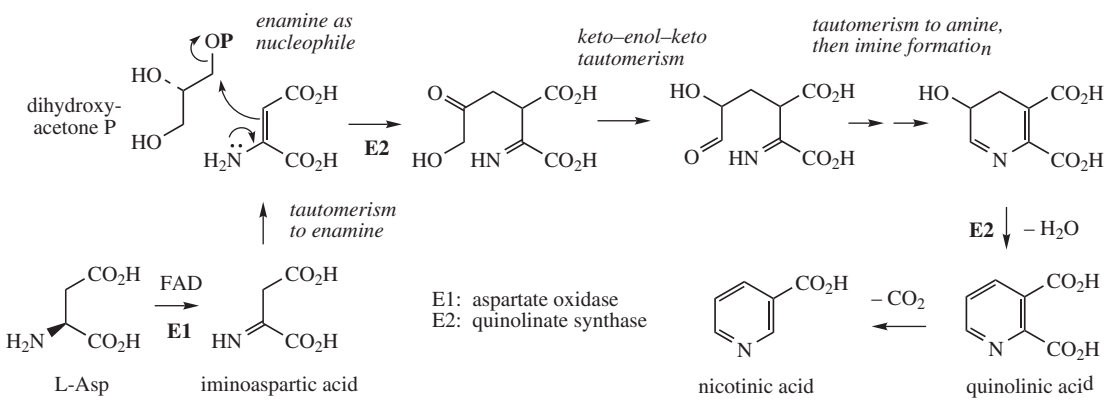


Figure 6.33

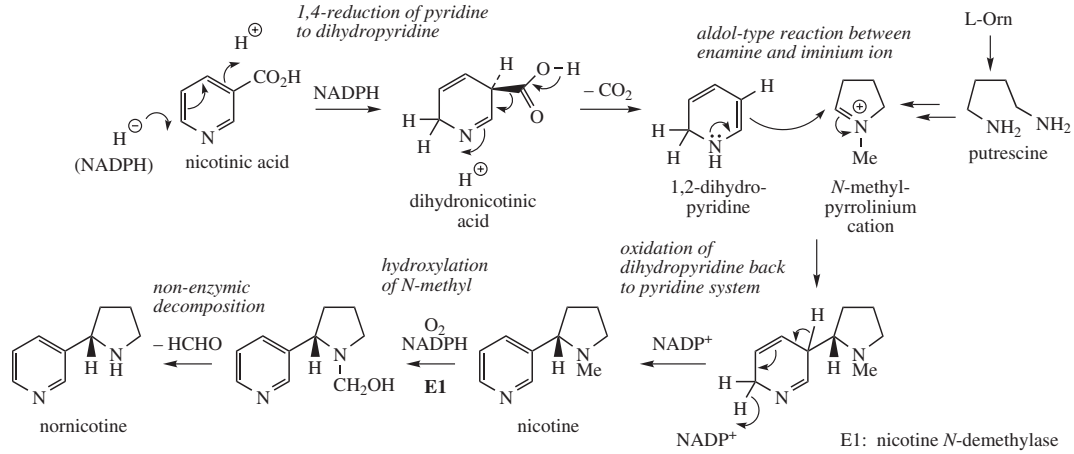


Figure 6.34

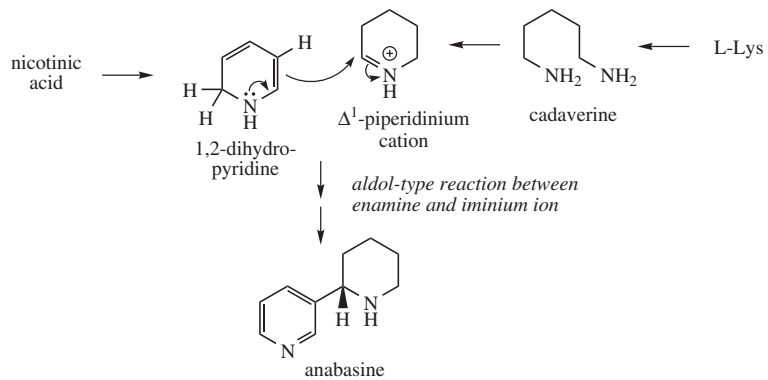


Figure 6.35

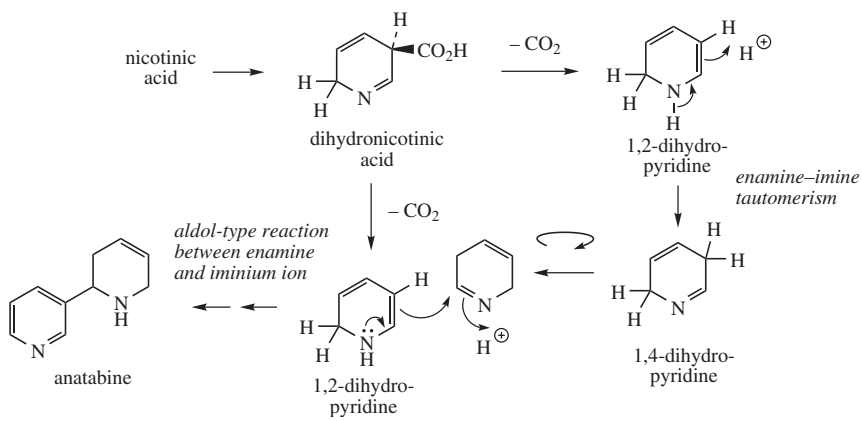


Figure 6.36

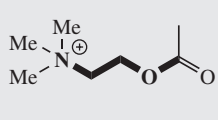
is produced from nicotinic acid and lysine via the Δ^1-piperidinium cation in an essentially analogous manner (Figure 6.35). A subtle anomaly has been exposed, in that a further *Nicotiana* alkaloid **anatabine** appears to be derived by combination of two nicotinic acid units, and the Δ^3-piperideine ring is *not* supplied by lysine (Figure 6.36).

Box 6.3

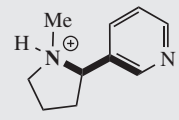
Tobacco

Tobacco is the cured and dried leaves of *Nicotiana tabacum* (Solanaceae), an annual herb indigenous to tropical America, but cultivated widely for smoking. Tobacco leaves may contain from 0.6 to 9% of (−)-nicotine (Figure 6.31), an oily, volatile liquid as the major alkaloid (about 93%), together with smaller amounts of structurally related alkaloids, e.g. nornicotine (about 3% of alkaloids), anabasine (about 0.5%; Figure 6.31), and anatabine (about 4%; Figure 6.36). In the leaf, the alkaloids are typically present as salts with malic and citric acids. Nicotine in small doses can act as a respiratory stimulant, though in larger doses it causes respiratory depression. Despite the vast array of evidence linking tobacco smoking and cancer, the smoking habit continues throughout the world, and tobacco remains a major crop plant. Tobacco smoke contains over 4000 compounds, including more than 60 known carcinogens formed by incomplete combustion. Amongst these are polycyclic aromatic hydrocarbons, e.g. benzopyrene, nitrosamines, aromatic amines, aldehydes, and other volatile compounds. Metabolism by the body's P-450 system leads to further reactive intermediates which can combine with DNA and cause mutations. Tobacco smoking also contributes to atherosclerosis, chronic bronchitis, and emphysema and is regarded as the single most preventable cause of death in modern society. Smoking tobacco is an addictive habit; unlike other addictive drugs, however, tobacco is legally and widely accessible. Nevertheless, in many parts of the world, it is rapidly becoming an antisocial activity, in that 'passive smoking' (inhaling smoke from users in confined spaces) can also lead to health problems. **Nicotine** is used by smokers who wish to stop the habit. It is available in the form of chewing gum or nasal sprays, or can be absorbed transdermally from nicotine-impregnated patches.

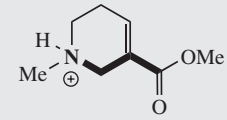
Powdered tobacco leaves have long been used as an insecticide, and nicotine from *Nicotiana tabacum* or *Nicotiana rustica* has been formulated for agricultural and horticultural use. The free base is considerably more toxic than salts, and soaps may be included in the formulations to ensure a basic pH and to provide a surfactant. Other *Nicotiana* alkaloids, e.g. anabasine and nornicotine, share this insecticidal activity. Although an effective insecticide, nicotine has generally been replaced by other agents considered to be safer. Nicotine is toxic to man due to its effect on the nervous system, interacting with the nicotinic acetylcholine receptors, though the tight binding observed is only partially accounted for by the structural similarity between acetylcholine and nicotine (Figure 6.37). Recent studies suggest that nicotine can improve memory by stimulating the transmission of nerve impulses, and this finding may account for the lower incidence of Alzheimer's disease in smokers. Any health benefits conferred by smoking are more than outweighed by the increased risk of heart, lung, and respiratory diseases.



acetylcholine



nicotine
(as conjugate acid)



arecoline
(as conjugate acid)

Figure 6.37

Arecoline (Figure 6.38) is a tetrahydronicotinic acid derivative found in betel-nuts (*Areca catechu*: Palmae/Arecaceae); no biosynthetic information has been reported. Betel-nuts are chewed in India and Asia for the stimulant effect of arecoline [Box 6.4].

Box 6.4

Areca

Areca nuts (betel-nuts) are the seeds of *Areca catechu* (Palmae/Arecaceae), a tall palm cultivated in the Indian and Asian continents. These nuts are mixed with lime, wrapped in leaves of the betel pepper (*Piper betle*) and then chewed for their stimulant effect, and subsequent feeling of well-being and mild intoxication. The teeth and saliva of chewers stain bright red. The major stimulant alkaloid is arecoline (up to 0.2%; Figure 6.38), the remainder of the alkaloid content (total about 0.45%) being composed of structurally related reduced pyridine structures, e.g. arecaidine, guvacine (tetrahydronicotinic acid), and guvacoline (Figure 6.38). Arecoline is an agonist for muscarinic acetylcholine receptors (see Figure 6.37), although the ester function is reversed compared with acetylcholine. **Arecoline** has been employed in veterinary practice as a vermicide to eradicate worms.

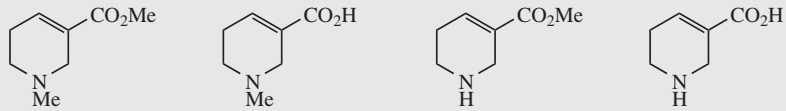
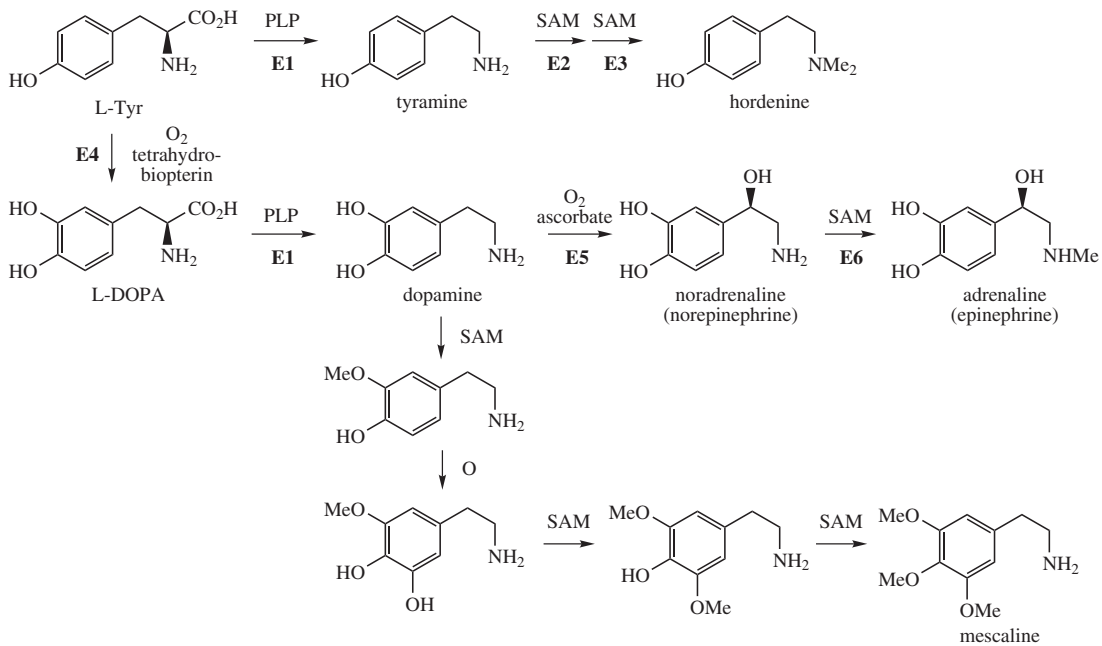


Figure 6.38



E1: aromatic L-amino acid decarboxylase (tyrosine decarboxylase; DOPA decarboxylase)
E2: tyramine *N*-methyltransferase
E3: *N*-methyltyramine *N*-methyltransferase
E4: tyrosine hydroxylase
E5: dopamine β-monooxygenase
E6: phenylethanolamine *N*-methyltransferase

Figure 6.39

ALKALOIDS DERIVED FROM TYROSINE

Phenylethylamines and Simple Tetrahydroisoquinoline Alkaloids

PLP-dependent decarboxylation of L-**tyrosine** gives the simple phenylethylamine derivative **tyramine**, which on di-*N*-methylation yields **hordenine**, a germination inhibitory alkaloid from barley (*Hordeum vulgare*; Graminae/Poaceae; Figure 6.39). More commonly, phenylethylamine derivatives possess 3,4-di- or 3,4,5-tri-hydroxylation patterns and are derived via **dopamine** (Figure 6.39), the decarboxylation product from L-**DOPA** (see page 147). The enzyme aromatic amino acid decarboxylase is relatively non-specific and can catalyse decarboxylation of other aromatic amino acids, e.g. tryptophan and histidine. Pre-eminent amongst the simple phenylethylamine derivatives are the catecholamines **noradrenaline** (**norepinephrine**), a mammalian neurotransmitter, and **adrenaline** (**epinephrine**), the 'fight or flight' hormone released in animals from the adrenal gland as a result of stress [Box 6.5]. These compounds are synthesized by sucessive β-hydroxylation and *N*-methylation reactions on dopamine (Figure 6.39). Aromatic hydroxylation and *O*-methylation reactions in the cactus *Lophophora williamsii* (Cactaceae) convert dopamine into **mescaline** (Figure 6.39), an alkaloid with pyschoactive and hallucinogenic properties [Box 6.5]. Note that the sequence of hydroxylations and methylations exactly parallels that described for the cinnamic acids (see page 149).

Box 6.5

Catecholamines

The catecholamines dopamine, noradrenaline (norepinephrine), and adrenaline (epinephrine) are produced in the adrenal glands and nervous tissue and act as neurotransmitters in mammals. Several adrenergic receptors have been identified. α-Receptors are usually excitatory and produce a constricting effect on vascular, uterine, and intestinal muscles. β-Receptors are usually inhibitory on smooth muscle, but stimulatory on heart muscles. **Dopamine** (Figure 6.39) can act on both vascular α_1 and cardiac β_1 receptors, but also has its own receptors in several other structures. In Parkinson's disease, there is a deficiency of dopamine due to neural degeneration, affecting the balance between excitatory and inhibitory transmitters. Treatment with **L-DOPA** (**levodopa**; Figure 6.39) helps to increase the dopamine levels in the brain. Unlike dopamine, DOPA can cross the blood–brain barrier, but needs to be administered with a DOPA-decarboxylase inhibitor, e.g. **carbidopa** (Figure 6.40), to prevent rapid decarboxylation in the bloodstream. Injections of dopamine or **dobutamine** (Figure 6.40) are valuable as cardiac stimulants in cases of cardiogenic shock. These agents act on β_1 receptors; **dopexamine** (Figure 6.40) is also used for chronic heart failure, but acts on β_2 receptors in cardiac muscle.

Noradrenaline (**norepinephrine**) (Figure 6.39) is a powerful peripheral vasoconstrictor predominantly acting on α-adrenergic receptors, and is useful in restoring blood pressure in cases of acute hypotension. The structurally related alkaloid **ephedrine** (see page 403) may be used in the same way, and synthetic analogues, e.g. **phenylephrine** and **metaraminol** (Figure 6.40), have also been developed. **Methyldopa** is used to treat hypertension; it is a centrally acting agent that becomes decarboxylated and hydroxylated to form the false transmitter α-methylnoradrenaline which competes with noradrenaline.

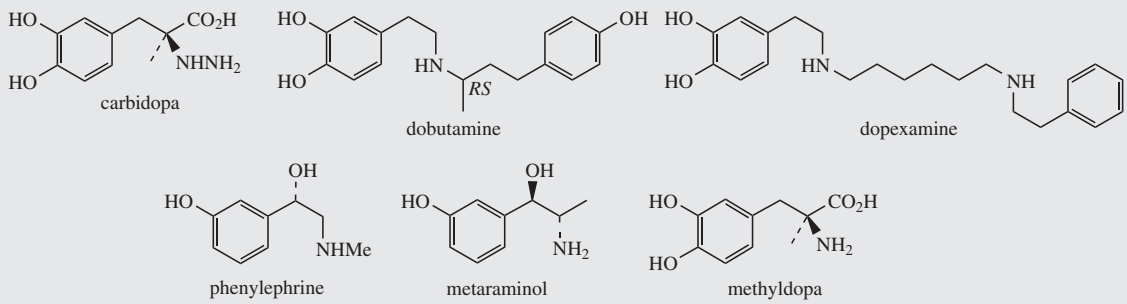


Figure 6.40

Box 6.5 (continued)

Adrenaline (**epinephrine**; Figure 6.39) is released from the adrenal glands when an animal is confronted with an emergency situation, markedly stimulating glycogen breakdown in muscle, increasing respiration, and triggering catabolic processes that result in energy release. Adrenaline interacts with both α- and β-receptors, an α-response being vasoconstriction of smooth muscle in the skin. β-Responses include mediation of cardiac muscle contractions and the relaxation of smooth muscle in the bronchioles of the lung. Injection of adrenaline is thus of value in cases of cardiac arrest, or in allergic emergencies such as bronchospasm or severe allergy (anaphylactic shock). It is not effective orally. A wide range of cardioactive β-adrenoceptor blocking agents (**beta-blockers**) has been developed to selectively bind to β-receptors to control the rate and force of cardiac contractions in the management of hypertension and other heart conditions. The prototype of the beta-blocker drugs is **propranolol** (Figure 6.41), in which the catechol ring system has been modified to a naphthalene ether and a bulky *N*-alkyl substituent has been incorporated. Many structural variants have been produced, and there is now a huge, perhaps bewildering, variety of beta-blockers in regular use, with subtle differences in properties and action affecting the choice of drug for a particular condition or individual patient. These are shown in Figure 6.41. **Atenolol, bisoprolol, metoprolol, nebivolol**, and, to a lesser extent, **acebutolol** have less effect on the β_2 bronchial receptors and are thus relatively cardioselective, but not cardiospecific. Most other agents are non-cardioselective, and could also provoke breathing difficulties. **Esmolol** and **sotalol** are used only in the management of arrhythmias.

Other β-agonists are mainly selective towards the β_2-receptors and are valuable as antiasthmatic drugs. Important examples include **salbutamol** (**albuterol**) and **terbutaline**, which are very widely prescribed principally for administration by inhalation at the onset of an asthma attack, but as with cardioactive beta-blockers, a range of agents is in current use (Figure 6.41).

non-selective beta-adrenoceptor blockers

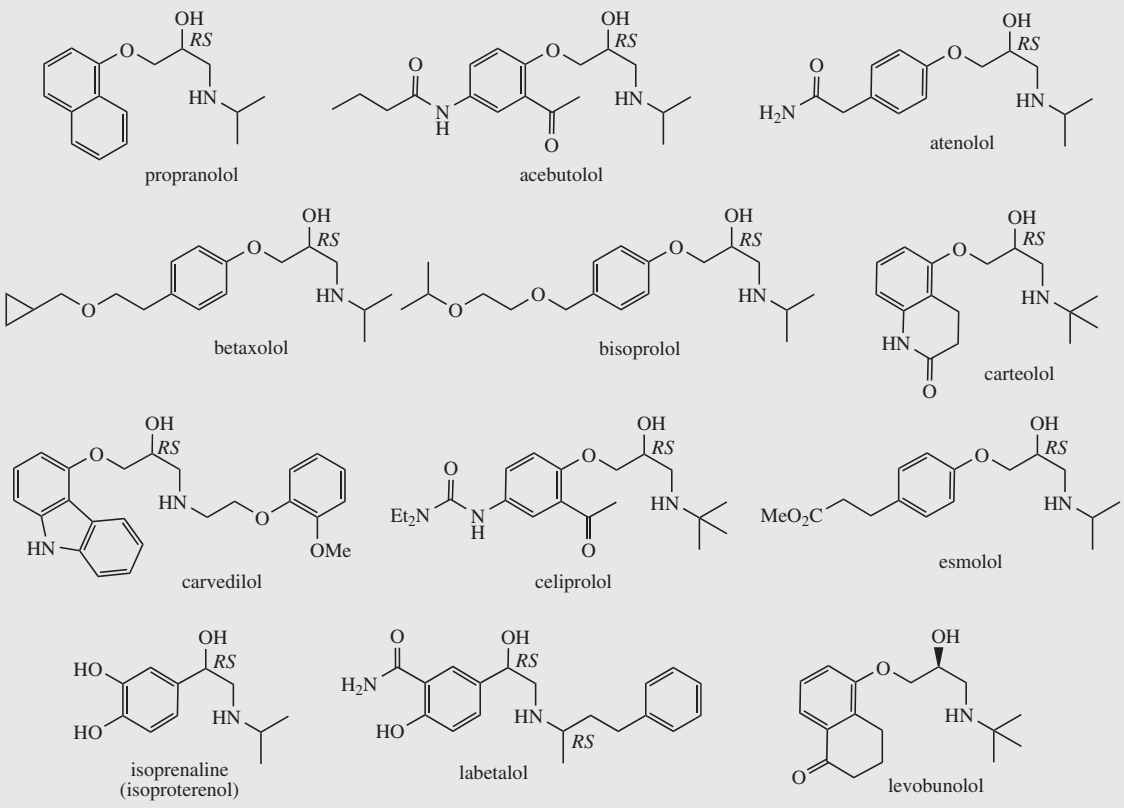
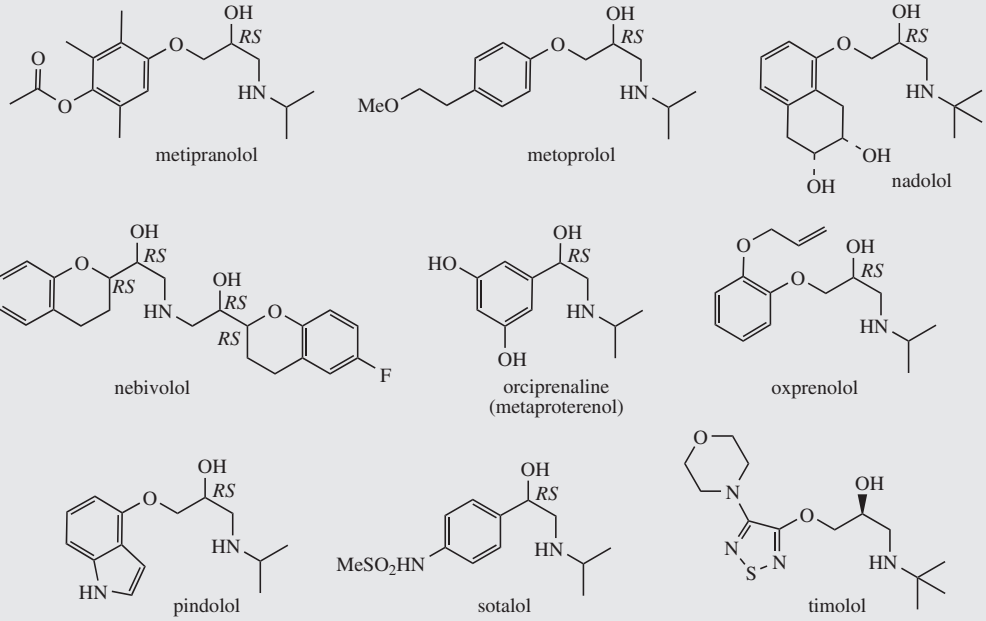


Figure 6.41 (*continued overleaf*)

Box 6.5 (continued)

non-selective beta-adrenoceptor blockers



selective β_2-adrenoceptor blockers

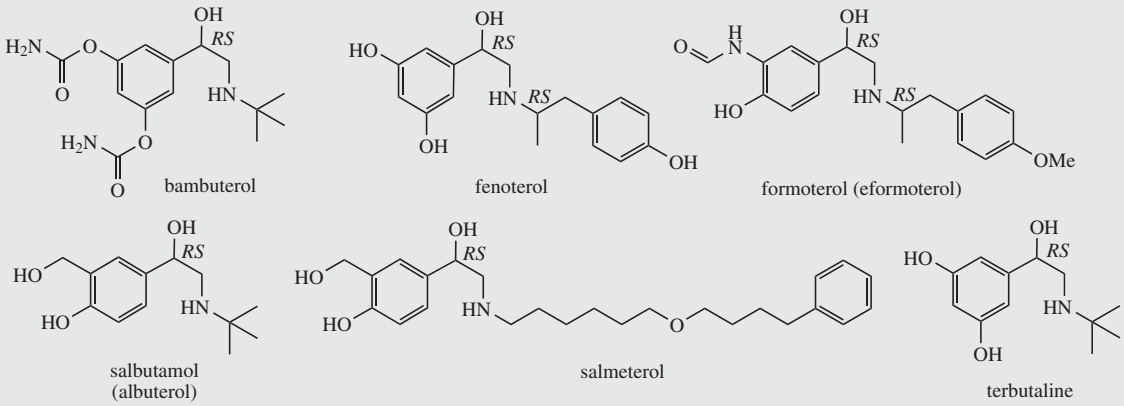


Figure 6.41 (*continued*)

These agents supersede the earlier less-selective bronchodilator drugs such as **isoprenaline** (**isoproterenol**) and **orciprenaline** (**metaproterenol**) (Figure 6.41). Topical application of a beta-blocker to the eye reduces intra-ocular pressure by reducing the rate of production of aqueous humour. Some drugs in this class, namely **betaxolol, carteolol, levobunolol, metipranolol**, and **timolol**, are thus useful in treating glaucoma. **Propranolol, metoprolol, nadolol**, and **timolol** also have additional application in the prophylaxis of migraine.

Catecholamine neurotransmitters are subsequently inactivated by enzymic methylation of the 3-hydroxyl (via catechol-*O*-methyltransferase) or by oxidative removal of the amine group via monoamine oxidase. Monoamine oxidase

Box 6.5 (continued)

inhibitors are sometimes used to treat depression, and these drugs cause an accumulation of amine neurotransmitters. Under such drug treatment, simple amines such as tyramine (in cheese, beans, fish, and yeast extract) are also not metabolized and can cause dangerous potentiation of neurotransmitter activity.

Though generally thought of as animal neurotransmitters, catecholamines are also fairly widespread in plants. Significant levels of dopamine accumulate in the flesh of banana (*Musa* species; Araceae), and adrenaline and noradrenaline occur in the peyote cactus (*Lophophora williamsii*; Cactaceae) (see below).

Lophophora

Lophophora or peyote consists of the dried sliced tops of *Lophophora williamsii* (Cactaceae), a small cactus from Mexico and the southwestern United States. The plant has been used by the Aztecs and then by the Mexican Indians for many years, especially in religious ceremonies to produce hallucinations and establish contact with the gods. The so-called mescal buttons were ingested, and this caused unusual and bizarre coloured images. The plant is still used by people seeking drug-induced experiences. The most active of the range of alkaloids found in lophophora (total 8–9% alkaloids in the dried mescal buttons) is mescaline (Figure 6.39), a simple phenylethylamine derivative. Other constituents include anhalamine, anhalonidine, and anhalonine (Figure 6.42). **Mescaline** has been used as a hallucinogen in experimental psychiatry. The dosage required is quite large (300–500 mg), but the alkaloid can readily be obtained by total synthesis, which is relatively uncomplicated. Mescaline is also found in other species of cactus, e.g. *Trichocereus pachanoi*, a substantially larger columnar plant that can grow up to 20 feet tall and found mainly in the Andes.

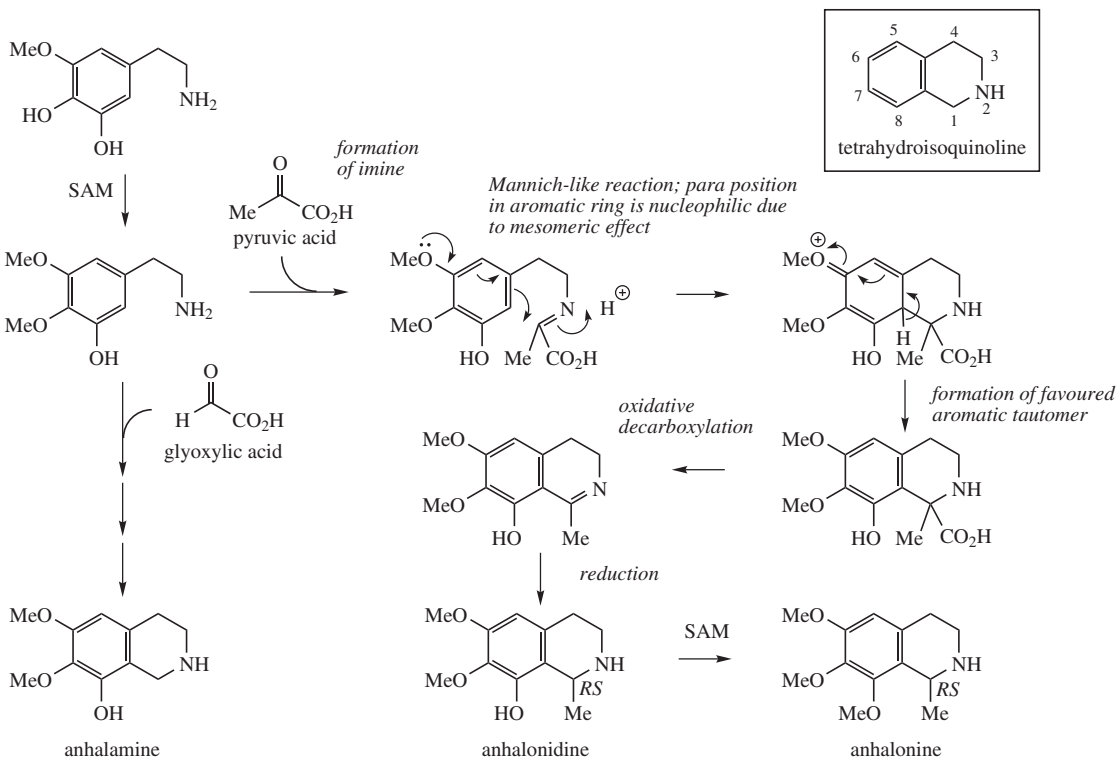


Figure 6.42

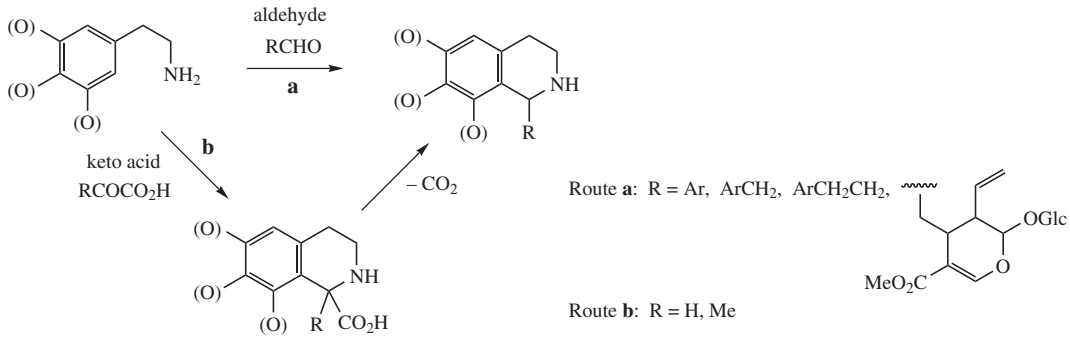


Figure 6.43

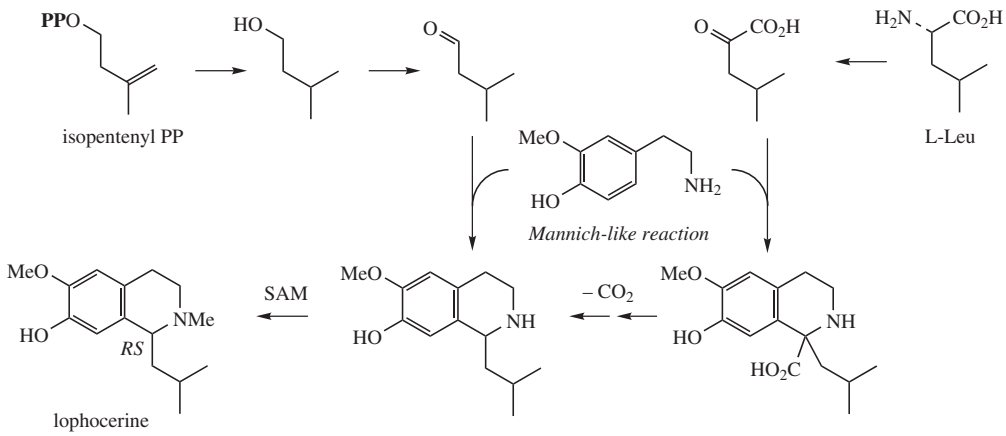


Figure 6.44

Closely related alkaloids co-occurring with mescaline are **anhalamine, anhalonine**, and **anhalonidine** (Figure 6.42), which are representatives of simple tetrahydroisoquinoline derivatives. The additional carbon atoms, two in the case of anhalonidine and anhalonine, and one for anhalamine, are supplied by pyruvate and glyoxylate respectively. In each case, a carboxyl group is lost from this additional precursor. The keto acid pyruvate reacts with a suitable phenylethylamine, in this case the dimethoxy-hydroxy derivative, giving an imine (Figure 6.42). In a Mannich-like mechanism, cyclization occurs to generate the isoquinoline system, the mesomeric effect from an oxygen substituent providing the nucleophilic site on the aromatic ring. Restoration of aromaticity via proton loss gives the tetrahydroisoquinoline, overall a biosynthetic equivalent of the Pictet–Spengler synthesis. The carboxyl group is then removed, not by a simple decarboxylation, but via an unusual oxidative decarboxylation first generating an intermediate imine. Reduction then leads to **anhalonidine**, with further methylation giving **anhalonine. Anhalamine** is derived from the same phenylethylamine precursor, but utilizing glyoxylic acid (Figure 6.42).

Chemical synthesis of tetrahydroisoquinolines by the Pictet–Spengler reaction does not usually employ keto acids like pyruvate or aldehyde acids like glyoxylate. Instead, simple aldehydes, e.g. acetaldehyde

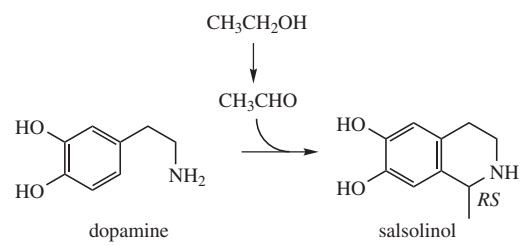
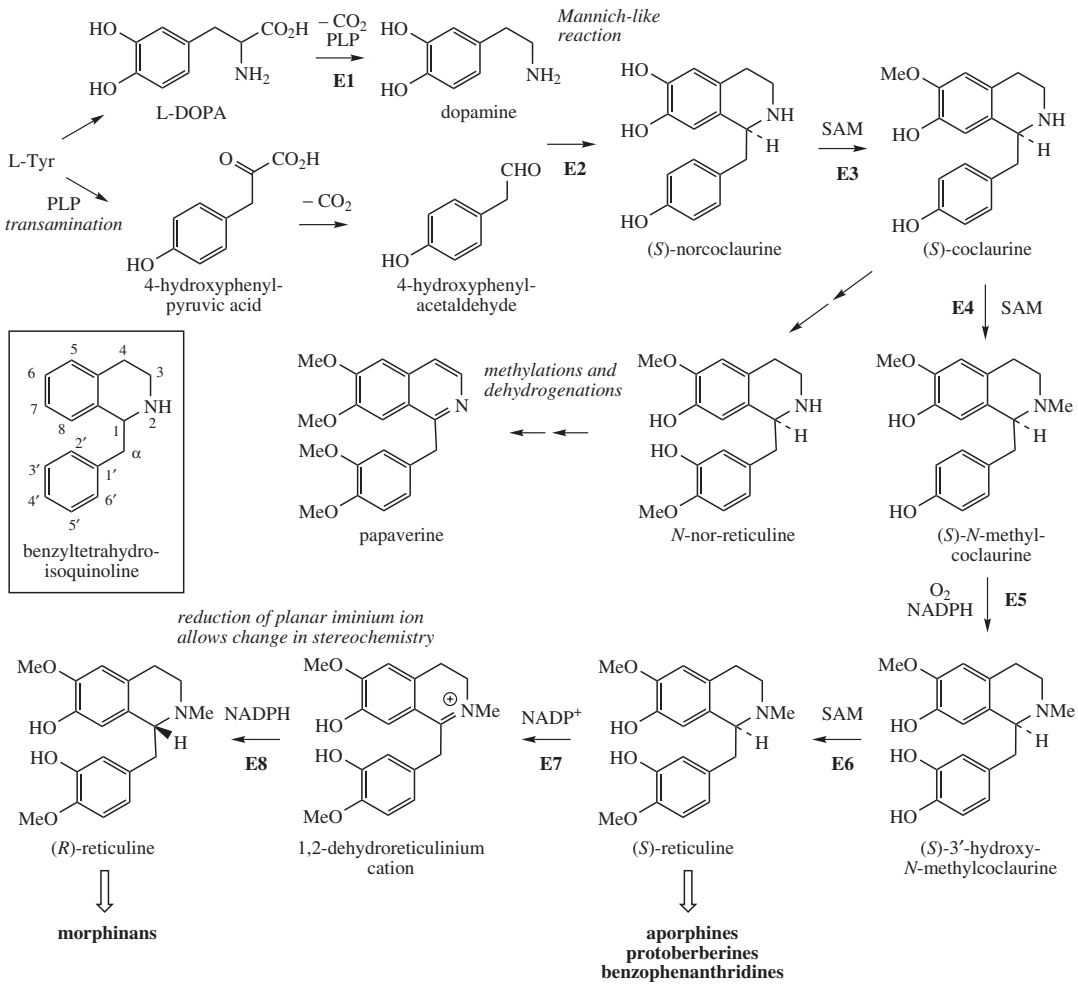


Figure 6.45

or formaldehyde, could be used (Figure 6.43, route **a**), giving the same product directly without the need for a decarboxylation step to convert the intermediate tetrahydroisoquinolinecarboxylic acid (Figure 6.43, route **b**). In nature, both routes are in fact found to operate, depending on the complexity of the R group. Thus, the keto acid (route **b**) is used for relatively simple substrates (R = H, Me), whilst more complex precursors (R = $ArCH_2$, $ArCH_2CH_2$, etc.) are incorporated via the corresponding aldehydes (route **a**). The stereochemistry in the product is thus controlled by the condensation/Mannich reactions (route **a**) or by the final reduction reaction (route **b**). Occasionally, both types of transformation have been demonstrated in the production of a single compound, an example being the *Lophophora schotti* alkaloid **lophocerine** (Figure 6.44). This requires utilization of a C_5 isoprene unit, incorporated via an aldehyde. However, a second route using the keto acid derived from the amino acid L-leucine by transamination has also been demonstrated. The alkaloid **salsolinol** (Figure 6.45) is found in plants, e.g. *Corydalis* spp. (Papaveraceae), but can also be detected in the urine of humans, where it is



E1: aromatic amino acid decarboxylase (DOPA decarboxylase)
E2: norcoclaurine synthase
E3: norcoclaurine 6-*O*-methyltransferase
E4: (*RS*)-coclaurine *N*-methyltransferase
E5: (*S*)-*N*-methylcoclaurine 3′-hydroxylase
E6: (*RS*)-3′-hydroxy-*N*-methylcoclaurine 4′-*O*-methyltransferase
E7: 1,2-dehydroreticuline synthase
E8: 1,2-dehydroreticuline reductase

Figure 6.46

a product from dopamine and acetaldehyde formed via a Pictet–Spengler reaction. Acetaldehyde is typically a metabolite produced after ingestion of ethanol.

Incorporation of a phenylethyl unit into the phenylethylamine gives rise to a benzyltetrahydroisoquinoline skeleton (Figure 6.46), which can undergo further modifications to produce a wide range of plant alkaloids, many of which feature as important drug materials. Fundamental changes to the basic skeleton increase the diversity of structural types, as described under 'modified benzyltetrahydroisoquinolines'. Though found mainly in five plant families (the Papaveraceae, Fumariaceae, Ranunculaceae, Berberidaceae, and Menispermaceae), over 2500 alkaloids can be assigned to this group. In recent years, a considerable amount of data covering enzymes and the genes encoding them has been accumulated for these alkaloids.

Most examples of benzyltetrahydroisoquinoline alkaloids and modified structures contain *ortho* dioxygenation in each aromatic ring, a pattern that is potentially derivable from the utilization of two DOPA molecules. Although two tyrosine molecules are used in the biosynthetic pathway, only the phenylethylamine fragment of the tetrahydroisoquinoline ring system is formed via DOPA, the remaining carbon atoms coming from tyrosine via 4-hydroxyphenylacetaldehyde (Figure 6.46). The product from the Mannich-like reaction is thus the trihydroxy alkaloid **norcoclaurine**, formed stereospecifically as the (*S*)-enantiomer. The tetrahydroxy substitution pattern is built up by further hydroxylation in the benzyl ring, though *O*-methylation (giving (*S*)-**coclaurine**) and *N*-methylation steps precede this. Eventually, (*S*)-**reticuline**, a pivotal intermediate to other alkaloids, is attained by *N*-methylation. Surprisingly, some alkaloids, such as the opium alkaloids morphine, codeine, and thebaine (see page 348), are elaborated from (*R*)-reticuline rather than the first-formed (*S*)-isomer. The change in configuration is known to be achieved by an oxidation–reduction process through the intermediate 1,2-dehydroreticulinium cation, as shown in Figure 6.46. **Papaverine**, a benzylisoquinoline alkaloid found in opium (see page 350), is formed from *N*-nor-reticuline by successive *O*-methylations and oxidation in the heterocyclic ring (Figure 6.46).

The potential of genetic engineering has been demonstrated by producing substantial quantities of reticuline in a transgenic system, incorporating plant genes from *Coptis japonica* (Ranunculaceae) into *E. coli* (Figure 6.47). By also incorporating a bacterial gene encoding monoamine oxidase, dopamine supplied to the culture was also converted into 3,4-dihydroxyphenylacetaldehyde. The broad substrate specificity of the plant norcoclaurine synthase and methyltransferase enzymes was then exploited by creating an alternative pathway to reticuline via **norlaudanosoline**, thus avoiding the late hydroxylation step. The *Escherichia coli* host system naturally provided the methylating agent SAM.

Structures in which two (or more) benzyltetrahydroisoquinoline units are linked together are readily explained by a phenolic oxidative coupling mechanism (see page 28). Thus, **tetrandrine** (Figure 6.48), a bis-benzyltetrahydroisoquinoline alkaloid isolated from *Stephania tetrandra* (Menispermaceae), is easily recognized as a coupling product from two molecules of (*S*)-*N*-methylcoclaurine (Figure 6.48). The two diradicals, formed by one-electron oxidations of a free phenol group in each ring, couple to give ether bridges, and the product is then methylated to tetrandrine. The pathway is much more likely to follow a stepwise

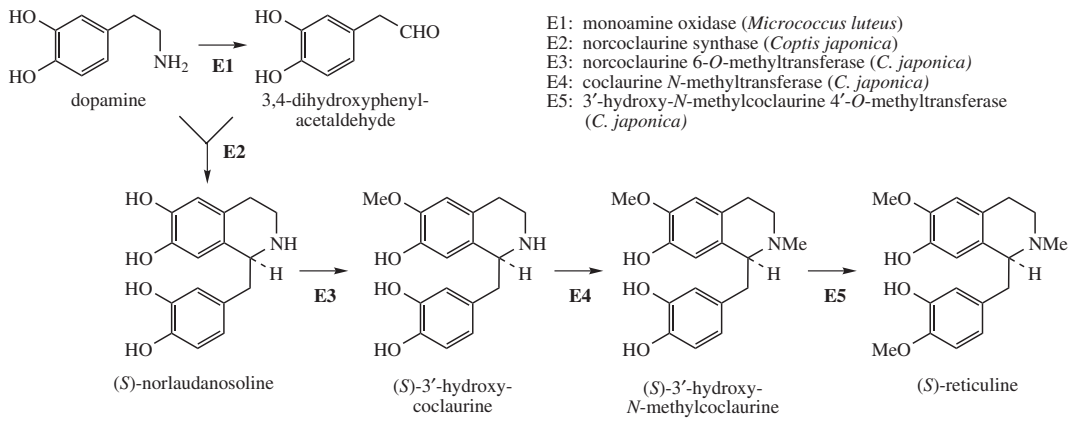


Figure 6.47

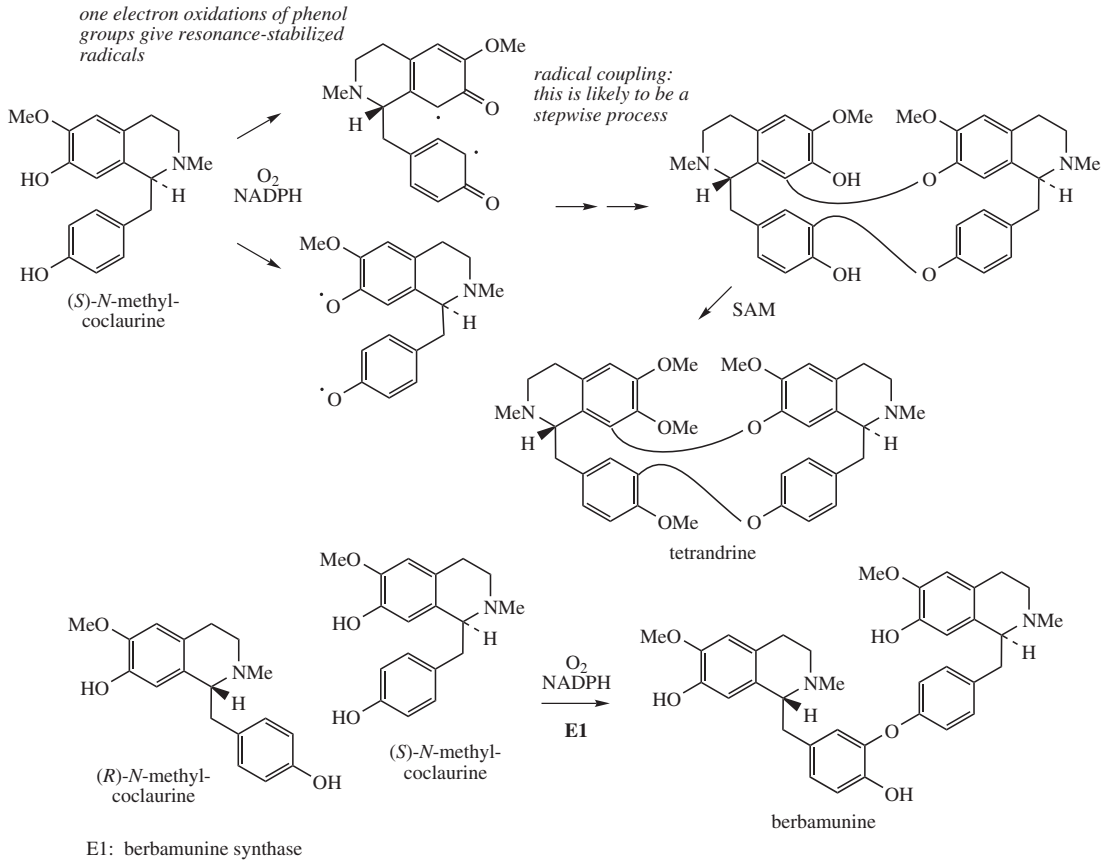


Figure 6.48

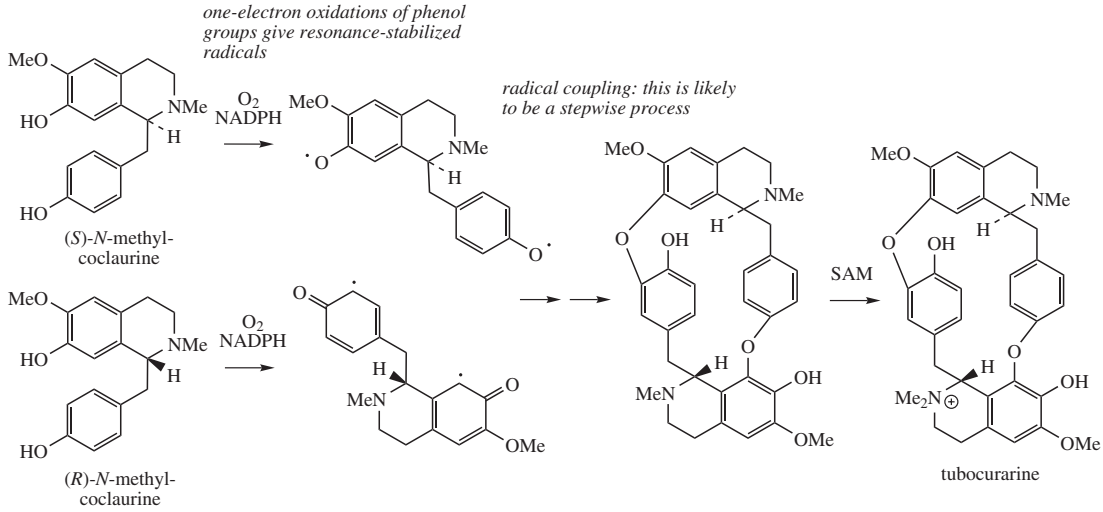


Figure 6.49

coupling process requiring two oxidative enzymes rather than the combined one suggested in Figure 6.48. Indeed, a cytochrome P-450-dependent enzyme from *Berberis stolonifera* couples one molecule each of (*S*)- and (*R*)-*N*-methylcoclaurine in a regiospecific and stereospecific manner to produce **berbamunine** (Figure 6.48) containing a single ether linkage between the two units. Tetrandrine is currently of interest for its ability to block calcium channels, and may have applications in the treatment of cardiovascular disorders. By a similar mechanism, **tubocurarine** (Figure 6.49) can be elaborated by a different coupling of (*S*)- and (*R*)-*N*-methylcoclaurine (Figure 6.49). Tubocurarine from *Chondrodendron tomentosum* (Menispermaceae) is the principal active component in the arrow poison curare [Box 6.6].

Box 6.6

Curare

Curare is the arrow poison of the South American Indians, and it may contain as many as 30 different plant ingredients, which may vary widely from tribe to tribe according to local custom. Curare is prepared in the rain forests of the Amazon and Orinoco and represents the crude dried extract from the bark and stems of various plants. The young bark is scraped off, pounded, and the fibrous mass percolated with water in a leaf funnel. The liquor so obtained is then concentrated by evaporation over a fire. Further vegetable material may be added to make the preparation more glutinous so that it will stick to the arrows or darts. The product is dark brown or black, and tar-like.

In the 1880s, it was found that the traditional container used for curare was fairly indicative of the main ingredients that had gone into its preparation. Three main types were distinguished. Tube curare was packed in hollow bamboo canes, and its principal ingredient was the climbing plant *Chondrodendron tomentosum* (Menispermaceae). Calabash curare was packed in gourds, and was derived from *Strychnos toxifera* (Loganiaceae). Pot curare was almost always derived from a mixture of loganiaceous and menispermaceous plants, and was packed in small earthenware pots. Current supplies of curare are mainly of the menispermaceous type, i.e. derived from *Chondrodendron*.

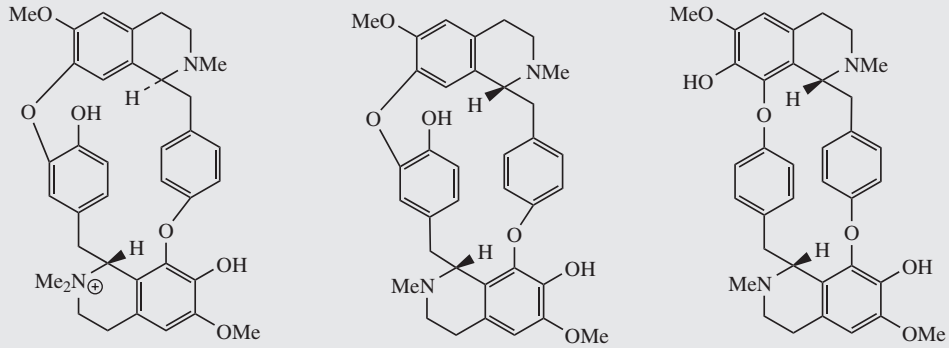
The potency of curare as an arrow poison is variable and consequently needs testing. A frequently quoted description of this testing is as follows: 'If a monkey hit by a dart is only able to get from one tree to the next before it falls dead, this is 'one-tree curare', the superior grade. 'Two-tree curare' is less satisfactory, and 'three-tree curare' is so weak that it can be used to bring down live animals that the Indians wish to keep in captivity.' Thus, the poison does not necessarily cause death; it depends on the potency. Curare is only effective if it enters the bloodstream, and small amounts taken orally give no ill effects provided there are no open sores in the mouth or throat. Animals killed by the poison could still be safely eaten.

Curare kills by producing paralysis, a limp relaxation of voluntary muscles. It achieves this by competing with acetylcholine at nicotinic receptor sites (see page 334), thus blocking nerve impulses at the neuromuscular junction. Death occurs because the muscles of respiration cease to operate, and artificial respiration is an effective treatment prior to the effects gradually wearing off through normal metabolism of the drug. Anti-acetylcholinesterase drugs, such as physostigmine and neostigmine (see page 386), are specific antidotes for moderate curare poisoning. Curare thus found medicinal use as a muscle relaxant, especially in surgical operations such as abdominal surgery, tonsillectomy, etc., where tense muscles needed to be relaxed. Curare was also found to be of value in certain neurological conditions, e.g. multiple sclerosis, tetanus, and Parkinson's disease, to temporarily relax rigid muscles and control convulsions, but was not a curative. However, the potency of curare varied markedly, and supplies were sometimes limited.

The alkaloid content of curare is from 4 to 7%. The most important constituent in menispermaceous curare is the bis-benzyltetrahydroisoquinoline alkaloid (+)-tubocurarine (Figure 6.50). This is a monoquaternary ammonium salt, and is water-soluble. Other main alkaloids include non-quaternary dimeric structures, e.g. curine (bebeerine) and isochondrodendrine (Figure 6.50), which appear to be derived from two molecules of (*R*)-*N*-methylcoclaurine, with the latter also displaying a different coupling mode. The constituents in loganiaceous curare (from calabash curare, i.e. *Strychnos toxifera*) are even more complex, and a series of 12 quaternary dimeric strychnine-like alkaloids has been identified, e.g. toxiferine-1 (see page 378).

Until recently, **tubocurarine** (Figure 6.50) was still extracted from menispermaceous curare and injected as a muscle relaxant in surgical operations, reducing the need for deep anaesthesia. Artificial respiration is required until the drug has been inactivated (about 30 min) or antagonized (e.g. with neostigmine). However, the limited availability of tubocurarine has led to the development of a series of synthetic analogues as neuromuscular blocking drugs, some of which have improved characteristics and have effectively superseded the natural product. Interestingly, the structure of tubocurarine was originally formulated incorrectly as a diquaternary salt, rather than a monoquaternary salt, and analogues were based on the pretext that curare-like effects might be obtained from compounds containing two quaternary nitrogens separated by a polymethylene chain. This was borne out in

Box 6.6 (continued)



(+)-tubocurarine

(–)-curine
[(–)-(*R,R*)-bebeerine]

(+)-(*R,R*)-isochondrodendrine

Figure 6.50

practice, and the separation of the quaternary centres was found to be optimal at about 10 carbon atoms. **Decamethonium** (Figure 6.51) was the first synthetic curare-like muscle relaxant, but it has been superseded too. In tubocurarine, the two nitrogen atoms are also separated by 10 atoms, and at physiological pH values it is likely that both centres will be positively charged. Obviously, the interatomic distance (1.4 nm in tubocurarine) is very dependent on the structure and stereochemistry rather than just the number of atoms separating the centres, but an extended conformation of decamethonium approximates to this distance. **Suxamethonium** (Figure 6.51) is an effective agent with a very short duration of action, due to the two ester functions which are rapidly metabolized in the body by an esterase (pseudocholinesterase: a non-specific serum cholinesterase), and this means the period during which artificial respiration is required is considerably reduced. It also has a 10-atom separation between the quaternary nitrogen atoms.

Atracurium (Figure 6.51) is a more recent development, containing two quaternary nitrogen atoms in benzyltetrahydroisoquinoline structures separated by 13 atoms. In addition to enzymic ester hydrolysis, atrocurium is also degraded in the body by non-enzymic Hofmann elimination (Figure 6.51), which is independent of liver or kidney function. Normally, this elimination would require strongly alkaline conditions and a high temperature, but the presence of the carbonyl group increases the acidity of the proton and thus facilitates its loss. The elimination proceeds readily under physiological conditions. This is particularly valuable in patients with low or atypical pseudocholinesterase enzymes. Atracurium contains four chiral centres (including the quaternary nitrogen atoms) and is supplied as a mixture of stereoisomers; the single isomer **cisatracurium** has now been introduced. This isomer is more potent than the mixture, has a slightly longer duration of action, and produces less cardiovascular side-effects. **Mivacurium** (Figure 6.51) has similar benzyltetrahydroisoquinoline structures to provide the quaternary centres, but their separation has now been increased to 16 atoms. In **pancuronium**, separation of the two quaternary centres is achieved by a steroidal skeleton. This agent is about five times as potent as tubocurarine. **Vecuronium** is the equivalent monoquaternary structure and has the fewest side-effects. **Rocuronium** is also based on an aminosteroid skeleton and provides rapid action with no cardiovascular effects. Neuromuscular blocking drugs are classified according to their duration of action as ultra-short (8 min, e.g. suxamethonium), short acting (15–30 min, e.g. mivacurium), intermediate (30–40 min, e.g. atracurium), and long acting (60–120 min, e.g. pancuronium).

The *Strychnos*-derived toxiferines (see Figure 6.89, page 378) also share the diquaternary character. **Alcuronium** is a semi-synthetic skeletal muscle relaxant containing a dimeric strychnine-like structure and is produced chemically from toxiferine-1 (see page 378).

These neuromuscular blocking agents are competitive antagonists at nicotinic acetylcholine (Figure 6.51) receptor sites. All the structures have two acetylcholine-like portions which can interact with the receptor. Where these are built into a rigid framework, e.g. tubocurarine and pancuronium, the molecule probably spans and effectively blocks several receptor sites without activating them. Tubocurarine and the heterocyclic analogues are termed non-depolarizing or competitive muscle relaxants; their action may be reversed with anticholinesterase agents such as neostigmine that increase acetylcholine concentration at the neuromuscular junction by inhibiting its breakdown. The straight-chain structures, e.g. decamethonium and suxamethonium, initially mimic the action of acetylcholine but then persist at the receptor site, and are termed depolarizing blocking agents. Thus, they trigger a

Box 6.6 (continued)

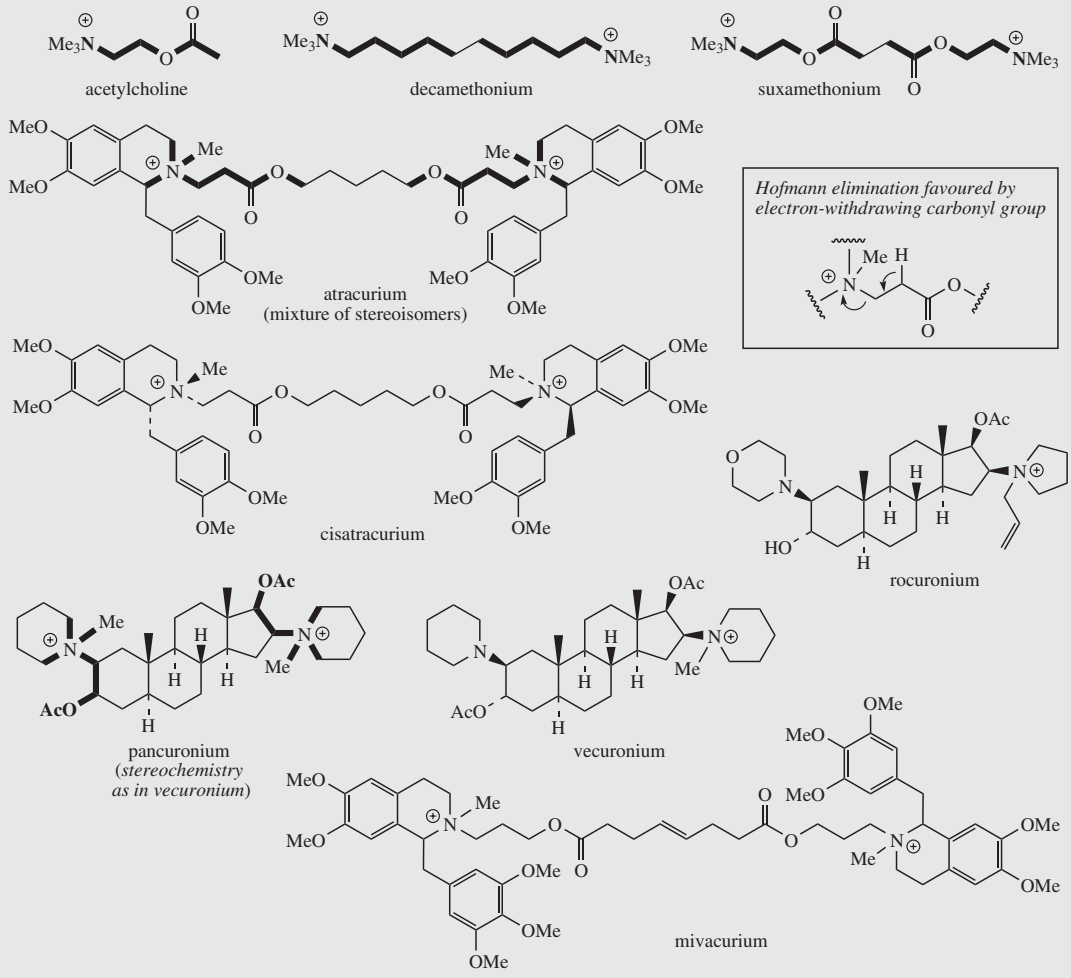


Figure 6.51

response, a brief contraction of the muscle, which is then followed by a prolonged period of muscular paralysis until the compound is metabolized. Their action cannot be reversed by anticholinesterase drugs. Because of their mode of action, depolarizing muscle relaxants may produce more side-effects than non-depolarizing agents, but no alternative ultra-short-acting agents are currently available.

Modified Benzyltetrahydroisoquinoline Alkaloids

The concept of phenolic oxidative coupling is a crucial theme in modifying the basic benzyltetrahydroisoquinoline skeleton to many other types of alkaloids. Tetrandrine (Figure 6.48) and tubocurarine (Figure 6.49) represent coupling of two benzyltetrahydroisoquinoline molecules by ether bridges, but this form of coupling is perhaps less frequent than that involving intramolecular carbon–carbon bonding between aromatic rings. **Morphine, codeine** and

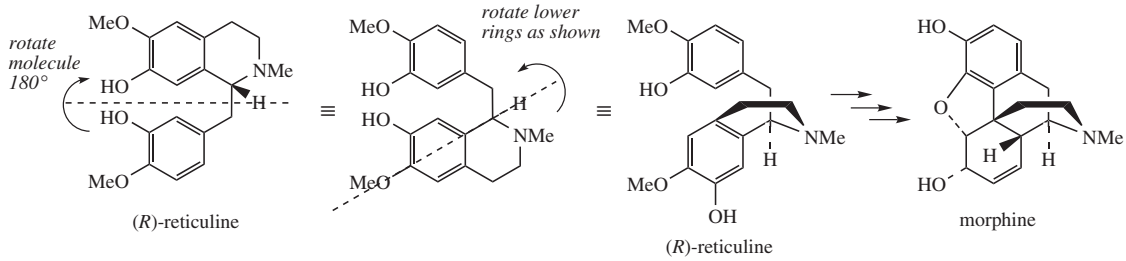


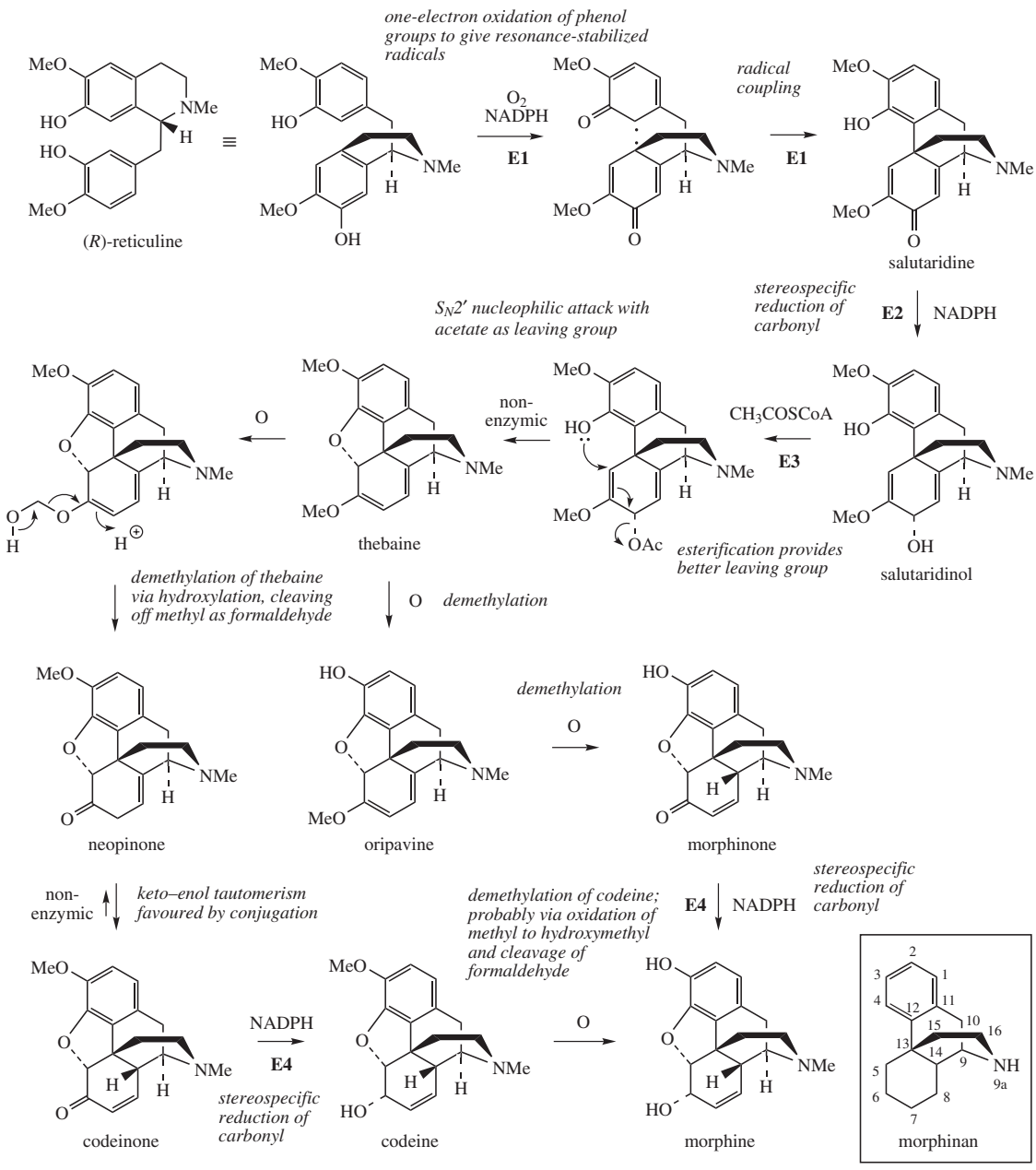
Figure 6.52

thebaine (see Figure 6.53), the principal opium alkaloids [Box 6.7], are derived by this type of coupling, though the subsequent reduction of one aromatic ring to some extent disguises their benzyltetrahydroisoquinoline origins. (*R*)-**Reticuline** (Figure 6.47) is firmly established as the precursor of these morphinan alkaloids; the structural relationship between these groups can be appreciated by careful manipulation of the precursor molecule (Figure 6.52).

(*R*)-Reticuline, redrawn as in Figure 6.53, is the substrate for one-electron oxidations of the phenol group in each ring, giving the diradical. Coupling *ortho* to the phenol group in the tetrahydroisoquinoline and *para* to the phenol in the benzyl substituent then yields the dienone **salutaridine**, found as a minor alkaloid constituent in the opium poppy *Papaver somniferum* (Papaveraceae). The coupling enzyme salutaridine synthase is a cytochrome P-450-dependent monooxygenase. Only the original benzyl aromatic ring can be restored to aromaticity, since the tetrahydroisoquinoline fragment is coupled *para* to the phenol function, a position which is already substituted. The alkaloid **thebaine** is obtained by way of **salutaridinol**, formed from salutaridine by stereospecific reduction of the carbonyl group. Ring closure to form the ether linkage in thebaine would be the result of nucleophilic attack of the phenol group onto the dienol system and subsequent displacement of the hydroxyl. This cyclization step can be demonstrated chemically by treatment of salutaridinol with acid. *In vivo*, however, an additional reaction is used to improve the nature of the leaving group, and this is achieved by acetylation with acetyl-CoA. The cyclization then occurs readily, and without any enzyme participation.

Subsequent reactions involve conversion of thebaine into **morphine** by way of **codeine**, a process which modifies the oxidation state of the diene ring, but most significantly removes two *O*-methyl groups. One is present as an enol ether, removal generating **neopinone**, which gives **codeinone** and then codeine by non-enzymic keto–enol tautomerism and NADPH-dependent reduction respectively. The final step, demethylation of the phenol ether codeine to the phenol morphine, is the type of reaction only achievable in the laboratory by the use of powerful and reactive demethylating agents, e.g. HBr or BBr_3. Because of the other functional groups present, chemical conversion of codeine into morphine is not usually a satisfactory process. However, the enzyme-mediated conversion in *Papaver somniferum* proceeds smoothly and efficiently. The enzymic demethylations of both the enol ether (in thebaine) and the phenol ether (in codeine) most probably involve initial cytochrome P-450-dependent hydroxylation followed by loss of the methyl groups as formaldehyde (Figure 6.53).

The involvement of these *O*-demethylation reactions is rather unusual; secondary metabolic pathways tend to increase the complexity of the product by adding methyl groups rather than removing them. In this pathway, it is convenient to view the methyl groups in reticuline as protecting groups, which reduce the possible coupling modes available during the oxidative coupling process, and these groups are then removed towards the end of the synthetic sequence. There is also some evidence that the later stages of the pathway in Figure 6.53 are modified in some strains of opium poppy. In such strains, thebaine is converted by way of **oripavine** and **morphinone**, this pathway removing the phenolic *O*-methyl before that of the enol ether, i.e. carrying out the same steps but in a different order. The enzymic transformation of thebaine into morphine and the conversion of (*R*)-reticuline into salutaridinol have also been observed in mammalian tissues, giving strong evidence that the trace amounts of morphine and related alkaloids which can sometimes be found in mammals are actually of endogenous origin rather than dietary.



E1: salutaridine synthase
E2: salutaridine:NADPH 7-oxidoreductase
E3: salutaridinol 7-*O*-acetyltransferase
E4: codeinone reductase

Figure 6.53

Box 6.7

Opium

Opium is the air-dried milky exudate, or latex, obtained by incising the unripe capsules of the opium poppy *Papaver somniferum* (Papaveraceae). The plant is an annual herb with large solitary flowers, of white, pink, or dull red–purple colour. For opium production, the ripening capsules, just changing colour from blue–green to yellow, are carefully incised with a knife to open the latex tubes, but not to cut through to the interior of the capsule. These latex tubes open into one another, so it is not necessary to incise them all. Cuts are made transversely or longitudinally according to custom. The initially white milky latex quickly oozes out, but rapidly turns brown and coagulates. This material, the raw opium, is then removed early the following morning, being collected by scraping from the capsule. Further incising and collection may be carried out over a period of about a week. The raw opium is moulded into balls or blocks, and typically these are wrapped in poppy leaves and shade-dried. The blocks may be dusted with various plant materials to prevent cohering. Fresh opium is pale to dark brown and plastic, but it becomes hard and brittle when stored.

Opium has been known and used for 4000 years or more. In recent times, attempts have been made at governmental and international levels to control the cultivation of the opium poppy, but with only limited success. In endeavours to reduce drug problems involving opium-derived materials, especially heroin, where extremely large profits can be made from smuggling relatively small amounts of opium, much pharmaceutical production has been replaced by the processing of the bulkier 'poppy straw'. The entire plant tops are harvested and dried, then extracted for their alkaloid content in the pharmaceutical industry. Poppy straw now accounts for most of the medicinal opium alkaloid production, but there is still considerable trade in illicit opium. In addition to opium, the opium poppy yields seeds which are used in baking and are also pressed to give poppy seed oil. The remaining seed cake is used as cattle feed, and it is held that these poppy seed products cover all the growing expenses, with opium providing the profit. Poppy seeds do not contain any significant amounts of alkaloids.

The main producer of medicinal opium for the world market is India, with China producing supplies for its own domestic use. Poppy straw is cultivated in Australia, France, Hungary, Spain, and Turkey, and more recently in the United Kingdom. Almost all (more than 90%) of the opium destined for the black market now originates from Afghanistan; other sources include Southeast Asia, (mainly in Myanmar (Burma) and Laos) and Latin America (principally Mexico and Colombia).

Crude opium has been used since antiquity as an analgesic, sleep-inducer (narcotic), and for the treatment of coughs. It has been formulated in a number of simple preparations for general use, though these are now uncommon. Laudanum, or opium tincture, was once a standard analgesic and narcotic mixture. Paregoric, or camphorated opium tincture, was used in the treatment of severe diarrhoea and dysentery, but is still an ingredient in the cough and cold preparation Gee's linctus. In Dover's powder, powdered opium was combined with powdered ipecacuanha (see page 363) to give a popular sedative and diaphoretic (promotes perspiration) to take at the onset of colds and influenza. Opium has traditionally been smoked for pleasure, but habitual users developed a craving for the drug followed by addiction. An unpleasant abstinence syndrome was experienced if the drug was withdrawn.

In modern medicine, only the purified opium alkaloids and their derivatives are commonly employed. Indeed, the analgesic preparation '**papaveretum**' (see below) which once contained the hydrochlorides of total opium alkaloids is now formulated from selected purified alkaloids, in the proportions likely to be found in opium. Although the ripe poppy capsule can contain up to 0.5% total alkaloids, opium represents a much concentrated form, and up to 25% of its mass is composed of alkaloids. Of the many (>40) alkaloids identified, some six represent almost all of the total alkaloid content. Actual amounts vary widely, as shown by the following figures: morphine (4–21%; Figure 6.53); codeine (0.8–2.5%; Figure 6.53); thebaine (0.5–2.0%; Figure 6.53); papaverine (0.5–2.5%; Figure 6.47); noscapine (narcotine; 4–8%; Figure 6.54); narceine (0.1–2%; Figure 6.54, and see also Figure 6.65, page 359). A typical commercial sample of opium would probably have a morphine content of about 12%. **Powdered opium** is standardized to contain 10% of anhydrous morphine, usually by dilution with an approved diluent, e.g. lactose or cocoa husk powder. The alkaloids are largely combined in salt form with meconic acid (poppy acid; Figure 6.54), opium containing some 3–5% of this material. Meconic acid is invariably found in opium, but apart from its presence in other *Papaver* species it has not been detected elsewhere. It gives a deep red-coloured complex with ferric chloride, and this has thus been used as a rapid and reasonably specific test for opium. In the past, the urine of suspected opium smokers could also be tested in this way. Of the main opium alkaloids, only morphine and narceine display acidic properties as well as the basic properties due to the tertiary amine. Narceine has a carboxylic acid function, whilst morphine is acidic due to its phenolic hydroxyl. This acidity can be exploited for the preferential extraction of these alkaloids (principally morphine) from an organic solvent by partitioning with aqueous base.

Morphine (Figure 6.53) is a powerful analgesic and narcotic, and remains one of the most valuable analgesics for relief of severe pain. It also induces a state of euphoria and mental detachment, together with nausea, vomiting, constipation, tolerance, and addiction. Regular users experience withdrawal symptoms, including agitation, severe abdominal cramps, diarrhoea, nausea, and vomiting, which may last for 10–14 days unless a further dose of morphine is taken. This leads to physical dependence

Box 6.7 (continued)

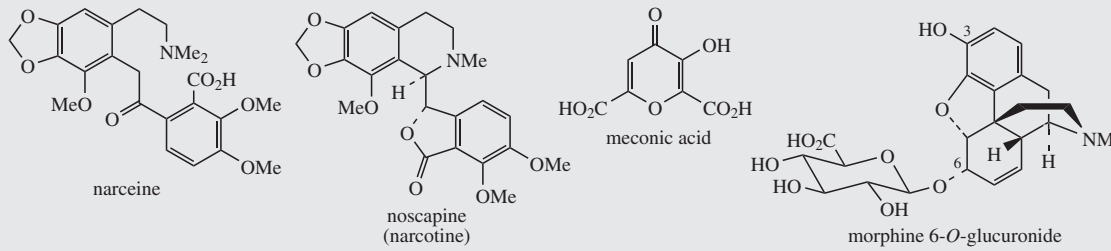


Figure 6.54

which is difficult to overcome, so that the major current use of morphine is thus in the relief of terminal pain. Although orally active, to obtain rapid relief of acute pain it is usually injected. The side-effect of constipation is utilized in some anti-diarrhoea preparations, e.g. kaolin and morphine. Morphine is metabolized in the body to glucuronides which are readily excreted. Whilst morphine 3-*O*-glucuronide is antagonistic to the analgesic effects of morphine, **morphine 6-*O*-glucuronide** (Figure 6.54) is actually a more effective and longer lasting analgesic than morphine, with fewer side-effects, such as nausea and vomiting. This agent is in clinical trials for the treatment of cancer-related pain. Since it is significantly hydrolysed in the gut, it is much less effective taken orally than when administered by injection.

Codeine (Figure 6.53) is the 3-*O*-methyl ether of morphine and is the most widely used of the opium alkaloids. Because of the relatively small amounts found in opium, almost all of the material prescribed is manufactured by semi-synthesis from morphine. Its action is dependent on partial demethylation in the liver to produce morphine, so it produces morphine-like analgesic effects, but little if any euphoria. As an analgesic, codeine has about one-tenth the potency of morphine. Codeine is almost always taken orally and is a component of many compound analgesic preparations. It is a relatively safe non-addictive medium analgesic, but is still too constipating for long-term use. Codeine also has valuable antitussive action, helping to relieve and prevent coughing. It effectively depresses the cough centre, raising the threshold for sensory cough impulses.

Thebaine (Figure 6.53) differs structurally from morphine/codeine mainly by its possession of a conjugated diene ring system. It is almost devoid of analgesic activity, but may be used as a morphine antagonist. Its main value is as substrate for the semi-synthesis of other drugs (see below).

Papaverine (Figure 6.47) is a benzylisoquinoline alkaloid, and is structurally very different from the morphine, codeine, thebaine group of alkaloids (morphinans). It has little or no analgesic or hypnotic properties, but it relaxes smooth muscle in blood vessels. It is sometimes used as an effective treatment for male impotence, being administered by direct injection to achieve erection of the penis. The advent of orally active agents such as sildenafil (Viagra®) has presumably diminished this application.

Noscapine (Figure 6.54) is a member of the phthalideisoquinoline alkaloids and provides a further structural variant in the opium alkaloids. Noscapine has good antitussive and cough suppressant activity comparable to that of codeine, but no analgesic or narcotic action. Its original name 'narcotine' was changed to reflect this lack of narcotic action. Despite many years of use as a cough suppressant, the finding that noscapine may have teratogenic properties (i.e. may deform a fetus) has resulted in noscapine preparations being deleted. In recent studies, antitumour activity has been noted from noscapine, which binds to tubulin as do podophyllotoxin and colchicine (see pages 155 and 361), thus arresting cells at mitosis. The chemotherapeutic potential of this orally effective agent merits further evaluation.

Papaveretum is a mixture of purified opium alkaloids, as their hydrochlorides, and is now formulated to contain only morphine (85.5%), codeine (7.8%) and papaverine (6.7%). It is used for pain relief during operations. It may be combined with the antisecretory tropane alkaloid hyoscine (see page 318).

A vast range of semi-synthetic or totally synthetic morphine-like derivatives have been produced. These are collectively referred to as 'opioids'. Many have similar narcotic and pain-relieving properties as morphine, but are less habit forming. Others possess the cough-relieving activity of codeine, but without the analgesic effect. More than 90% of the morphine extracted from opium (or poppy straw) is currently processed to give other derivatives (Figure 6.55). Most of the codeine is obtained by semi-synthesis from morphine, mono-*O*-methylation occurring at the acidic phenolic hydroxyl. Similarly, **pholcodine** (Figure 6.55), an effective and reliable antitussive, can be obtained by alkylation with *N*-(chloroethyl)morpholine. **Dihydrocodeine** (Figure 6.55) is a reduced form of codeine with similar analgesic properties, the double bond not being essential for activity. In **hydromorphone** (Figure 6.55), the double bond of morphine has been reduced, and in addition the 6-hydroxyl has been oxidized to a ketone. This increases the analgesic effects, but also the side-effects; the drug is used for severe pain associated with cancer. **Diamorphine** (or

Box 6.7 (continued)

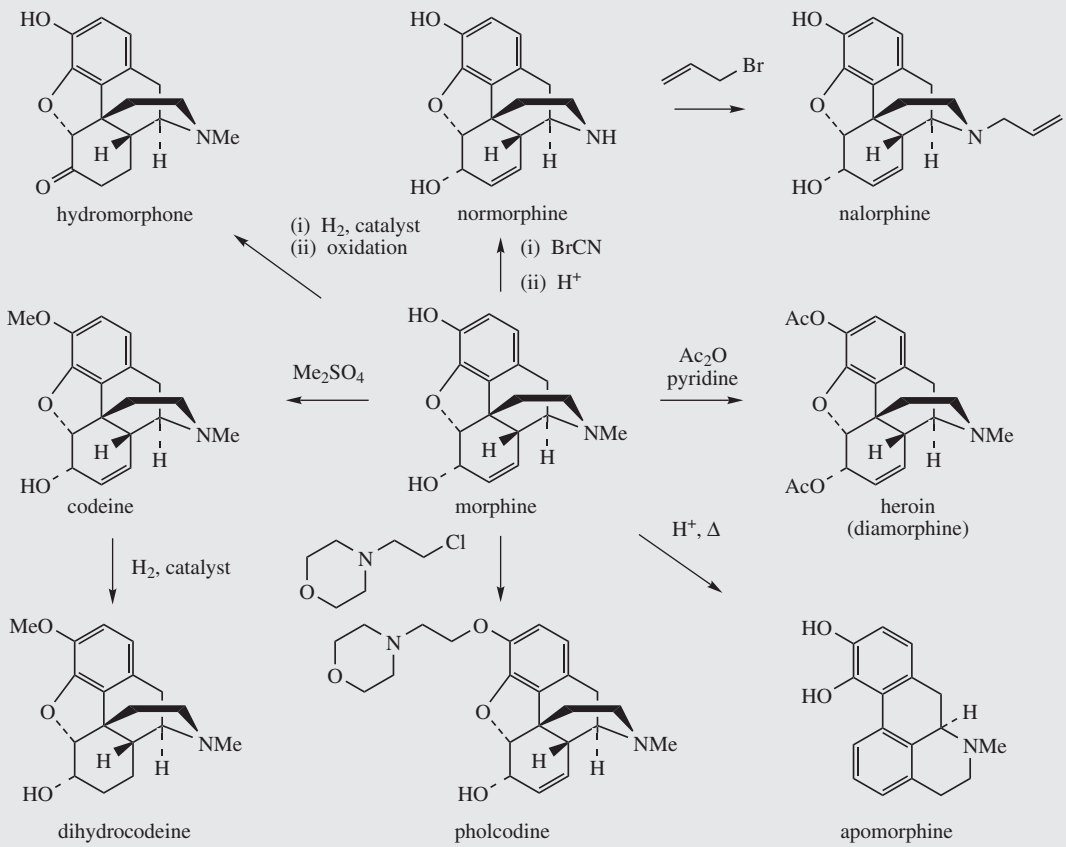


Figure 6.55

heroin; Figure 6.55), is merely the diacetate of morphine; it is a highly addictive analgesic and hypnotic. The increased lipophilic character of heroin over morphine results in improved solubility, with better transport and absorption, though the active agent is probably the 6-acetate, the 3-acetate group being hydrolysed by esterases in the brain. Heroin was synthesized originally as a cough suppressant; and though most effective in this role, it has unpleasant addictive properties, with users developing a psychological craving for the drug. It is widely used for terminal care, e.g. cancer sufferers, both as an analgesic and cough suppressant. The euphoria induced by injection of heroin has resulted in much abuse of the drug and creation of a worldwide major drug problem.

The *N*-methyl group of morphine can be removed by treatment with cyanogen bromide, then hydrolysis. A variety of *N*-alkyl derivatives, e.g. *N*-allyl-normorphine (**nalorphine**; Figure 6.55) may be produced by use of appropriate alkyl bromides. Nalorphine has some analgesic activity, but it was also found to counter the effects of morphine and is thus a mixed agonist–antagonist. It has been used as a narcotic antagonist, but is principally regarded as the forerunner of pure opiate antagonists such as naloxone (see below). Treatment of morphine with hot acid induces a rearrangement process, resulting in a highly modified structural skeleton, a representative of the aporphine group of alkaloids (see page 355). The product **apomorphine** (Figure 6.55) has no analgesic properties, but morphine's side-effects of nausea and vomiting are highly emphasized. Apomorphine is a powerful emetic and can be injected for emergency treatment of poisoning. This is now regarded as dangerous, but apomorphine is currently valuable to control the symptoms of Parkinson's disease, being a stimulator of D_1 and D_2 dopamine receptors. Apomorphine's structure contains a dihydroxyphenylethylamine (dopamine) fragment conferring potent dopamine agonist properties to this agent (see page 336).

Box 6.7 (continued)

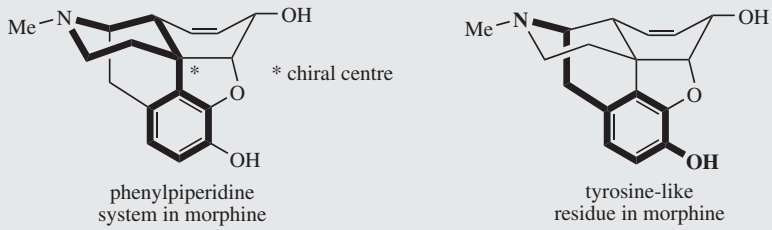


Figure 6.56

It has been found that a common structural feature required for centrally acting analgesic activity in the opioids is the combination of aromatic ring and a piperidine ring which maintain the stereochemistry at the chiral centre, as shown in Figure 6.56. The three-dimensional disposition of the nitrogen function to the aromatic ring allows morphine and other analgesics to bind to pain-reducing receptors in the brain. Three distinct classes of opioid receptors, μ, δ, and κ, have been distinguished; morphine acts primarily at μ-receptors. Morphine is not the natural ligand for opioid receptors; the natural agonists are peptides termed opioid peptides (see page 434). These include enkephalins, endorphins, dynorphins, and endomorphins. All contain a terminal tyrosine residue in their structures, and it this feature that is mimicked by the morphine structure, allowing binding to the appropriate receptor (Figure 6.56). The opioid peptides themselves are rapidly degraded in the body and are currently unsuitable for drug use.

Some totally synthetic opioid drugs modelled on morphine are shown in Figure 6.57. Removal of the ether bridge and the functionalities in the cyclohexene ring are exemplified in levomethorphan and **dextromethorphan**. Levomethorphan has analgesic properties, whilst both enantiomers possess the antitussive activity of codeine. In practice, the 'unnatural' isomer dextromethorphan is the preferred drug material, being completely non-addictive and possessing no analgesic activity. **Pentazocine** is an example of a morphine-like structure where the ether bridge has been omitted and the cyclohexene ring has been replaced by simple methyl groups. Pentazocine has both agonist and antagonist properties, and although it is a good analgesic, it can induce withdrawal symptoms. Even more drastic simplification of the morphine structure is found in **pethidine** (**meperidine**), one of the most widely used synthetic opiates. Only the aromatic ring and the piperidine systems are retained. Pethidine is less potent than morphine, but produces prompt, short-acting analgesia, and is also less constipating than morphine. It can be addictive. **Fentanyl** has a 4-anilino- rather than a 4-phenyl-piperidine structure, and is 50–100 times more active than morphine due to its high lipophilicity and excellent transport properties; it can be administered transdermally via a patch. **Alfentanil** and **remifentanil** are further variants on the fentanyl structure; all three drugs are rapid-acting and used during operative procedures. The piperidine ring system is no longer present in **methadone**, though this diphenylpropylamine derivative can be drawn in such a way as to mimic the piperidine ring conformation. Methadone is orally active, has similar activity to morphine, but is less euphorigenic and has a longer duration of action. Although it is as potentially addictive as morphine, the withdrawal symptoms are different and much less severe than with other drugs such as heroin, so that methadone is widely used for the treatment and rehabilitation of heroin addicts. However, it only replaces one addiction with another, albeit a less dangerous one. **Dipipanone** is a structural variant on methadone, and is used for moderate to severe pain; it is usually administered in combination with an anti-emetic. **Diphenoxylate** is used as an antidiarrhoeal; to minimize its habit-forming properties and potential abuse it is combined with a sub-therapeutic amount of the anticholinergic atropine (see page 318). **Dextropropoxyphene** contains an ester function but mimics the piperidine ring in a rather similar manner. This agent has only low analgesic activity, about half that of codeine, and finds application in combination formulations with aspirin or paracetamol. **Meptazinol** is structurally unlike the other opiate analgesics, in that it contains a seven-membered nitrogen heterocycle. It is an effective analgesic, and it produces relatively few side-effects with a low incidence of respiratory depression. **Tramadol** is a recent drug claimed to produce analgesia by two mechanisms, an opioid mechanism and also by enhancement of serotoninergic and adrenergic pathways. It produces few typical opioid side-effects.

Thebaine, for many years regarded as an unwanted by-product from opium, is now the raw material for semi-synthesis of several useful drugs. On treatment with hydrogen peroxide, the conjugated diene undergoes 1,4-addition, and hydrolysis results in formation of a 4-hydroxy cyclohexenone system (Figure 6.58). Reduction and demethylation lead respectively to **oxycodone** and **oxymorphone**, which are potent analgesics. The conjugated diene system in thebaine can also be exploited in a Diels–Alder reaction, building on yet another ring system (Figure 6.59). Some of these adducts have quite remarkable levels of analgesic activity, but are too powerful for human use. Some, e.g. **etorphine** (Figure 6.59), are used in veterinary practice to sedate large

Box 6.7 (continued)

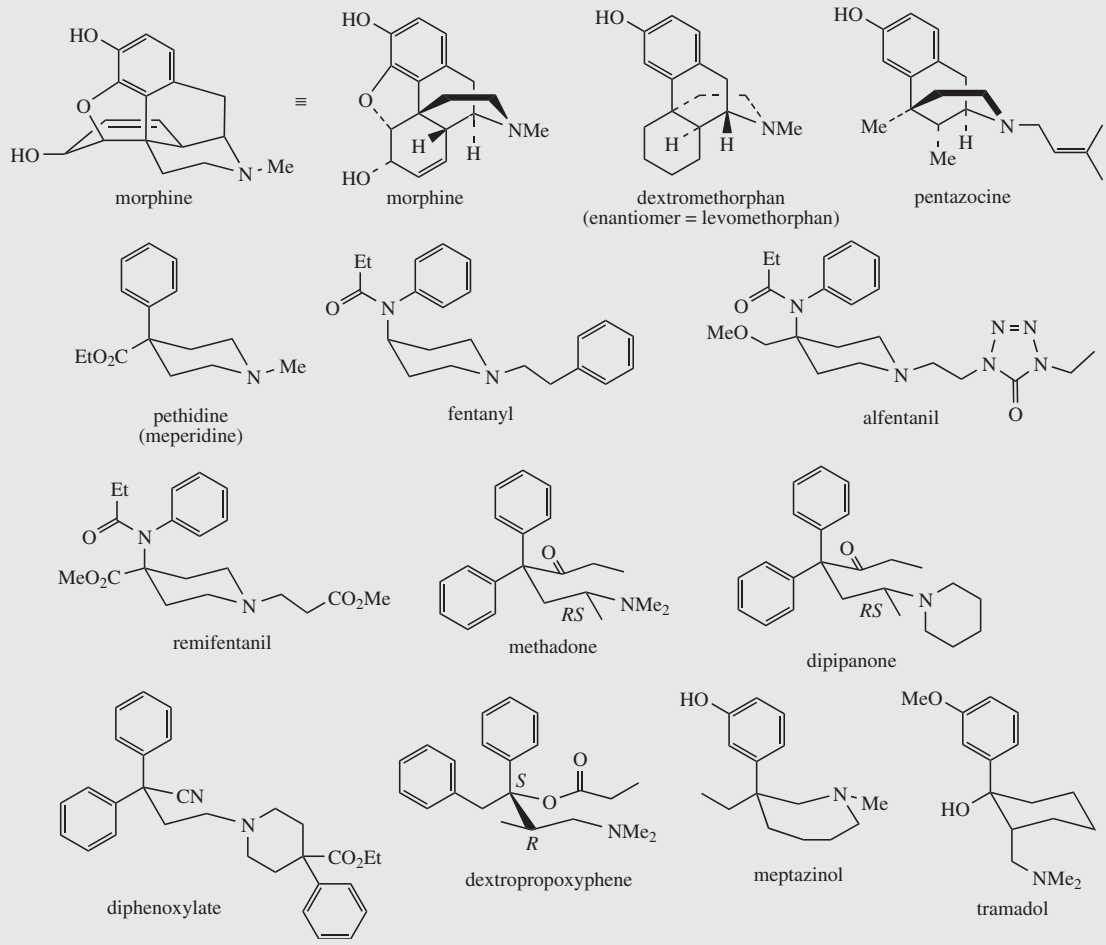


Figure 6.57

animals (elephants, rhinos) by means of tranquillizer darts. Etorphine is some 5000–10 000 times more potent than morphine. **Buprenorphine** (Figure 6.59) is an etorphine analogue with an *N*-cyclopropylmethyl substituent and *tert*-butyl instead of *n*-propyl in the side-chain. This material has both opioid agonist and antagonist properties. Mixed agonist–antagonist properties offer scope for producing analgesia whilst negating the effects of other opioids to which a patient may be addicted. Buprenorphine has a long duration of action and only low dependence potential, but it may precipitate withdrawal symptoms in patients dependent on other opioids. In addition to use as an analgesic, it is now being used as an alternative to methadone in the treatment of opioid dependence. **Nalbuphine** (Figure 6.58), produced semi-sythetically from thebaine, also displays mixed agonist–antagonist properties and has similar agonist activity as morphine, but it produces less side-effects and has less abuse potential. **Naloxone** (Figure 6.58) shows hardly any agonist activity, but it is a potent antagonist at all opioid receptors and is used to treat opiate poisoning, including that in children born to heroin addicts. **Naltrexone** (Figure 6.58) also has antagonist activity similar to naloxone. These agents are *N*-alkyl derivatives related to oxymorphone/oxycodone.

Thebaine may also be transformed very efficiently into codeine in about 75% yield (Figure 6.58). The two-stage synthesis involves acid-catalysed hydrolysis of the enol ether function to give codeinone (this being the more favoured tautomer of the

Box 6.7 (continued)

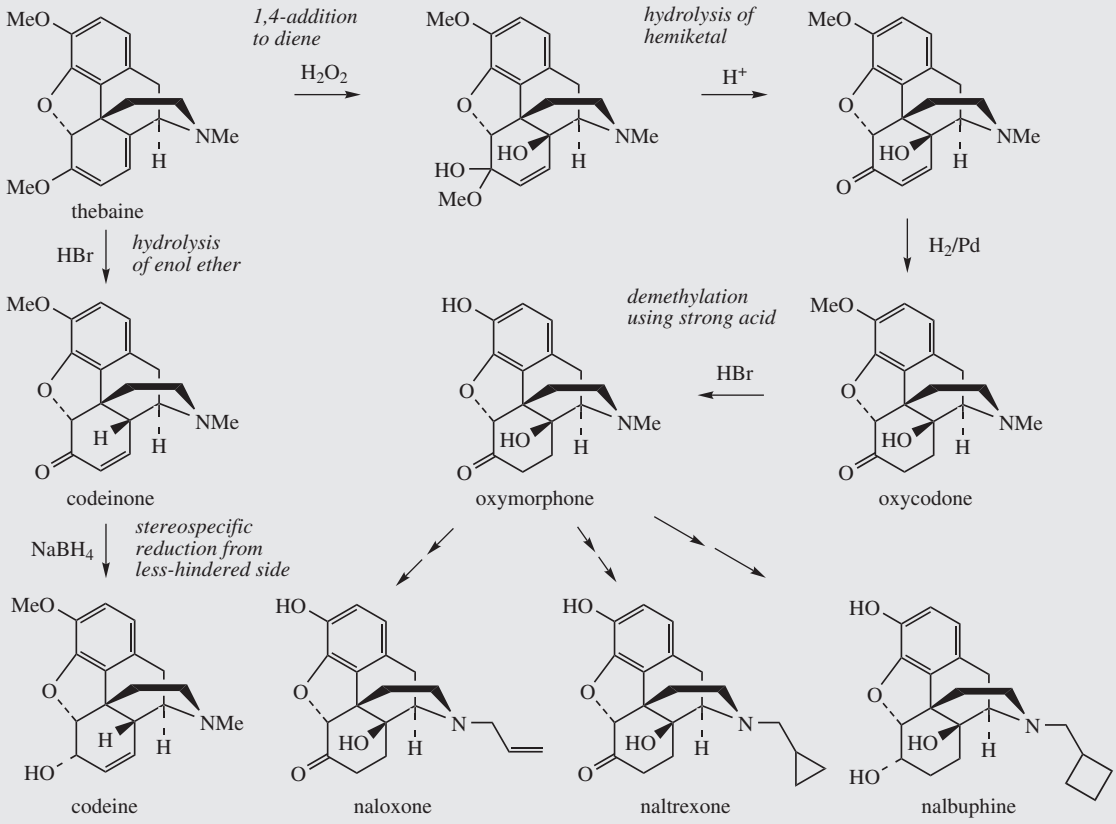


Figure 6.58

first-formed conjugated enol) followed by stereospecific borohydride reduction of the carbonyl. This opens up possibilities for producing codeine (the most widely used of the opium alkaloids) without using morphine. At present, almost all of the codeine used is synthesized by methylation of morphine. The advantage of using thebaine is that the raw material for the pharmaceutical industry could be shifted away from morphine and opium. This might then help in the battle to eliminate illicit morphine production and its subsequent conversion into heroin. Conversion of thebaine into morphine and heroin is much more difficult and low yielding. Thus, considerable effort has been put into selecting thebaine-rich varieties of *Papaver somniferum* and cultivating these for alkaloid production. A significant proportion of the poppy crop in Australia and France is now composed of thebaine-rich varieties. These strains produce thebaine and oripavine (Figurer 6.53) as main alkaloids, and appear to lack enzymes that carry out the late demethylation steps in Figure 6.53. Most species of *Papaver* seem to lack the enzyme that reduces salutaridine to salutaridinol (Figure 6.53) and, thus, they do not synthesize morphine-like alkaloids. Oripavine (3-demethylthebaine) may be used in the same way as thebaine in the synthesis of drugs. Metabolic engineering would seem to offer scope for modifying the alkaloid patterns in *Papaver somniferum*. In one study, the late step catalysed by codeinone reductase has been blocked by gene silencing through RNA interference: supplying a short strand of RNA to target that portion of mRNA responsible for a particular enzyme. Morphine and codeine production was markedly diminished, but other morphinan alkaloids, such as thebaine and oripavine, were also suppressed. Instead, the major alkaloids accumulating were reticuline and some of its methyl ethers. The metabolic block appeared to be several steps further back in the pathway than predicted.

Box 6.7 (continued)

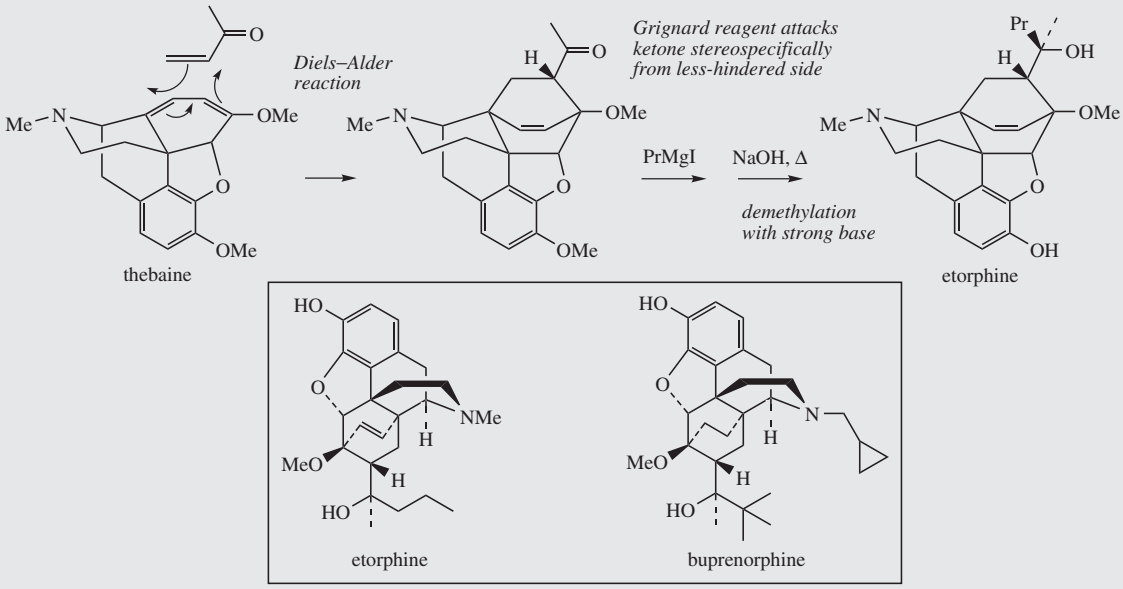


Figure 6.59

Remarkably, there is now considerable evidence that various animals, including humans and other mammals, are also able to synthesize morphine and related alkaloids in small amounts. These compounds have been detected in various tissues, including brain, liver, spleen, adrenal glands, and skin, and endogenous morphine may thus play a role in pain relief, combining its effects with those provided by the enkephalin peptides.

A minor constituent of *Papaver somniferum* is the aporphine alkaloid **isoboldine** (Figure 6.60). Other species of poppy, e.g. *Papaver orientale* and *Papaver pseudoorientale*, are known to synthesize aporphine alkaloids rather than morphinan structures as their principal constituents. Aporphines constitute one of the largest groups of isoquinoline alkaloids, with more than 500 representatives known. Apomorphine (Figure 6.55), the acid rearrangement product from morphine, is a member of this group, though is not a natural product. A cytochrome P-450-dependent enzyme catalysing the *ortho–ortho* oxidative coupling of **(*S*)-reticuline** to **(*S*)-corytuberine** (Figure 6.60) has been characterized from *Coptis japonica* (Ranunculaceae), a plant that also synthesizes berberine (see below). (*S*)-Isoboldine is readily appreciated to be the product of a similar oxidative coupling of (*S*)-reticuline, though coupling *ortho* to the phenol group in the tetrahydroisoquinoline portion and *para* to the phenol of the benzyl substituent (Figure 6.60). Some structures, e.g. **isothebaine** (Figure 6.60) from *Papaver orientale*, are not as easily rationalized. **(*S*)-Orientaline** is a precursor of isothebaine (Figure 6.61). This benzyltetrahydroisoquinoline, with a different methylation pattern to reticuline, is able to participate in oxidative coupling, but inspection of the structures indicates a phenol group appears to be lost in the transformation. The pathway (Figure 6.61) involves an unexpected rearrangement process, however. Thus, oxidative coupling *ortho–para* to the phenol groups gives a dienone **orientalinone** (compare the structure of salutaridine in Figure 6.53). After reduction of the carbonyl group, a rearrangement occurs, restoring aromaticity and expelling the hydroxyl (originally a phenol group) to produce **isothebaine**. This type of rearrangement, for which good chemical analogies are available, is a feature of many other alkaloid biosynthetic pathways and occurs because normal keto–enol

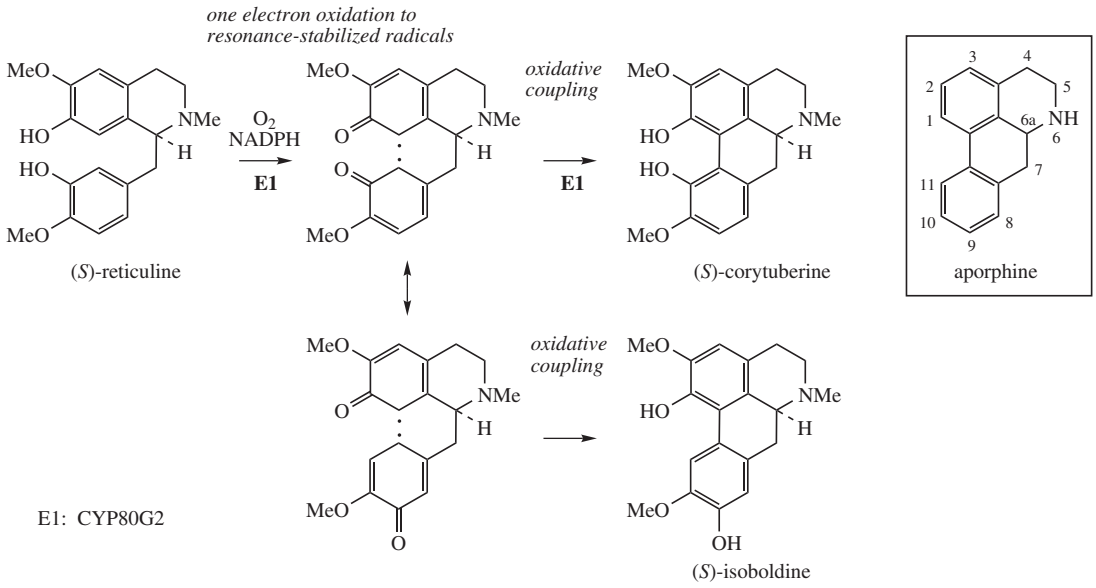


Figure 6.60

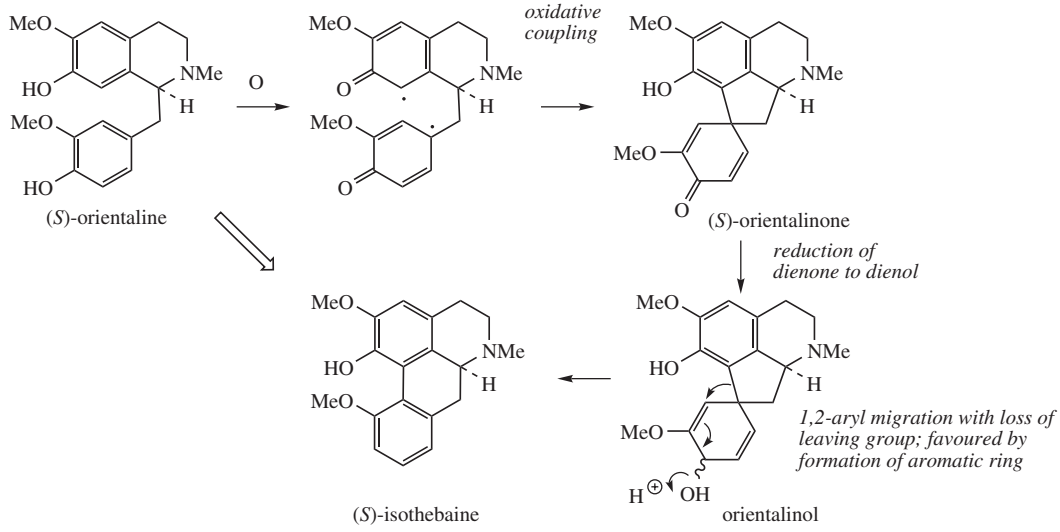


Figure 6.61

tautomerism is not possible for rearomatization when coupling positions are already substituted. The process is fully borne out by experimental evidence, including the subsequent isolation of orientalinone and orientalinol from *Papaver orientale*.

Stephanine (Figure 6.62) from *Stephania* species (Menispermaceae) is analogous to isothebaine and shares a similar pathway, though this time from (*R*)-**orientaline**. The different substitution pattern in stephanine compared with isothebaine is a consequence of the intermediate dienol suffering migration of the alkyl rather than aryl group (Figure 6.62). **Aristolochic acid** is a novel modified aporphine containing a nitro group and is produced from stephanine by oxidative reactions leading

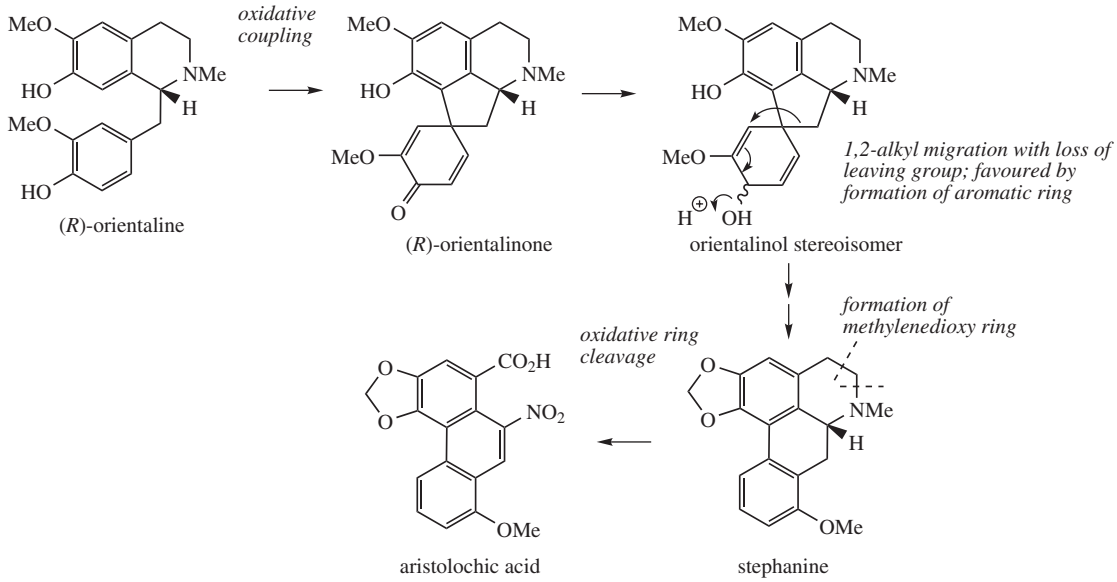


Figure 6.62

to ring cleavage (Figure 6.62). Aristolochic acid is present in many species of *Aristolochia* (Aristolochiaceae) used in traditional medicine, e.g. snake-root *Aristolochia serpentina*. However, because aristolochic acid is now known to be nephrotoxic and to cause acute kidney failure, the use of *Aristolochia* species in herbal medicines, especially Chinese remedies, has been banned in several countries.

The alkaloid **berberine** (Figure 6.63) is found in many members of the Berberidaceae (e.g. *Berberis, Mahonia*), the Ranunculaceae (e.g. *Hydrastis*), and other families. Berberine has antiamoebic, antibacterial, and anti-inflammatory properties, and plants containing berberine have long been used in traditional medicine. Its tetracyclic skeleton is derived from a benzyltetrahydroisoquinoline system with the incorporation of an extra carbon atom, supplied from SAM via an *N*-methyl group (Figure 6.63). This extra skeletal carbon is known as a 'berberine bridge'. Formation of the berberine bridge is readily rationalized as an oxidative process in which the *N*-methyl group is oxidized to an iminium ion, and a cyclization to the aromatic ring occurs by virtue of the phenolic group (Figure 6.64).

The oxidative cyclization process resembles formation of a methylenedioxy group (see page 27), whilst the mechanism of cyclization is exactly the same as that invoked in formation of a tetrahydroisoquinoline ring, i.e. a Mannich-like reaction (see page 19). The product from the enzymic transformation of (*S*)-**reticuline** is the protoberberine alkaloid (*S*)-**scoulerine**, the berberine bridge enzyme requiring molecular oxygen as oxidant and releasing H_2O_2 as by-product (Figure 6.64). Its role in

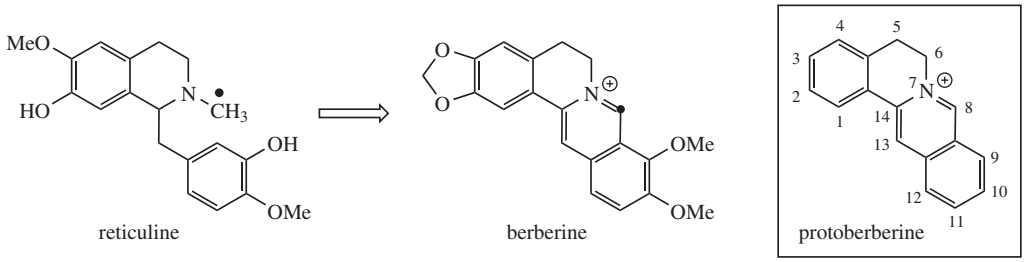


Figure 6.63

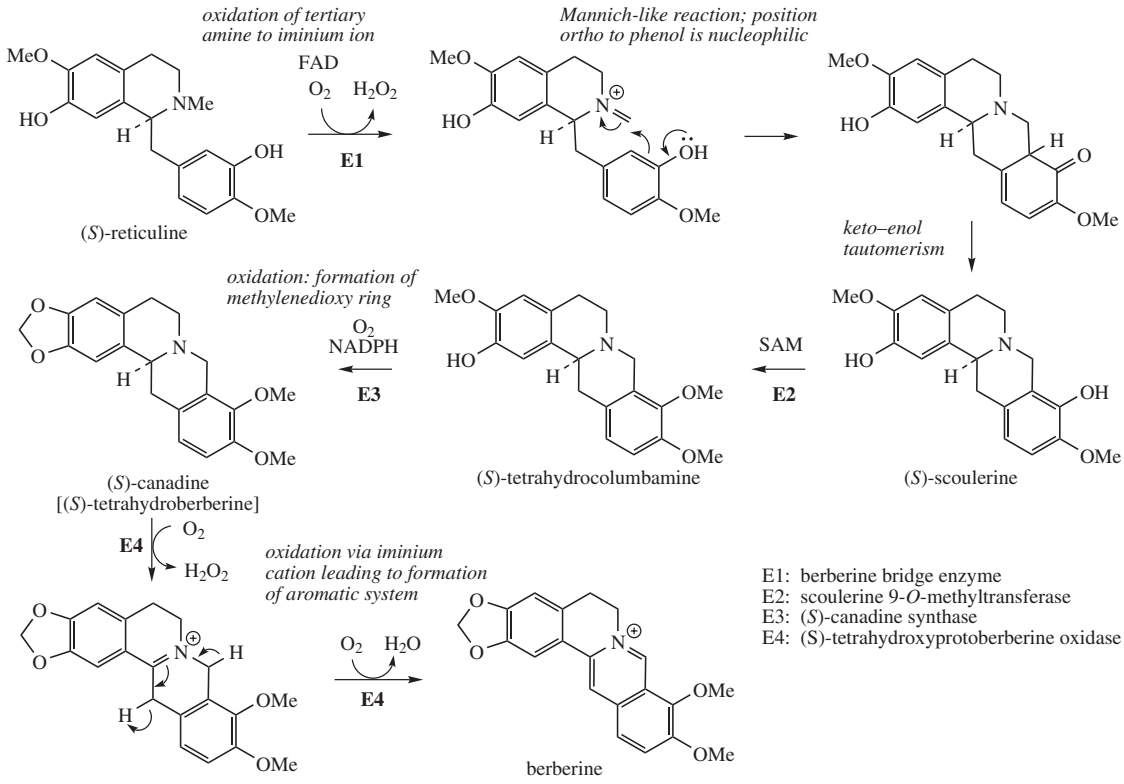


Figure 6.64

the cyclization reaction completed, the phenol group in scoulerine is then methylated to give **tetrahydrocolumbamine**, and this step is followed by construction of the methylenedioxy group from the *ortho*-methoxyphenol, via an O_2-, NADPH- and cytochrome P-450-dependent enzyme. **Canadine** is oxidized to give the quaternary isoquinolinium system of **berberine**. This appears to involve two separate oxidation steps, both requiring molecular oxygen, with H_2O_2 and H_2O produced in the successive processes. The mechanistic sequence through an iminium ion has been suggested to account for these observations.

The protoberberine skeleton of scoulerine may be subjected to further modifications, some of which are given in Figure 6.65. Cleavage of the heterocyclic ring systems adjacent to the nitrogen atom as shown give rise to new skeletal types: protopine, e.g. **protopine** from *Chelidonium majus* (Papaveraceae), phthalideisoquinoline, e.g. **hydrastine** from *Hydrastis canadensis* (Ranunculaceae), and benzophenanthridine, e.g. **chelidonine** also from *Chelidonium majus*. The non-heterocyclic system seen in the opium alkaloid **narceine** from *Papaver somniferum* can be visualized as the result of cleavage of two of these bonds. Some enzymes implicated in these modifications have been characterized. These include an *N*-methyltransferase, yielding the quaternary amine with defined stereochemistry at the new chiral centre (Figure 6.65), and a cytochrome P-450-dependent monooxygenase that hydroxylates at position 14. This initiates ring opening to the protopine-type systems. A similar process can be formulated to rationalize cleavage of other rings. Some alkaloids of the phthalide-type are medicinally important. **Noscapine** (Figure 6.66) is one of the opium alkaloids and although it lacks any analgesic activity it is an effective cough suppressant (see page 350). **Hydrastine** is beneficial as a traditional remedy in the control of uterine bleeding. *Hydrastis* also contains berberine, indicating the close biosynthetic relationship of the two types of alkaloid. **Bicuculline** (Figure 6.66) from species of *Corydalis* and *Dicentra* (Fumariaceae) and its quaternary methiodide have been identified as potent GABA antagonists and have found widespread application as pharmacological probes for convulsants acting at GABA neuroreceptors.

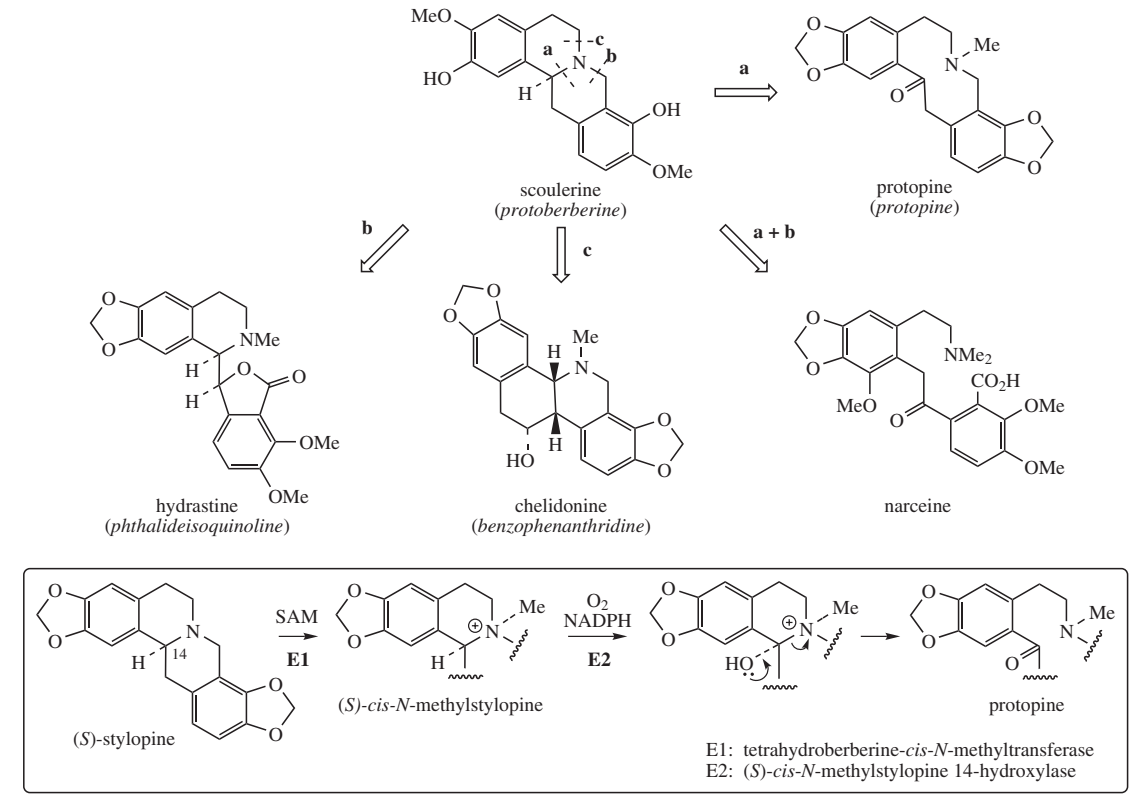


Figure 6.65

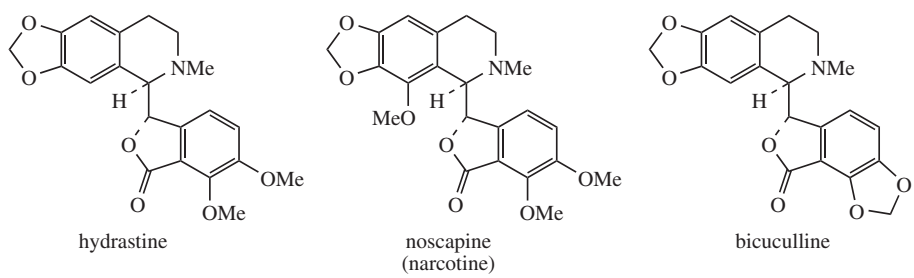


Figure 6.66

Phenethylisoquinoline Alkaloids

Several genera in the lily family (Liliaceae) are found to synthesize analogues of the benzyltetrahydroisoquinoline alkaloids, e.g. **autumnaline** (Figure 6.67), which contain an extra carbon between the tetrahydroisoquinoline and the pendant aromatic rings. This skeleton is formed in a similar way to that in the benzyltetrahydroisoquinolines from a phenylethylamine and an aldehyde (Figure 6.67), but a whole C_6C_3 unit rather than a C_6C_2 fragment functions as the reacting aldehyde. Typically, dopamine (from tyrosine) and 4-hydroxydihydrocinnamaldehyde (from phenylalanine) are involved in the initial condensation, and further hydroxylation and methylation steps then build up the substitution pattern to that of autumnaline. Phenolic oxidative coupling accounts for

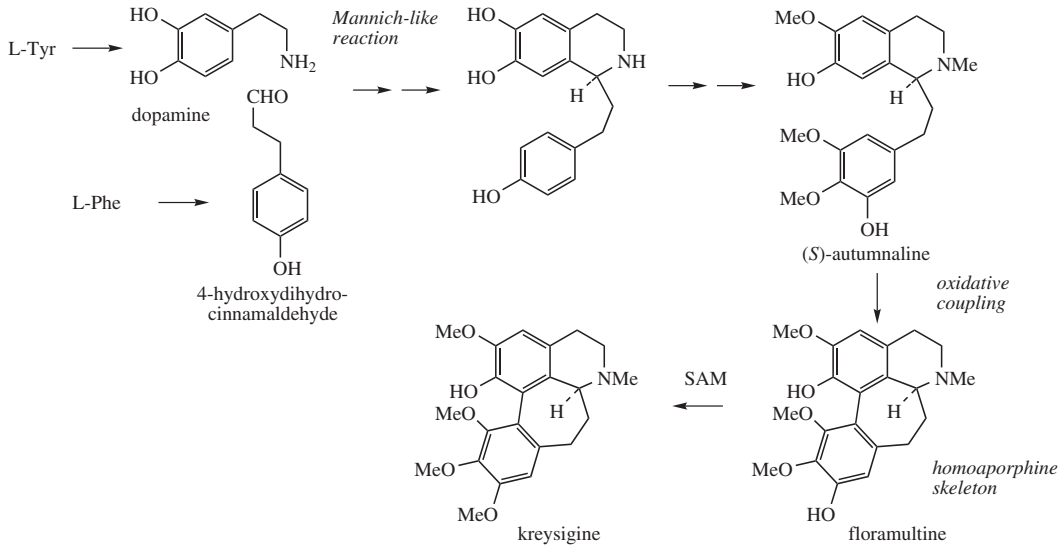


Figure 6.67

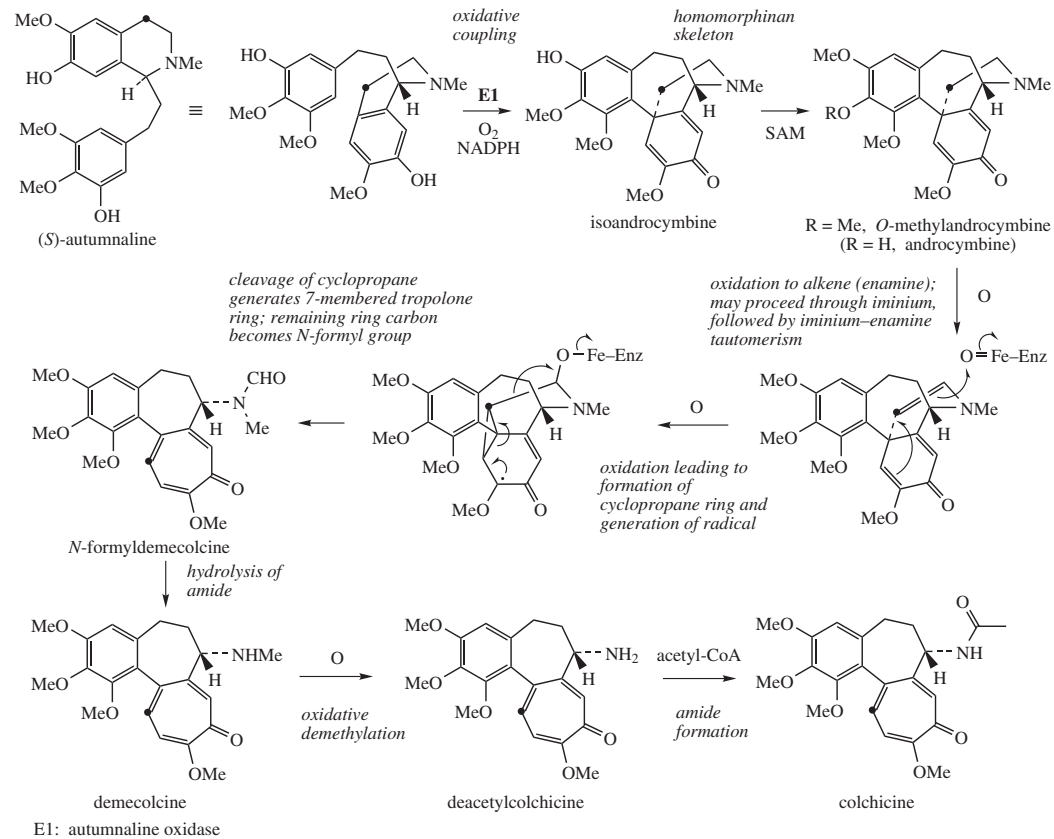


Figure 6.68

the occurrence of homoaporphine alkaloids such as **floramultine** and **kreysigine** in *Kreysigia multiflora* (Liliaceae/Convallariaceae).

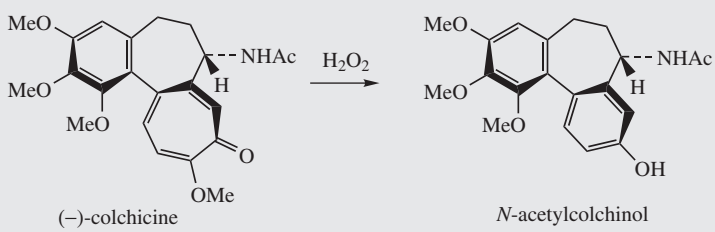
(*S*)-**Autumnaline** has also been found to act as a precursor for **colchicine** (Figure 6.68), an alkaloid containing an unusual tropolone ring. Colchicine is found in species of *Colchicum*, e.g. *Colchicum autumnale* (Liliaceae/Colchicaceae), as well as many other plants in the Liliaceae [Box 6.8]. Colchicine no longer has its nitrogen atom in a ring system, and extensive reorganization of the autumnaline structure has occurred. The seven-membered tropolone ring was shown by labelling experiments to originate by ring expansion of the tyrosine-derived aromatic ring taking in the adjacent benzylic carbon (Figure 6.68). Prior to these remarkable rearrangements, oxidative coupling of autumnaline in the *para–para* sense features in the pathway giving the dienone **isoandrocymbine**, which has a homomorphinan skeleton (compare salutaridine, Figure 6.53). The isomer **androcymbine** (Figure 6.68) had been isolated earlier from *Androcymbium melanthioides* (Liliaceae/Colchicaceae), thus giving a clue to the biosynthetic pathway. Methylation follows, giving *O*-methylandrocymbine, and it is then proposed that enzymic oxidation to an enamine yields the substrate for ring modification. Experimental labelling studies are then best explained by a cytochrome P-450-dependent process in which formation of a cyclopropane ring is followed by ring opening to generate the 6π electron aromatic tropolone system. This incorporates the original tyrosine benzylic carbon into the seven-membered ring, and also breaks the original phenylethylamine side-chain between the carbon atoms. One carbon is left on the nitrogen as a formyl group, and this can be lost by hydrolysis. **Colchicine** is produced by exchanging the *N*-methyl group for an *N*-acetyl group, by way of an oxidative demethylation followed by acetylation using acetyl-CoA. **Demecolcine** and **deacetylcolchicine** are intermediates in the process.

Box 6.8

Colchicum

Colchicum seed and corm are obtained from *Colchicum autumnale* (Liliaceae/Colchicaceae), the autumn crocus or meadow saffron. The plant, though not a crocus, produces crocus-like flowers in the autumn, the leaves not emerging until the spring. It is a native of Europe, is widely cultivated as an ornamental garden plant, and is grown for drug use, mainly in Europe and North Africa. The principal alkaloid is colchicine (Figure 6.68), which occurs to the level of about 0.8% in the seed, and 0.6% in the corm. The nitrogen in colchicine is part of an amide function, so colchicine does not display any significant basicity and does not form well-defined salts. Demecolcine (*N*-deacetyl-*N*-methylcolchicine; Figure 6.68) is a minor constituent in both corm and seeds.

Extracts of *Colchicum autumnale*, and later **colchicine** itself, have been used in the treatment of gout, a painful condition in which impaired purine metabolism leads to a build-up of uric acid crystals in the joints. Colchicine is an effective treatment for acute attacks, but it is very toxic, and this restricts its general use. It appears to act primarily as an anti-inflammatory agent and does not itself affect uric acid metabolism, which needs to be treated with other agents, e.g. a xanthine oxidase inhibitor such as allopurinol. The cytotoxic properties of colchicine and related alkaloid structures from *Colchicum autumnale* led to their being tested as potential anticancer agents. Colchicine binds to tubulin in the mitotic spindle, preventing polymerization and assembly into microtubules, as do podophyllotoxin (see page 155) and vincristine (see page 375), and provides a useful biochemical probe for this process. A feature of colchicine's structure is that the two aromatic rings are not coplanar, but are twisted relative to each other with a dihedral angle of 54° (Figure 6.69). This is essential for binding to tubulin. Colchicine and most related compounds are too toxic for medicinal use as anticancer agents, though research still progresses. One derivative under active development



(−)-colchicine

N-acetylcolchinol

Figure 6.69

Box 6.8 (continued)

is a water-soluble phosphate pro-drug of ***N*-acetylcolchinol**, a compound which was initially synthesized from colchicine by an oxidation reaction that prompts a ring contraction process (Figure 6.69).

The ability of colchicine to act as a mitotic poison is exploited in plant breeding, since the interference with mitosis results in multiplication of chromosomes in the cell nucleus without the process of cell division. Cell division recommences on cessation of treatment. This allows generation of mutations (polyploids) and possible new varieties of plant. Colchicine is also found in other species of *Colchicum*, as well as in many other plants in the Liliaceae (e.g. *Bulbocodium, Gloriosa, Merendera*, and *Sandersonia*), a group of plants now classified as the family Colchicaceae. *Gloriosa superba* is currently a commercial source of colchicine.

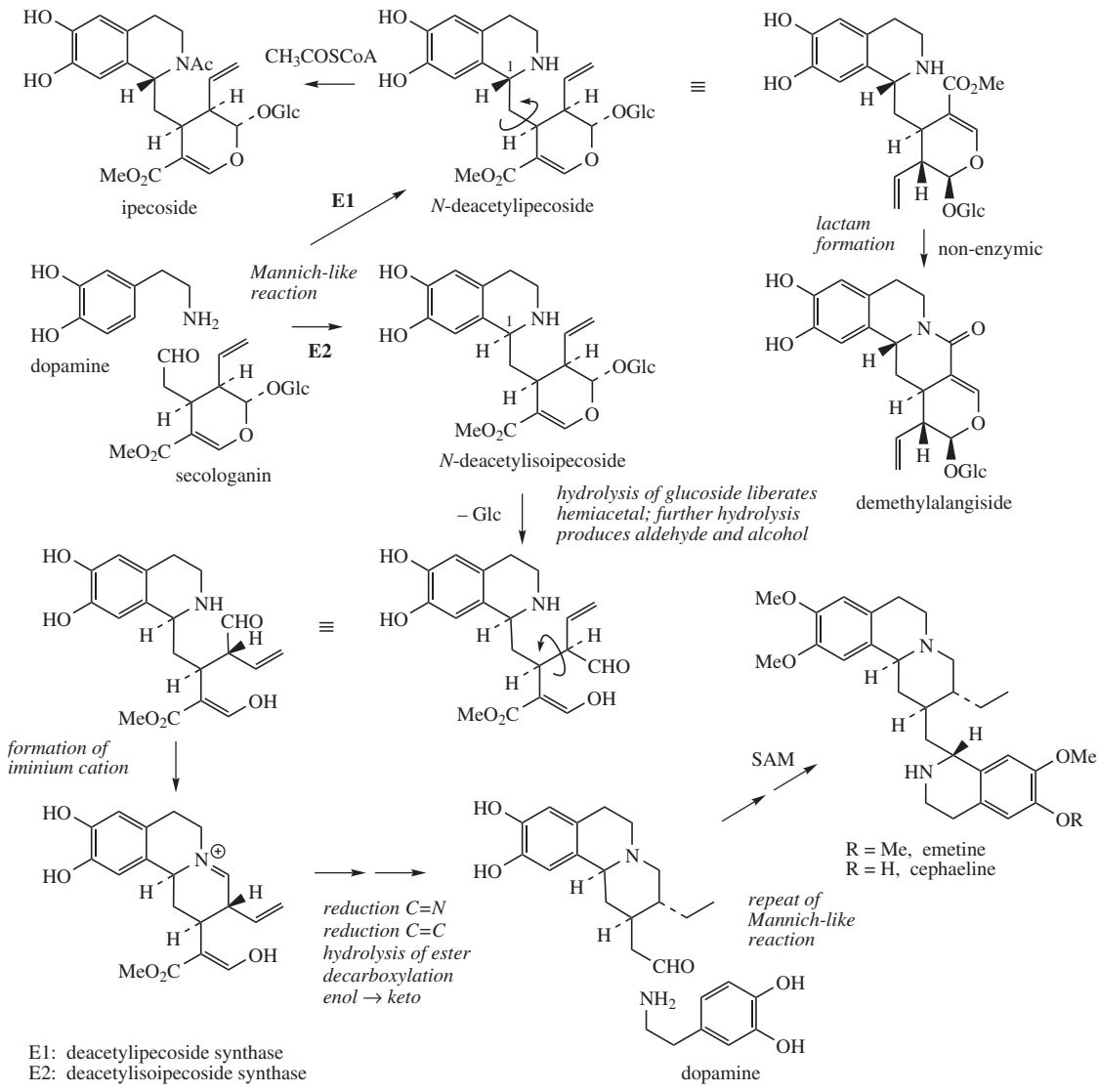


Figure 6.70

Terpenoid Tetrahydroisoquinoline Alkaloids

The alkaloids found in ipecacuanha [Box 6.9], the dried rhizome and roots of *Cepahaelis ipecacuanha* (Rubiaceae), have a long history of use in the treatment of amoebic dysentery, and provide unusual examples of tetrahydroisoquinoline structures. The principal alkaloids, e.g. **emetine** and **cephaeline** (Figure 6.70), possess a skeleton with two tetrahydroisoquinoline ring systems plus a further fragment which has its origin in a terpenoid-derived molecule. This terpenoid substrate is the secoiridoid **secologanin** (see page 207), a compound which also features in the biosynthesis of many complex indole alkaloids (see page 370).

Secologanin is an aldehyde and can condense with dopamine in a Mannich-like reaction to give the tetrahydroisoquinoline alkaloids *N*-deacetylisoipecoside or *N*-deacetylipecoside with different configurations at C-1 (Figure 6.70). Indeed, dopamine and secologanin react readily under mildly acidic conditions to give a mixture of these two alkaloids; in nature, two different enzymes are involved. The *N*-acetate **ipecoside** is also

Box 6.9

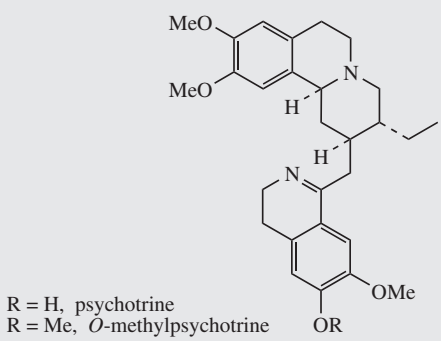
Ipecacuanha

Ipecacuanha or **ipecac** is derived from the dried rhizome and roots of *Cephaelis ipecacuanha* or *Cephaelis acuminata* (Rubiaceae). These are low, straggling shrubs possessing horizontal rhizomes with prominently ridged roots. *Cephaelis ipecacuanha* yields what is termed Rio or Brazilian ipecac, and is cultivated mainly in Brazil, whilst *Cephaelis acuminata* gives Cartagena, Nicaragua, or Panama ipecac, and comes principally from Colombia and Nicaragua. Most of the commercial ipecac now derives from *Cephaelis acuminata*. Ipecac is an age-old remedy of the South American Indians, who used it for the treatment of dysentery. More recently it was mixed with powdered opium to give Dover's powder (see page 349), where the ipecac content functioned as a diaphoretic (promotes perspiration).

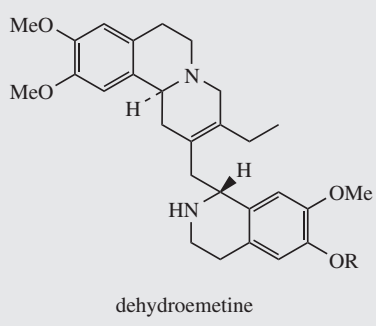
Ipecac contains 2–2.5% of alkaloids, the principal ones being emetine and cephaeline (Figure 6.70). Typically, in *Cephaelis ipecacuanha* the emetine to cephaeline ratio might be about 2:1, whereas in *Cephaelis acuminata* the ratio ranges from about 1:2 to 1:1. Minor alkaloids characterized include psychotrine and *O*-methylpsychotrine (Figure 6.71), which are dehydro variants of cephaeline and emetine respectively.

Both **emetine** and the synthetic **dehydroemetine** (Figure 6.71) have been useful as anti-amoebics, particularly in the treatment of amoebic dysentery. However, they also cause nausea, and this has now made other drugs preferable. The emetic action of the alkaloids is particularly valuable though, and the crude drug extract in the form of **ipecacuanha emetic mixture** is an important preparation used for drug overdose or poisoning. The emetic mixture is often a standard component in poison antidote kits. Ipecacuanha also has expectorant activity, and extracts are still components of a number of compound expectorant preparations. Emetine has more expectorant and less emetic action than cephaeline; thus, the Brazilian drug is preferred for such mixtures. If required, emetine may be obtained in larger amounts by methylating the cephaeline component of the plant material.

Emetine and cephaeline are both potent inhibitors of protein synthesis, inhibiting at the translocation stage. They display antitumour, antiviral, and antiamoebic activity, but they are too toxic for therapeutic use. In recent studies, *O*-methylpsychotrine has displayed fairly low effects on protein synthesis, but a quite potent ability to curb viral replication through inhibition of HIV reverse transcriptase. This may give it potential in the treatment of AIDS.



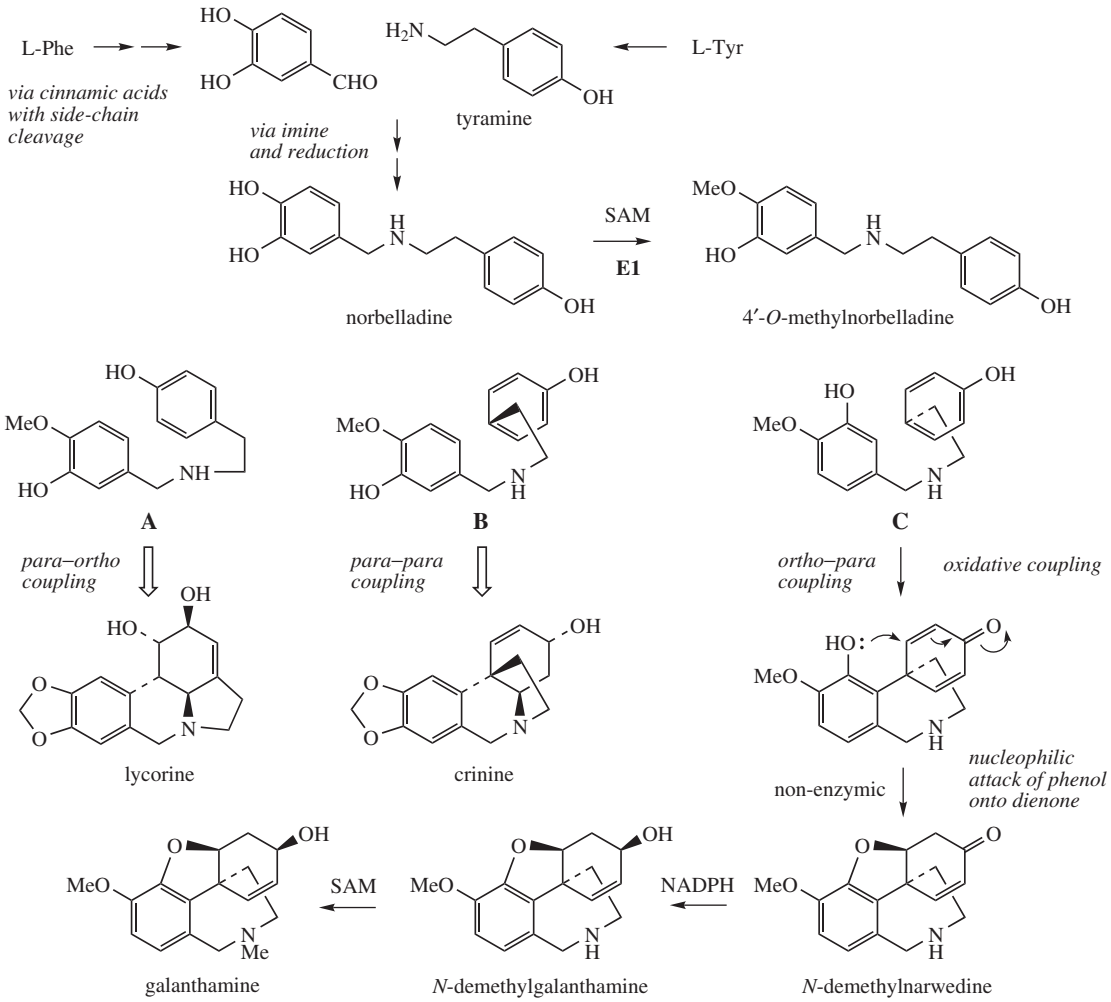
R = H, psychotrine
R = Me, *O*-methylpsychotrine



dehydroemetine

Figure 6.71

found in ipecacuanha; however, this has the opposite stereochemistry at C-1 to the biosynthetic intermediates leading to emetine. In the absence of the *N*-acetyl function, lactam (amide) formation between the amine and the carboxylic ester may occur spontaneously to give **demethylalangiside**; the C-1 epimer is also known. The pathway to emetine from *N*-deacetylisoipecoside may be postulated as follows. The secologanin fragment in *N*-deacetylisoipecoside contains an acetal function, which can be restored to its component aldehyde and alcohol fragments by hydrolysis of the glucosidic bond. The newly liberated aldehyde can then bond with the secondary amine to give the quaternary iminium cation. This intermediate is converted into an aldehyde by a sequence of reactions: reduction of iminium, reduction of alkene, plus hydrolysis of ester and subsequent decarboxylation, though not necessarily in that order. The decarboxylation step is facilitated by the β-aldehyde function, shown as an enol in Figure 6.71. Most of the reactions taking place in the secologanin-derived part of the structure are also met in discussions of terpenoid indole alkaloids (see page 370). The resultant aldehyde is now able to participate in formation of a second tetrahydroisoquinoline ring system, by reaction with a second dopamine molecule. Methylation gives **cephaeline** and **emetine**, though these methylation steps may well occur earlier in the pathway.



E1: catechol *O*-methyltransferase

Figure 6.72

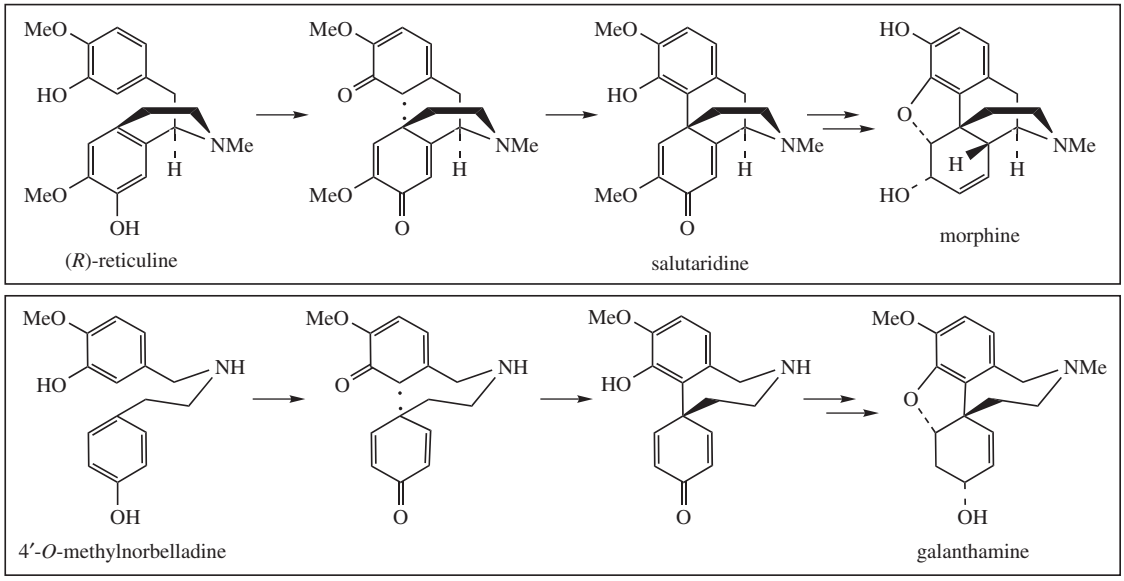


Figure 6.73

Amaryllidaceae Alkaloids

Various types of alkaloid structure are encountered in the daffodil/narcissus family, the Amaryllidaceae, and they can be rationalized better through biosynthesis than by structural type. The alkaloids arise by alternative modes of oxidative coupling of precursors related to **norbelladine** (Figure 6.72), which is formed through combination of 3,4-dihydroxybenzaldehyde with tyramine, these two precursors arising from phenylalanine and tyrosine respectively. Three structural types of alkaloid can be related to **4′-*O*-methylnorbelladine** by different alignments of the phenol rings allowing couplings *para–ortho* (A), *para–para* (B), or *ortho–para* (C), as shown in Figure 6.72.

For **galanthamine**, the dienone formed via oxidative coupling (C) undergoes a spontaneous nucleophilic addition from the phenol group, forming an ether linkage (compare opium alkaloids, page 348), and the sequence is completed by reduction and methylation reactions. The analogy with morphine biosynthesis is quite striking and can be appreciated from Figure 6.73. For **lycorine** and **crinine**, although details are not given in Figure 6.72,

Box 6.10

Galanthamine

Galantamine (galanthamine; Figure 6.72) can be isolated from a number of species of the Amaryllidaceae, including snowdrops (*Galanthus* species), daffodils (*Narcissus pseudonarcissus*), and snowflakes (*Leucojum* species), where typical content varies from about 0.05 to 0.2% in the bulbs. Several structurally related alkaloids are also present. It is currently isolated for drug use from the bulbs of wild *Leucojum aestivum* and *Galanthus* species; longer term supplies will require development of cheaper synthetic procedures. Galantamine acts as a centrally acting competitive and reversible inhibitor of acetylcholinesterase, and significantly enhances cognitive function in the treatment of Alzheimer's disease by raising acetylcholine levels in brain areas lacking cholinergic neurones. It is less toxic than other acetylcholinesterase inhibitors, such as physostigmine (see page 386). There is also evidence that galantamine displays an increased beneficial effect due to a sensitizing action on nicotinic acetylcholine receptors in the central nervous system. In common with other treatments for Alzheimer's disease, it does not cure the condition, but merely slows the rate of cognitive decline.

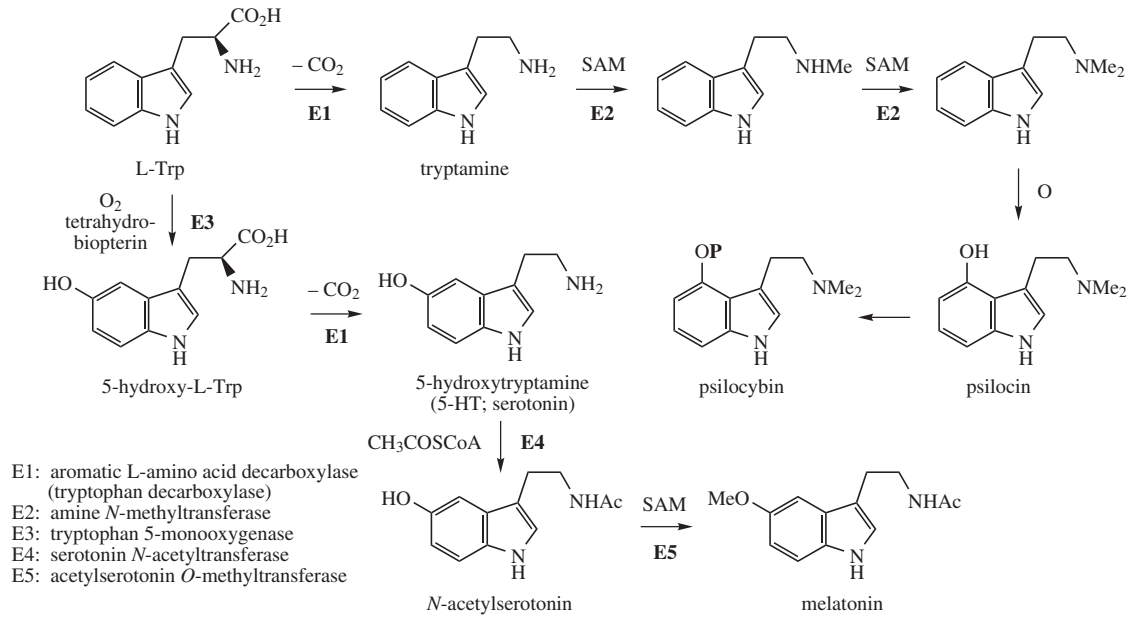


Figure 6.74

it is apparent that the nitrogen atom acts as a nucleophile towards the dienone system, generating the new heterocyclic ring systems. Alkaloids such as lycorine, crinine, and galanthamine can undergo further modifications, which include ring cleavage reactions, generating many more variations than can be considered here. The Amaryllidaceae family includes *Amaryllis, Narcissus*, and *Galanthus*, and the alkaloid content of bulbs from most members makes these toxic. Lycorine was first isolated from *Lycorus radiata*, but is common and found throughout the family. **Galanthamine** from snowdrops (*Galanthus* species) is currently an important drug material of value in treating Alzheimer's disease [Box 6.10].

ALKALOIDS DERIVED FROM TRYPTOPHAN

L-Tryptophan is an aromatic amino acid containing an indole ring system, having its origins in the shikimate pathway (Chapter 4) via anthranilic acid. It acts as a precursor of a wide range of indole alkaloids, but there is also definite proof that major rearrangment reactions can convert the indole ring system into a quinoline ring, thus increasing further the ability of this amino acid to act as an alkaloid precursor (see page 380).

Simple Indole Alkaloids

Tryptamine and its *N*-methyl and *N*,*N*-dimethyl derivatives (Figure 6.74) are widely distributed in plants, as are simple hydroxylated derivatives such as **5-hydroxytryptamine (5-HT, serotonin)**. These are formed (Figure 6.74) by a series of decarboxylation, methylation, and hydroxylation reactions, though the sequences of these reactions are found to vary according to final product and/or organism involved. 5-HT is also found in mammalian tissue, where it acts as a neurotransmitter in the central nervous system [Box 6.11]. It is formed from tryptophan by hydroxylation and then decarboxylation, paralleling the tyrosine → dopamine pathway (see page 335). *N*-Acetylation of serotonin followed by *O*-methylation leads to **melatonin**, an animal hormone that regulates daily (circadian) rhythms: melatonin levels inform the organism about the time of day [Box 6.11]. Melatonin is also found in plants, however. In the formation of **psilocin** (Figure 6.74), decarboxylation precedes *N*-methylation, and hydroxylation occurs last. Phosphorylation of the hydroxyl in psilocin gives **psilocybin**. These two compounds are responsible for the hallucinogenic properties of so-called magic mushrooms, which include species of *Psilocybe, Panaeolus*, etc. [Box 6.11].

Box 6.11

5-Hydroxytryptamine (Serotonin)

5-Hydroxytryptamine (5-HT, serotonin) is a monoamine neurotransmitter found in cardiovascular tissue, the peripheral nervous system, blood cells, and the central nervous system. It mediates many central and peripheral physiological functions, including contraction of smooth muscle, vasoconstriction, food intake, sleep, pain perception, and memory, a consequence of it acting on several distinct receptor types; currently, seven types have been identified. Although 5-HT may be metabolized by monoamine oxidase, platelets and neurons possess a high affinity 5-HT reuptake mechanism. This mechanism may be inhibited, thereby increasing levels of 5-HT in the central nervous system, by widely prescribed antidepressant drugs termed selective serotonin re-uptake inhibitors (SSRIs), e.g. fluoxetine (Prozac®).

Migraine headaches that do not respond to analgesics may be relieved by the use of an agonist of the 5-HT_1 receptor, since these receptors are known to mediate vasoconstriction. Though the causes of migraine are still not clear, they are characterized by dilation of cerebral blood vessels. 5-HT_1 agonists based on the 5-HT structure in current use include the sulfonamide derivative **sumatriptan**, and the more recent agents **almotriptan, eletriptan, frovatriptan, naratriptan, rizatriptan**, and **zolmitriptan** (Figure 6.75). These are of considerable value in treating acute attacks. Several of the ergot alkaloids (page 390) also interact with 5-HT receptors.

Melatonin

In animals, **melatonin** (Figure 6.74) is a hormone synthesized by the pineal gland in the brain. It plays a key role in the circadian rhythm, sleep regulation, and seasonal photoperiodic regulation. The duration of elevated melatonin levels is usually proportional to night length in vertebrates. Melatonin concentration and its daily rhythm can thus inform the organism about the time of day and about the season. It is also found in invertebrates and plants, though less is known about its role. Melatonin is claimed to be effective in helping to regulate disrupted circadian rhythms and sleep disorders. A slow-release formulation is available for treating insomnia in older patients; melatonin production is found to decrease with age. It is currently also popular to reduce the effects of jet-lag by resetting the internal body clock.

Psilocybe

The genus *Psilocybe* constitutes a group of small mushrooms with worldwide distribution. It has achieved notoriety on account of the hallucinogenic effects experienced following ingestion of several species, particularly those from Mexico, and has led to the collective description ‘magic mushrooms’. Over 80 species of *Psilocybe* have been found to be psychoactive, whereas over

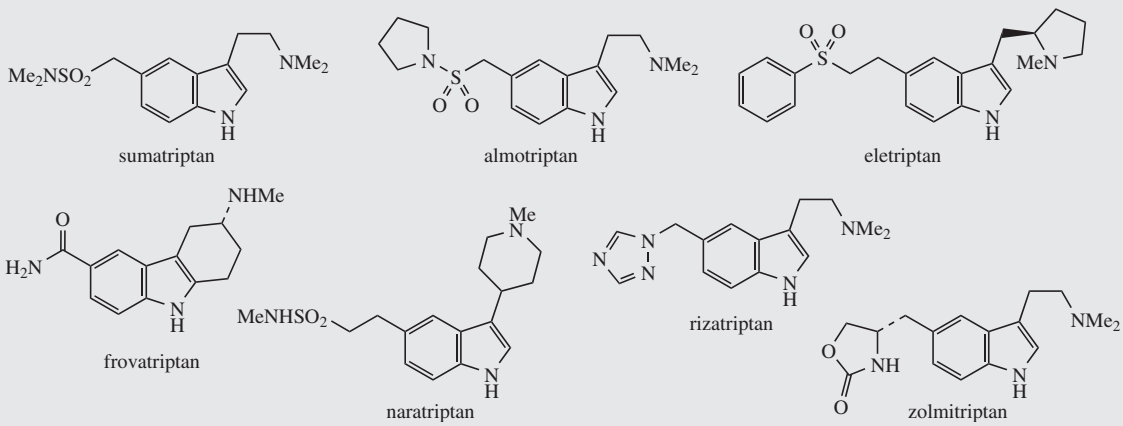


Figure 6.75

Box 6.11 (continued)

50 species are inactive. More than 30 of the hallucinogenic species have been identified in Mexico, but active species may be found in all areas of the world. *Psilocybe mexicana* has been used by the Mexican Indians in ancient ceremonies for many years, and its history can be traced back to the Aztecs. In temperate regions, *Psilocybe semilanceata*, the liberty cap, is a common species with similar activity. All the psychoactive members of the genus are said to stain blue when the fresh tissue, particularly that near the base of the stalk, is damaged, though the converse is not true. Ingestion of the fungus causes visual hallucinations with rapidly changing shapes and colours, and different perceptions of space and time, the effects gradually wearing off and causing no lasting damage or addiction.

The active hallucinogens, present at about 0.3%, are the tryptamine derivatives psilocybin and psilocin (Figure 6.74), which are structurally related to the neurotransmitter 5-HT, thus explaining their neurological effects. Psilocybin is probably the main active ingredient, though to produce hallucinations a dose of some 6–20 mg is required. In addition to species of *Psilocybe*, these compounds may be found in some fungi from other genera, including *Conocybe, Panaeolus*, and *Stropharia*. Misidentification of fungi can lead to the consumers experiencing possible unwanted toxic effects, especially gastrointestinal upsets, instead of the desired psychedelic visions.

Gramine (Figure 6.76) is a simple amine found in barley (*Hordeum vulgare*; Graminae/Poaceae) and is derived from tryptophan by a biosynthetic pathway which cleaves off two carbon atoms, yet surprisingly retains the tryptophan nitrogen atom. It has been suggested that the nitrogen reacts with a cofactor, e.g. pyridoxal phosphate, and is subsequently transferred back to the indolemethyl group after the chain shortening. Only the *N*-methyltransferases have been characterized.

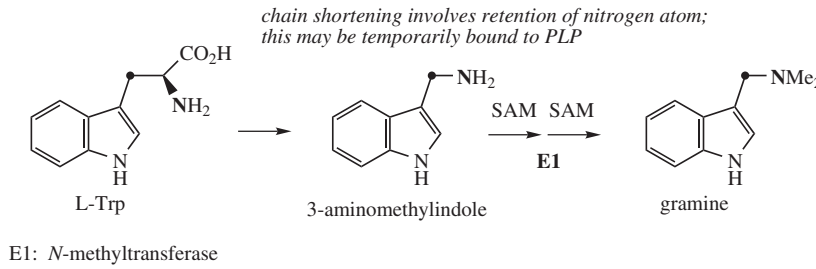


Figure 6.76

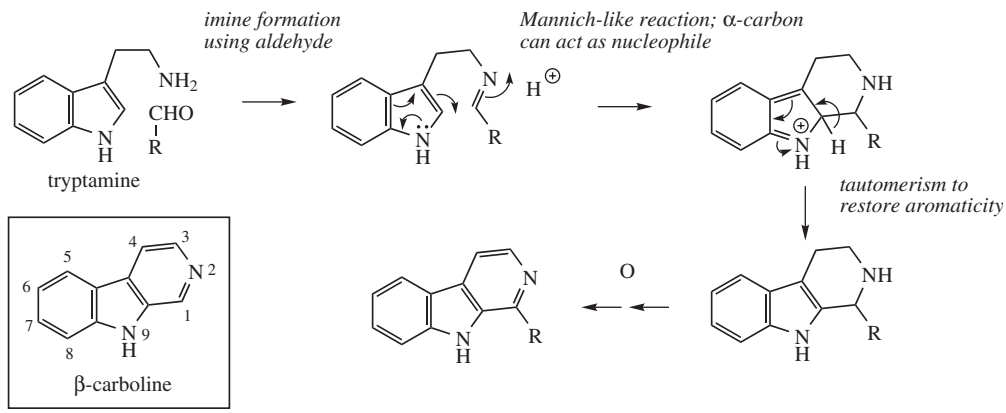


Figure 6.77

Simple β-Carboline Alkaloids

Alkaloids based on a β-carboline system (Figure 6.77) exemplify the formation of a new six-membered heterocyclic ring using the ethylamine side-chain of tryptamine in a process analogous to generation of tetrahydroisoquinoline alkaloids (see page 340). Position 2 of the indole system is nucleophilic, due to the adjacent nitrogen, and can participate in a Mannich/Pictet–Spengler-type reaction, attacking an imine generated from tryptamine and an aldehyde (or keto acid) (Figure 6.77). Aromaticity is restored by subsequent loss of the C-2 proton. (It should be noted that the analogous chemical reaction actually involves nucleophilic attack from C-3, and then a subsequent rearrangement occurs to give bonding at C-2; this type of process does not appear to participate in biosynthetic pathways.)

Extra carbon atoms are supplied by aldehyes or keto acids, according to the complexity of the substrate (compare tetrahydroisoquinoline alkaloids, page 340). Thus, complex β-carbolines, e.g. the terpenoid indole alkaloid ajmalicine (see page 371), are produced by a pathway using an aldehyde such as secologanin. Simpler structures employ keto acids; for example, **harmine** (Figure 6.78) incorporates two extra carbon atoms from pyruvate. In such cases, an intermediate acid is involved, and oxidative decarboxylation gives the dihydro-β-carboline, from which reduced tetrahydro-β-carboline structures, e.g. **elaeagnine** from *Elaeagnus angustifolia* (Elaeagnaceae), or fully aromatic β-carboline structures, e.g. **harman** and **harmine** from *Peganum harmala* (Zygophyllaceae), are derived (Figure 6.78). The methoxy substitution in the indole system of harmine is introduced at some stage in the pathway by successive hydroxylation and methylation reactions. A sequence from 6-hydroxytryptamine is also feasible. The reported psychoactive properties of the plants *Peganum harmala* and *Banisteriopsis caapi* (Malpighiaceae) is due to β-carboline alkaloids such as harmine, harmaline, and tetrahydroharmine (Figure 6.78), which are potent serotonin antagonists.

Terpenoid Indole Alkaloids

More than 3000 terpenoid indole alkaloids are recognized, making this one of the major groups of alkaloids in plants. They are found mainly in eight plant families, of which the Apocynaceae, the Loganiaceae, and the Rubiaceae provide the best sources. In terms of structural complexity, many of these alkaloids are quite outstanding, and it is a tribute to the painstaking experimental studies of various groups of workers that we are able to rationalize these structures in terms of their biochemical origins. Many of the steps have now been characterized at the enzymic level, and appropriate genes have been identified.

In virtually all structures, a tryptamine portion can be recognized. The remaining fragment is usually a C_9 or C_{10} residue, and three main structural types are discernible according to the arrangement of atoms in this fragment. These are termed the *Corynanthe* type, as in **ajmalicine** and **akuammicine**, the *Aspidosperma* type, as in **tabersonine**, and the *Iboga* type, exemplified by **catharanthine** (Figure 6.79). The C_9 or C_{10} fragment was shown to be of terpenoid origin, and the secoiridoid **secologanin** (see page 207) was identified as the terpenoid derivative which initially combined with the tryptamine portion of the molecule. Furthermore, the *Corynanthe, Aspidosperma* and *Iboga* groups of alkaloids could then be related and rationalized in terms of rearrangements occurring in the terpenoid part of the structures (Figure 6.79). Secologanin itself contains the 10-carbon framework that is typical of the *Corynanthe* group. The *Aspidosperma* and *Iboga* groups could then arise by rearrangement of

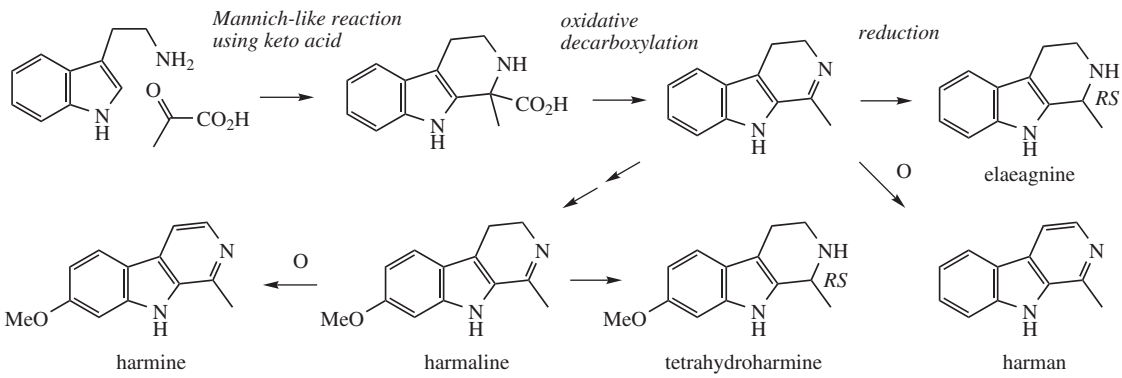


Figure 6.78

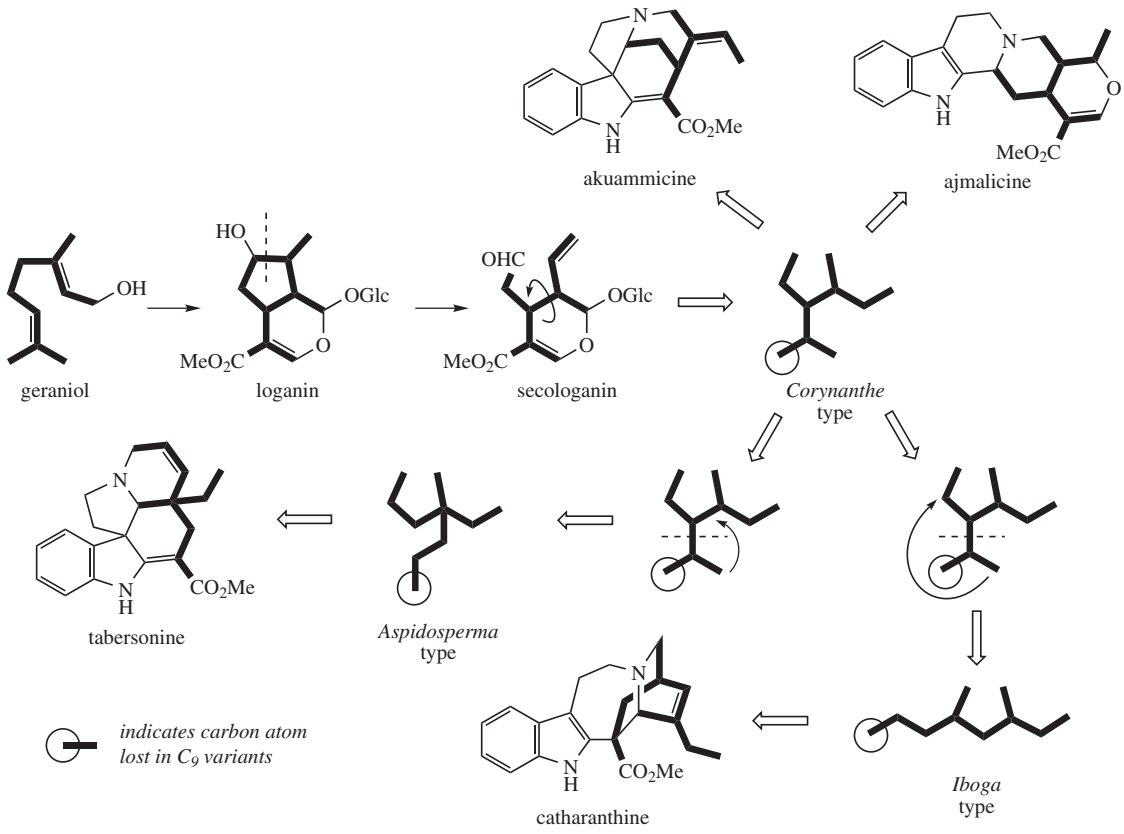


Figure 6.79

the *Corynanthe* skeleton as shown. This is represented by detachment of a three-carbon unit which is then rejoined to the remaining C_7 fragment in one of two different ways. Where C_9 terpenoid units are observed, the alkaloids normally appear to have lost the carbon atom indicated in the circle. This corresponds to the carboxylate function of secologanin and its loss by hydrolysis/decarboxylation is now understandable.

The origins of loganin and secologanin have already been discussed in Chapter 5 (see page 207). Condensation of secologanin with tryptamine in a Mannich-like reaction generates the tetrahydro-β-carboline system and produces **strictosidine** (Figure 6.80). Hydrolysis of the glycoside function allows opening of the hemiacetal and exposure of an aldehyde group which can react with the secondary amine function to give a quaternary iminium cation. These reactions are also seen in the pathway to ipecac alkaloids (see page 362). Allylic isomerization, moving the vinyl double bond into conjugation with the iminium, generates **dehydrogeissoschizine**, and cyclization to **cathenamine** follows. Cathenamine is reduced to **ajmalicine** in the presence of NADPH. Oxidation of ajmalicine to **serpentine** is catalysed by a peroxidase.

Although details are not confirmed, carbocyclic variants related to ajmalicine, such as **yohimbine**, are likely to arise from dehydrogeissoschizine by the mechanism indicated in Figure 6.81. Yohimbine is found in yohimbe bark (*Pausinystalia yohimbe*; Rubiaceae) and also aspidosperma bark (*Aspidosperma* species; Apocynaceae) and has been used in folk medicine as an aphrodisiac. It does have some pharmacological activity and is known to dilate blood vessels. More important examples containing the same carbocyclic ring system are the alkaloids found in species of *Rauwolfia*, especially *Rauwolfia serpentina* (Apocynaceae) [Box 6.12]. **Reserpine** and **deserpidine** (Figure 6.82) are trimethoxybenzoyl esters of yohimbine-like alkaloids, whilst **rescinnamine** is a trimethoxycinnamoyl ester. Both reserpine and rescinnamine contain an additional methoxyl substituent on the indole system at position 11, the result of hydroxylation

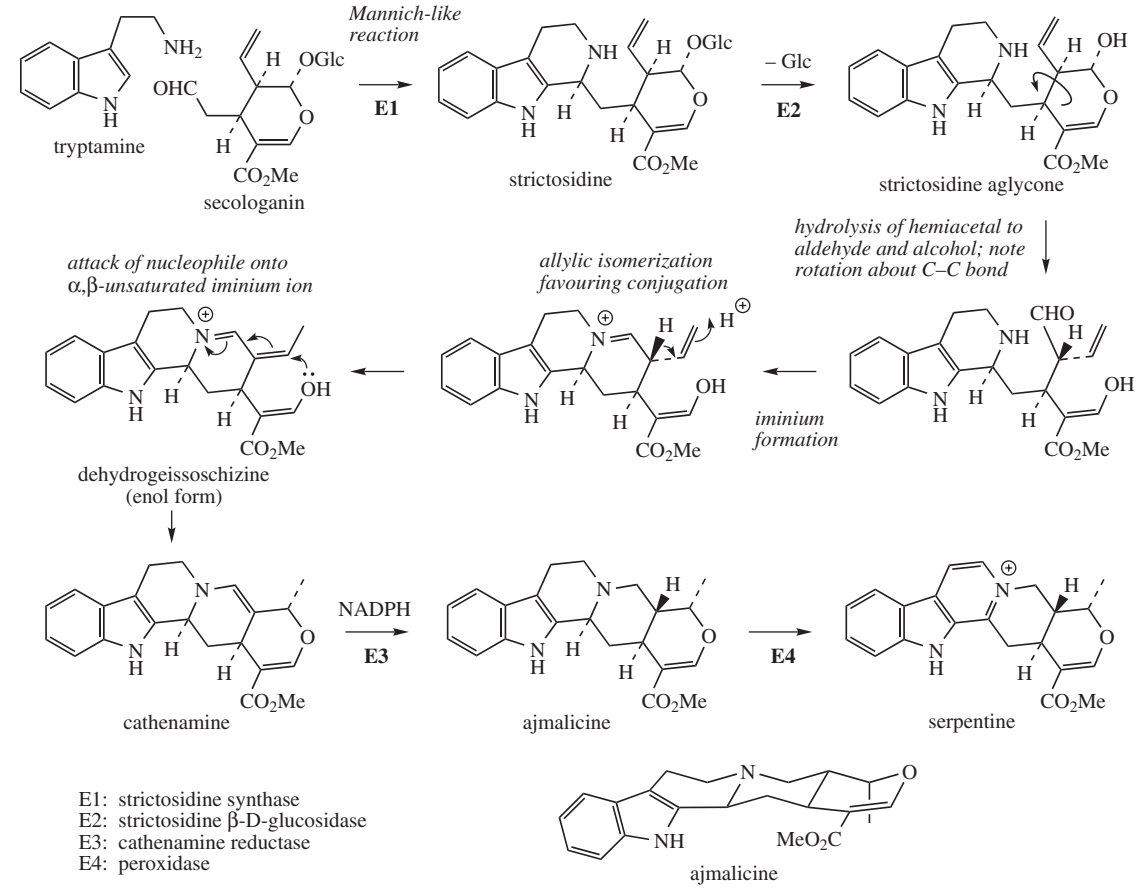


Figure 6.80

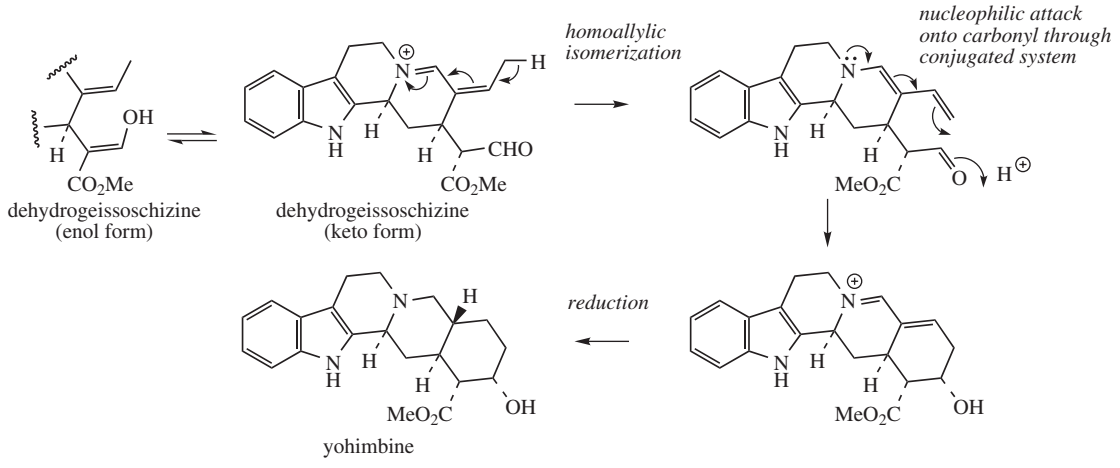


Figure 6.81

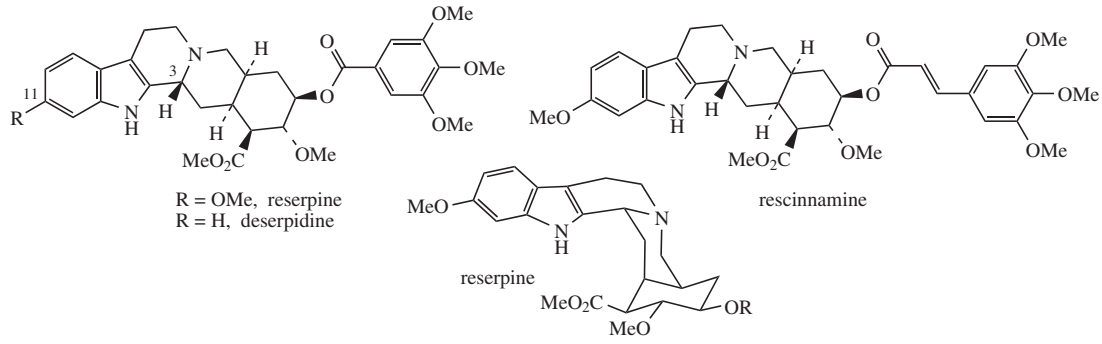


Figure 6.82

and methylation at a late stage in the pathway. A feature of these alkaloids is that they have the opposite stereochemistry at position 3 to yohimbine and strictosidine, and it is likely they are formed from the C-3 epimer of strictosidine. *Rauwolfia serpentina* also contains significant amounts of ajmalicine (Figure 6.80), emphasizing the structural and biosynthetic relationships between the two types of alkaloid.

Box 6.12

Rauwolfia

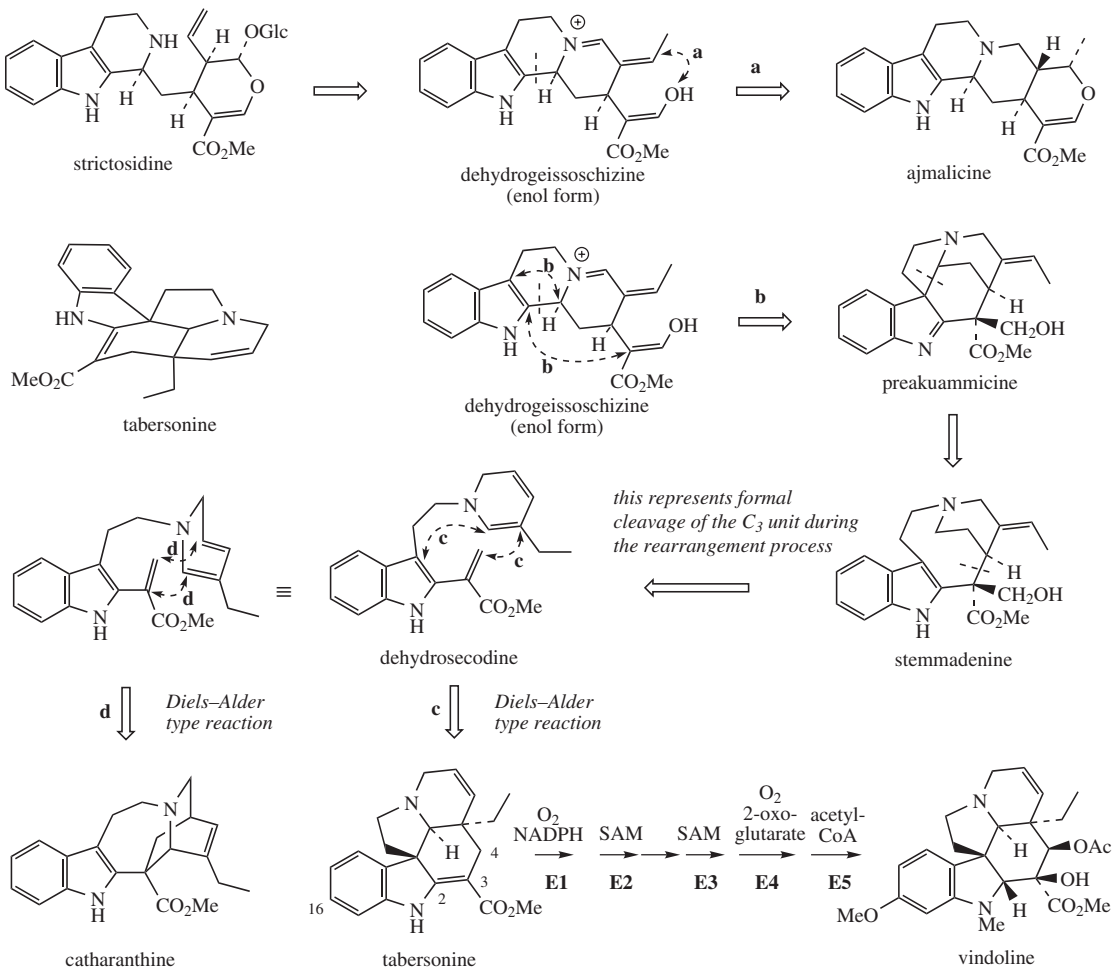
Rauwolfia has been used in Africa for hundreds of years, and in India for at least 3000 years. It was used as an antidote to snake-bite, to remove white spots in the eyes, against stomach pains, fever, vomiting, and headache, and to treat insanity. It appeared to be a universal panacea, and was not considered seriously by Western scientists until the late 1940s/early 1950s. Clinical tests showed the drug to have excellent antihypertensive and sedative activity. It was then rapidly and extensively employed in treating high blood pressure and to help mental conditions, relieving anxiety and restlessness, and thus initiated the tranquillizer era. The 'cure for insanity' was thus partially justified, and rauwolfia was instrumental in showing that mental disturbance has a chemical basis and may be helped by the administration of drugs.

Rauwolfia is the dried rhizome and roots of *Rauwolfia* (sometimes *Rauvolfia*) *serpentina* (Apocynaceae) or snakeroot, a small shrub from India, Pakistan, Burma, and Thailand. Other species used in commerce include *Rauwolfia vomitoria* from tropical Africa, a small tree whose leaves after ingestion cause violent vomiting, and *Rauwolfia canescens* (= *Rauwolfia tetraphylla*) from India and the Caribbean. Most of the drug material has been collected from the wild. *Rauwolfia serpentina* contains a wide range of indole alkaloids, totalling 0.7–2.4%, though only 0.15–0.2% consists of desirable therapeutically active compounds, principally reserpine, rescinnamine, and deserpidine (Figure 6.82). Other alkaloids of note are serpentine (Figure 6.80), ajmalicine (Figure 6.80), and ajmaline (see Figure 6.86). Reserpine and deserpidine are major alkaloids in *Rauwolfia canescens*, and *Rauwolfia vomitoria* contains large amounts of rescinnamine and reserpine.

Reserpine and **deserpidine** (Figure 6.82) have been widely used as antihypertensives and mild tranquillizers. They act by interfering with catecholamine storage, depleting levels of available neurotransmitters. Prolonged use of the pure alkaloids, reserpine in particular, has been shown to lead to severe depression in some patients, a feature not so prevalent when the powdered root was employed. The complex nature of the alkaloidal mixture means the medicinal action is somewhat different from that of reserpine alone. Accordingly, crude powdered rauwolfia remained an important drug for many years, and selected alkaloid fractions from the crude extract have also been widely used. The alkaloids can be fractionated according to basicity. Thus, serpentine and similar structures are strongly basic, whilst reserpine, rescinnamine, deserpidine, and ajmalicine are weak bases. Ajmaline and related compounds have intermediate basicity.

The rauwolfia alkaloids are now hardly ever prescribed in the UK, either as antihypertensives or as tranquillizers. Over a period of a few years, they have been rapidly superseded by synthetic alternatives. Reserpine has also been suggested to play a role in the promotion of breast cancers. Both **ajmalicine** (= raubasine) (Figure 6.80) and **ajmaline** (see Figure 6.86) are used clinically in Europe, though not in the UK. Ajmalicine is employed as an antihypertensive, whilst ajmaline is of value in the treatment of cardiac arrhythmias. Ajmalicine is also extracted commercially from *Catharanthus roseus* (see page 376).

The structural changes involved in converting the *Corynanthe*-type skeleton into those of the *Aspidosperma* and *Iboga* groups are quite complex, and are summarized in Figure 6.83. In parts, the pathways have yet to be confirmed. Only the tabersonine to vindoline conversion has been characterized in any detail. Early intermediates are alkaloids such as **preakuammicine**, which, although clearly of the *Corynanthe* type, is sometimes designated as *Strychnos* type (compare strychnine, page 377). This is because the *Corynanthe* terpenoid unit, originally attached to the indole α-carbon (as in ajmalicine), is now bonded to the β-carbon, and a new bonding between the rearrangeable C_3 unit and C-α is in place (see transformation **b**). **Stemmadenine** arises through fission of the bond to C-β, and then further fission yields **dehydrosecodine**, the importance of which is that the rearrangeable C_3 unit has been cleaved from the rest of the terpenoid carbon atoms. Hypothetically, this compound could undergo Diels–Alder-type coupling (see page 96) in two different ways. Alkaloids of the *Aspidosperma* type, e.g. **tabersonine** and **vindoline**, and *Iboga* type, e.g. **catharanthine**, emerge from the different bonding modes (Figure 6.83).



E1: tabersonine 16-hydroxylase
E2: 16-hydroxytabersonine 16-*O*-methyltransferase
E3: 16-methoxy-2,3-dihydro-3-hydroxytabersonine *N*-methyltransferase
E4: deacetoxyvindoline 4-hydroxylase
E5: deacetylvindoline *O*-acetyltransferase

Figure 6.82

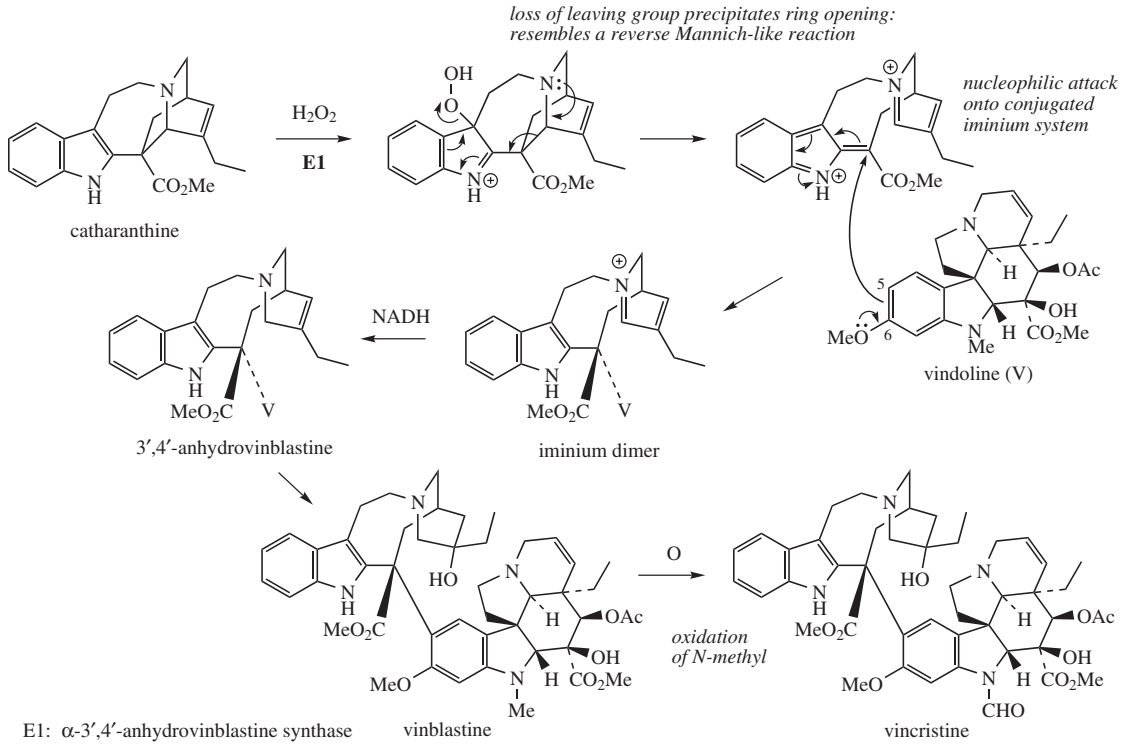


Figure 6.82

Many of the experimental studies which have led to an understanding of terpenoid indole alkaloid biosynthesis have been carried out using plants of the Madagascar periwinkle (*Catharanthus roseus*, formerly *Vinca rosea*; Apocynaceae) [Box 6.13]. Representatives of all the main classes of these alkaloids are produced, including **ajmalicine** (*Corynanthe*), **catharanthine** (*Iboga*), and **vindoline** (*Aspidosperma*). The sequence of alkaloid formation has been established initially by noting which alkaloids become labelled as a feeding experiment progresses, and more recently by appropriate enzyme and gene studies. However, the extensive investigations of the *Catharanthus roseus* alkaloids have also been prompted by the anticancer activity detected in a group of bisindole alkaloids. Two of these, **vinblastine** and **vincristine** (Figure 6.84), have been introduced into cancer chemotherapy and feature as some of the most effective anticancer agents available. These structures are seen to contain the elements of catharanthine and vindoline; indeed, they are derived by coupling of these two alkaloids. A peroxidase enzyme catalyses the coupling, and the product generated is **anhydrovinblastine**. It is proposed that **catharanthine** is oxidized to an iminium cation via a peroxide which loses the peroxide as a leaving group, breaking a carbon–carbon bond as shown (Figure 6.84). This intermediate electrophilic ion is then attacked by the

Box 6.13

Catharanthus

The Madagascar periwinkle *Catharanthus roseus* (= *Vinca rosea*) (Apocynaceae) is a small herb or shrub originating in Madagascar, but now common in the tropics and widely cultivated as an ornamental for its shiny dark green leaves and pleasant five-lobed flowers. Drug material is now cultivated in many parts of the world, including the USA, Europe, India, Australia, and South America.

Box 6.13 (continued)

The plant was originally investigated for potential hypoglycaemic activity because of folklore usage as a tea for diabetics. Although plant extracts had no effects on blood sugar levels in rabbits, test animals succumbed to bacterial infection due to depleted white blood cell levels (leukopenia), though no other adverse effects were apparent. The selective action suggested anticancer potential for the plant, and an exhaustive study of the constituents was initiated. The activity was found in the alkaloid fraction, and more than 150 alkaloids have since been characterized in the plant. These are principally terpenoid indole alkaloids, many of which are known in other plants, especially from the same family. Useful antitumour activity was demonstrated in a number of dimeric indole alkaloid structures (more correctly, bisindole alkaloids, since the 'monomers' are different), including vincaleukoblastine, leurosine, leurosidine, and leurocristine. These compounds became known as vinblastine, vinleurosine, vinrosidine, and vincristine respectively, the vin- prefix being a consequence of the earlier botanical nomenclature *Vinca rosea*, in common use at that time. The alkaloids vinblastine and vincristine (Figure 6.85) were introduced into cancer chemotherapy and have proved to be extremely valuable drugs.

Despite the minor difference in structure between vinblastine and vincristine, a significant difference exists in the spectrum of human cancers which respond to the drugs. **Vinblastine** (Figure 6.85) is used mainly in the treatment of Hodgkin's disease, a cancer affecting the lymph glands, spleen, and liver. **Vincristine** (Figure 6.85) has superior antitumour activity compared with vinblastine but is more neurotoxic. It is clinically more important than vinblastine, and is especially useful in the treatment of childhood leukaemia, giving a high rate of remission. Some other cancer conditions, including lymphomas, small-cell lung cancer, and cervical and breast cancers, also respond favourably. The alkaloids need to be injected, and both generally form part of a combination regimen with other anticancer drugs.

Vindesine (Figure 6.85) is a semi-synthetic amide derivative of vinblastine which has been introduced for the treatment of acute lymphoid leukaemia in children. **Vinorelbine** (Figure 6.85) is a newer semi-synthetic modification obtained from anhydrovinblastine (see below). In this structure, the indole.C_2N bridge in the catharanthine-derived unit has been shortened by one carbon; other agents feature structural modifications in the vindoline unit. It is orally active and has a broader anticancer activity, yet with lower neurotoxic side-effects than either vinblastine or vincristine. It is given intravenously for the treatment of advanced breast cancer and non-small-cell lung cancer, or orally to treat small-cell lung cancer. These compounds all inhibit cell mitosis, acting by binding to the protein tubulin in the mitotic spindle, preventing polymerization into microtubules, a mode of action shared with other natural agents, e.g. colchicine (see page 361) and podophyllotoxin (see page 155).

A major problem associated with the clinical use of vinblastine and vincristine is that only very small amounts of these desirable alkaloids are present in the plant. Although the total alkaloid content of the leaf can reach 1% or more, over 500 kg

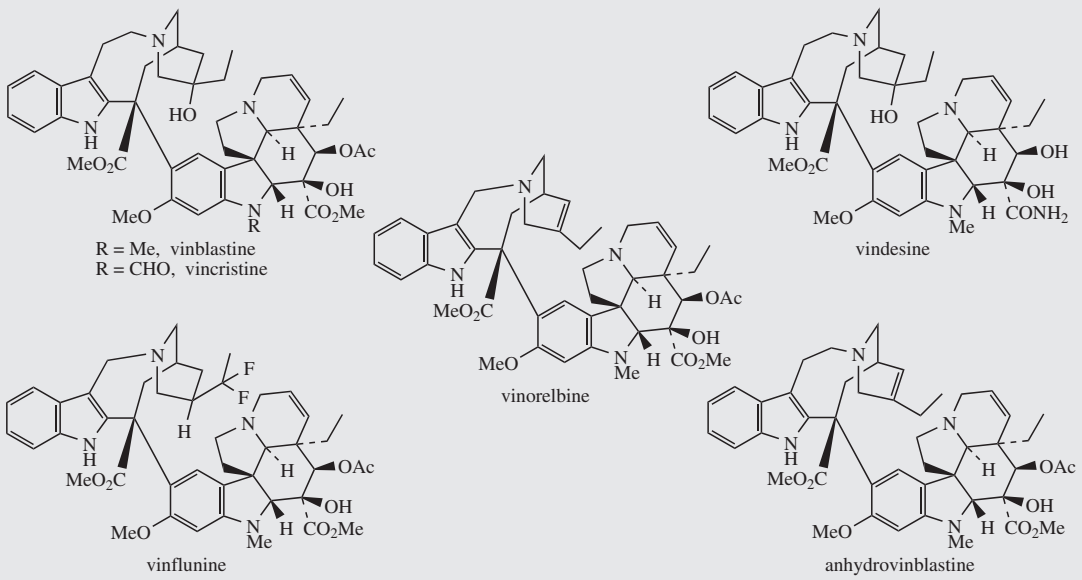


Figure 6.85

Box 6.13 (continued)

of catharanthus is needed to yield 1 g of vincristine. This yield (0.0002%) is the lowest of any medicinally important alkaloid isolated on a commercial basis. Extraction is both costly and tedious, requiring large quantities of raw material and extensive use of chromatographic fractionations. The plant also produces a much higher proportion of vinblastine than vincristine, and the latter drug is medicinally more valuable. Fortunately, it is possible to convert vinblastine into vincristine by controlled chromic acid oxidation, or by chemical formylation of demethylvinblastine. The latter compound occurs naturally or can be obtained from vinblastine via a microbiological *N*-demethylation using *Streptomyces albogriseolus*. Considerable effort has been expended on the semi-synthesis of the 'dimeric' alkaloids from 'monomers' such as catharanthine and vindoline, which are produced in *Catharanthus roseus* in much larger amounts. Efficient, stereospecific coupling has eventually been achieved, and it is now possible to convert catharanthine and vindoline into vinblastine in about 40% yield, though reliance on the natural monomers restricts its commercial application. Excellent yields of anhydrovinblastine (Figure 6.85) can also be obtained by electrochemical oxidation of a catharanthine–vindoline mixture; coupling can also be accomplished enzymically using commercial horseradish peroxidase. This is the starting material for vinorelbine production. This drug (noranhydrovinblastine) was the unexpected product from the attempted conversion of anhydrovinblastine into vinblastine, which resulted in loss of a carbon atom. Further testing demonstrated its drug potential.

Total synthesis is beginning to improve the supply of these alkaloids and derivatives, and also allow more detailed studies of structure–activity relationships to be undertaken. Synthesis is currently the approach being used to maintain supplies. So far, attempts to manipulate alkaloid levels in the plant, or in plant cell cultures, have had limited success, and metabolic engineering has yet to make a significant impact. Though several *Catharanthus roseus* biosynthetic genes have been overexpressed in other plants, overexpression in the host plant has only increased alkaloid production marginally. Heterologous constructs may provide greatest potential. Thus, *Catharanthus roseus* genes encoding strictosidine synthase and strictosidine glucosidase (Figure 6.80) have been expressed in yeast, and the enzymes were found in the culture medium and the cells respectively. When the culture was supplied with secologanin and tryptamine, yeast produced high levels of strictosidine in the medium; upon breaking the cells, strictosidine was then converted into cathenamine by the action of the released strictosidine glucosidase enzyme. In due course it may prove possible to construct even more of the plant metabolic pathway in a microbial host.

This group of compounds is still of very high interest, and development programmes for analogues continue. Indeed, **vinflunine** (Figure 6.85), a fluorinated analogue produced from vinorelbine, is undergoing clinical trials.

Ajmalicine (see rauwolfia, page 372) is present in the roots of *Catharanthus roseus* at a level of about 0.4%, and this plant is used as a commercial source in addition to *Rauwolfia serpentina*.

Iboga

The *Iboga* group of terpenoid indole alkaloids takes its name from *Tabernanthe iboga* (Apocynaceae), a shrub from the Congo and other parts of equatorial Africa. Extracts from the root bark of this plant have long been used by indigenous people in rituals, to combat fatigue, and as an aphrodisiac. The root bark contains up to 6% indole alkaloids, the principal component of which is ibogaine (Figure 6.86). Ibogaine is a central nervous system stimulant, and is also psychoactive. In large doses, it can cause paralysis and respiratory arrest. Ibogaine is of interest as a potential drug for treating cocaine, heroin, and alcohol addiction. A single administration is claimed to cause a reduction in drug withdrawal symptoms, and a significantly reduced drug craving, though it does cause hallucinations. A number of deaths resulting from the unsupervised use of ibogaine have led to it being banned in some countries.

nucleophilic vindoline, C-5 of the indole nucleus being suitably activated by the methoxyl at C-6 and also by the indole nitrogen. The coupled product, another iminium cation, is then reduced in the dihydropyridinium ring to give anhydrovinblastine. This intermediate is known to be transformed into **vinblastine** and **vincristine**. Vincristine, with its *N*-formyl group rather than *N*-methyl on the vindoline fragment, appears be an oxidized product derived from vinblastine.

Further variants on the terpenoid indole alkaloid skeleton (Figure 6.86) are found in **ibogaine** from *Tabernanthe iboga* [Box 6.13], **vincamine** from *Vinca minor*, and **ajmaline** from *Rauwolfia serpentina* [Box 6.12]. Ibogaine is simply a C_9 *Iboga*-type alkaloid, but is of interest as an experimental drug to treat drug addiction. In a number of European countries, vincamine is used clinically as a vasodilator to increase cerebral blood flow in cases of senility, and ajmaline is used to treat cardiac arrhythmias. Ajmaline contains a C_9 *Corynanthe*-type unit, and its relationship to **dehydrogeissoschizine** is indicated in Figure 6.86; most enzyme and gene aspects of the pathway have now been delineated. Vincamine still retains a

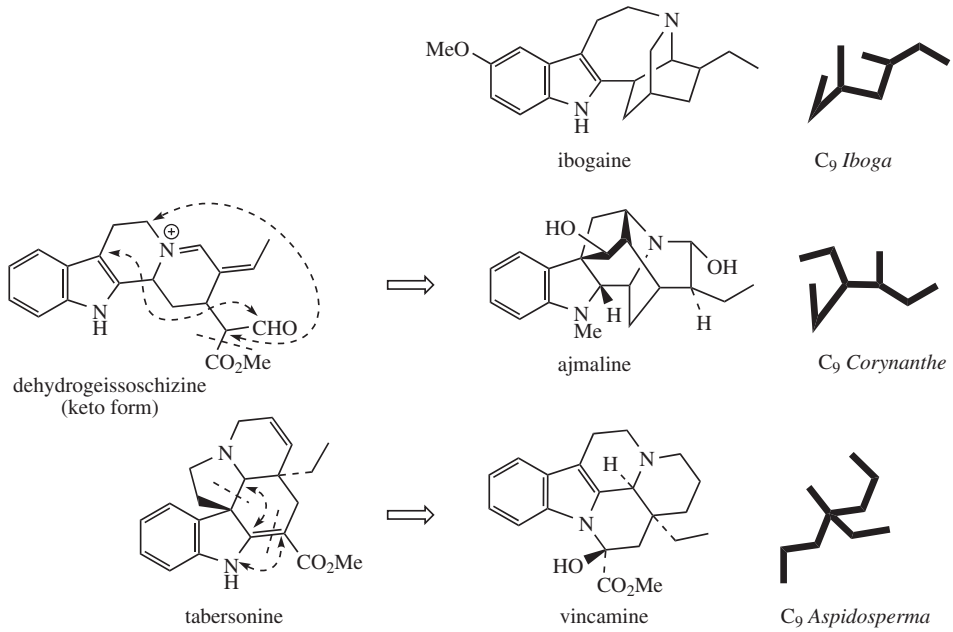


Figure 6.86

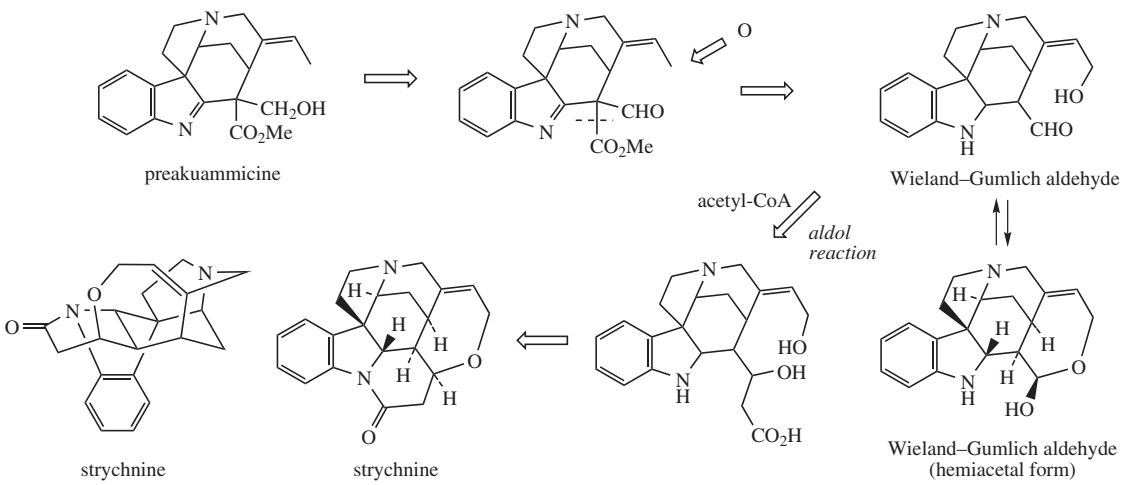


Figure 6.87

C_{10} *Aspidosperma* unit, and it originates from **tabersonine** by a series of reactions that involve cleavage of bonds to both α and β positions of the indole (Figure 6.86).

Alkaloids like **preakuammicine** (Figure 6.83) and **akuammicine** (Figure 6.79) contain the C_{10} and C_9 *Corynanthe*-type terpenoid units respectively. They are, however, representatives of a subgroup of *Corynanthe* alkaloids termed the *Strychnos* type because of their structural similarity to many of the alkaloids found in *Strychnos* species (Loganiaceae), e.g. *Strychnos nux-vomica* [Box 6.14], noteworthy examples being the extremely poisonous **strychnine** (Figure 6.87) and its dimethoxy analogue **brucine** (Figure 6.88). The structures of these natural products are regarded as the most complex possible for

Box 6.14

Nux-vomica

Nux-vomica consists of the dried ripe seeds of *Strychnos nux-vomica* (Loganiaceae), a small tree found in a wide area of East Asia extending from India to northern Australia. The fruit is a large berry with a hard coat and a pulpy interior containing three to five flattish grey seeds. These seeds contain 1.5–5% of alkaloids, chiefly strychnine (about 1.2%; Figure 6.87) and brucine (about 1.6%; Figure 6.88). **Strychnine** is very toxic, affecting the central nervous system and causing convulsions. This is a result of binding to receptor sites in the spinal cord which normally accommodate glycine. Fatal poisoning (consumption of about 100 mg by an adult) would lead to asphyxia following contraction of the diaphragm. It has found use as a vermin killer, especially for moles. Its only medicinal use is in very small doses as an appetite stimulant and general tonic, sometimes with iron salts if the patient is anaemic. Brucine is considerably less toxic. Both compounds have been regularly used in synthetic chemistry as optically active bases to achieve optical resolution of racemic acids. Seeds of the related *Strychnos ignatii* have also served as a commercial source of strychnine and brucine.

Of biochemical interest is the presence of quite significant amounts (up to 5%) of the iridoid glycoside loganin (see page 207) in the fruit pulp of *Strychnos nux-vomica*. This compound is, of course, an intermediate in the biosynthesis of strychnine and other terpenoid indole alkaloids.

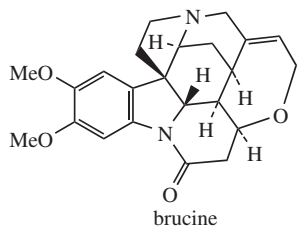


Figure 6.88

compounds of their molecular size. The non-tryptamine portion of these compounds contains 11 carbon atoms, and is constructed from an iridoid-derived C_9 unit plus two further carbon atoms supplied from acetate. The suggested pathway to **strychnine** in Figure 6.87 involves loss of one carbon from a preakuammicine-like structure via hydrolysis/decarboxylation to give the so-called Wieland–Gumlich aldehyde, a demonstrated precursor. The Wieland–Gumlich aldehyde normally exists as a hemiacetal. Addition of the extra two carbon atoms requires aldol condensation with the formyl group, and subsequent formation of strychnine is merely construction of ether and amide linkages.

The arrow poison curare, when prepared from *Chondrodendron* species (Menispermaceae), contains principally the bis-benzyltetrahydroisoquinoline alkaloid tubocurarine (see page 343). Species of *Strychnos*,

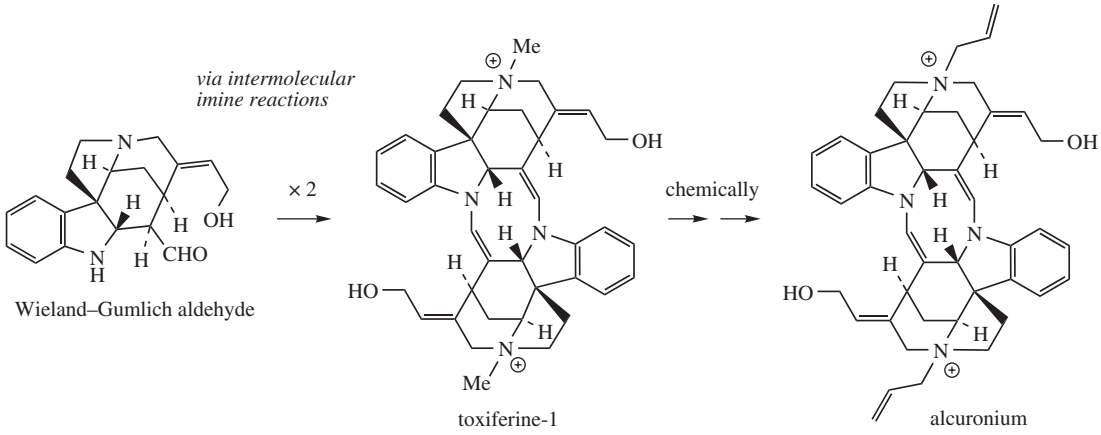


Figure 6.89

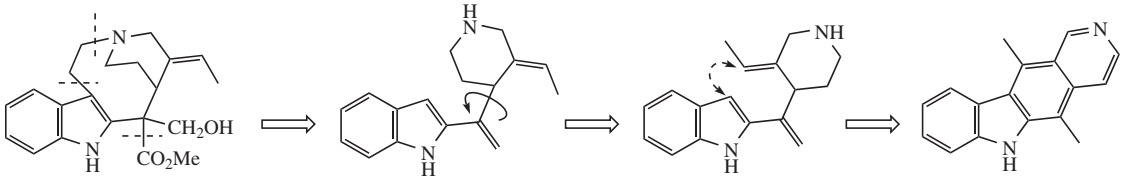


Figure 6.90

especially *Strychnos toxifera*, are employed in making loganiaceous curare, and biologically active alkaloids isolated from such preparations have been identified as a series of toxiferines, e.g. **toxiferine-1** (Figure 6.89). The structures appear remarkably complex, but may be envisaged as a combination of two Wieland–Gumlich aldehyde-like molecules (Figure 6.89). The presence of two quaternary nitrogen atoms, separated by an appropriate distance, is responsible for the curare-like activity (compare tubocurarine and synthetic analogues, page 344). **Alcuronium** (Figure 6.89) is a semi-synthetic skeletal muscle relaxant produced from toxiferine-1 (see curare, page 345).

Ellipticine (Figure 6.90) contains a pyridocarbazole skeleton which is also likely to be formed from a tryptamine–terpenoid precursor. Although little evidence is available, it is suggested that a precursor like **stemmadenine** may undergo transformations that effectively remove the two-carbon bridge originally linking the indole and the nitrogen in tryptamine (Figure 6.90). The remaining C_9 terpenoid fragment now containing the tryptamine nitrogen can then be used to generate the rest of the skeleton. Ellipticine is found in *Ochrosia elliptica* (Apocynaceae) and related species and has useful anticancer properties [Box 6.15].

Box 6.15

Ellipticine

Ellipticine (Figure 6.90) and related alkaloids, e.g. 9-methoxyellipticine (Figure 6.91), are found in the bark of *Ochrosia elliptica* (Apocynaceae) and other *Ochrosia* species. Clinical trials with these alkaloids and a number of synthetic analogues showed them to be potent inhibitors of several cancerous disorders, but preclinical toxicology indicated a number of side-effects, including haemolysis and cardiovascular effects. Ellipticines are planar molecules that intercalate between the base pairs of DNA and cause a partial unwinding of the helical array. Recent research suggests they also inhibit the enzyme topoisomerase II (see page 155). Ellipticine is oxidized *in vivo* mainly to 9-hydroxyellipticine, which has increased activity, and it is believed that this may in fact be the active agent. Poor water solubility of ellipticine and derivatives gave problems in formulation for clinical use, but quaternization of 9-hydroxyellipticine to give the water-soluble 9-hydroxy-2-*N*-methylellipticinium acetate (**elliptinium acetate**; Figure 6.91) has produced a highly active material, of value in some forms of breast cancer and perhaps also in renal cell cancer. Several other quaternized derivatives are being tested, and some water-soluble *N*-glycosides also show high activity.

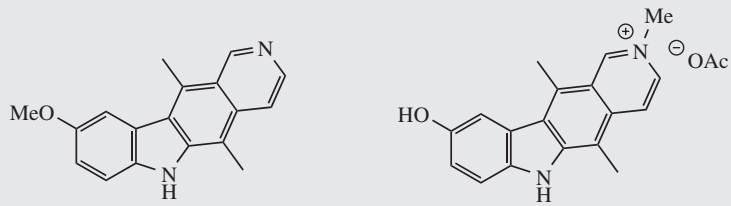


Figure 6.91

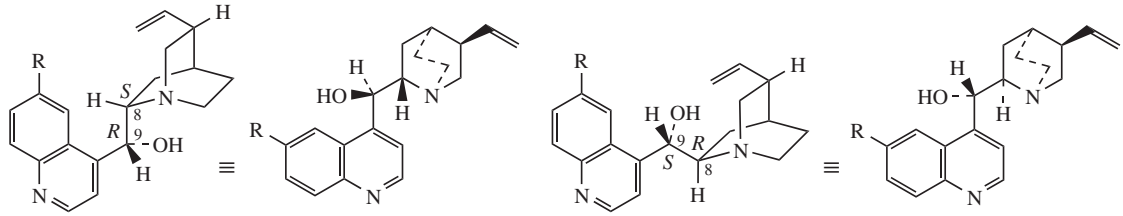


Figure 6.92

Quinoline Alkaloids

Some of the most remarkable examples of terpenoid indole alkaloid modifications are to be found in the genus *Cinchona* (Rubiaceae), in the alkaloids **quinine, quinidine, cinchonidine**, and **cinchonine** (Figure 6.92), long prized for their antimalarial properties [Box 6.16]. These structures are remarkable in that the indole nucleus is no longer present; it has been rearranged into a quinoline system (Figure 6.93). The relationship was suspected quite early on, however, since the indole derivative **cinchonamine** (Figure 6.94) was known to co-occur with these quinoline alkaloids.

An outline of the pathway from the *Corynanthe*-type indole alkaloids to cinchonidine is shown in Figure 6.94. The conversion is dependent on the reversible processes by which amines plus aldehydes or ketones, imines, and amine reduction products of imines are related in nature (see page 19).

Suitable modification of strictosidine leads to an aldehyde (compare the early reactions in the ajmalicine pathway; Figure 6.80). Hydrolysis/decarboxylation would initially remove one carbon from the iridoid portion and produce **corynantheal**; the demonstrated involvement of this compound shows that the methoxycarbonyl group is lost at an early stage. **Cinchonaminal**, an oxidized version of cinchonamine, would result if the tryptamine side-chain were cleaved adjacent to the nitrogen, and if this nitrogen was then bonded to the acetaldehyde function. Ring opening in the indole heterocyclic ring could generate new amine and keto functions. The new quinoline heterocycle would then be formed by combining this amine with the aldehyde produced in the tryptamine side-chain cleavage, giving **cinchonidinone**. The two epimers **cinchonidinone** and **cinchoninone** equilibrate readily in the plant through keto–enol tautomerism. Finally, reduction of the ketone group gives **cinchonidine** or **cinchonine**. Hydroxylation and methylation at some stage allows biosynthesis of **quinine** and **quinidine**.

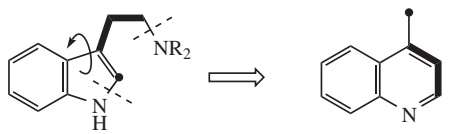


Figure 6.93

Quinine and quinidine, or cinchonidine and cinchonine, are pairs of diastereoisomers, and have opposite chiralities at two centres, C-8 and C-9 (Figure 6.92). The stereochemistry at C-8 is easily reversed by tautomerism in cinchonidinone and cinchoninone as described above. The stereochemistry adjacent to the quinoline ring (C-9) is controlled by the reduction step. An enzyme catalysing the reduction of cinchoninone produces an unequal mixture of cinchonine and cinchonidine, showing that the stereochemistry of reduction may somehow depend upon the substrate (Figure 6.94).

Box 6.16

Cinchona

Cinchona bark is the dried bark from the stem and root of species of *Cinchona* (Rubiaceae), which are large trees indigenous to South America. Trees are cultivated in many parts of the world, including Bolivia, Guatemala, India, Indonesia, Zaire, Tanzania, and Kenya. About a dozen different *Cinchona* species have been used as commercial sources, but the great variation in alkaloid

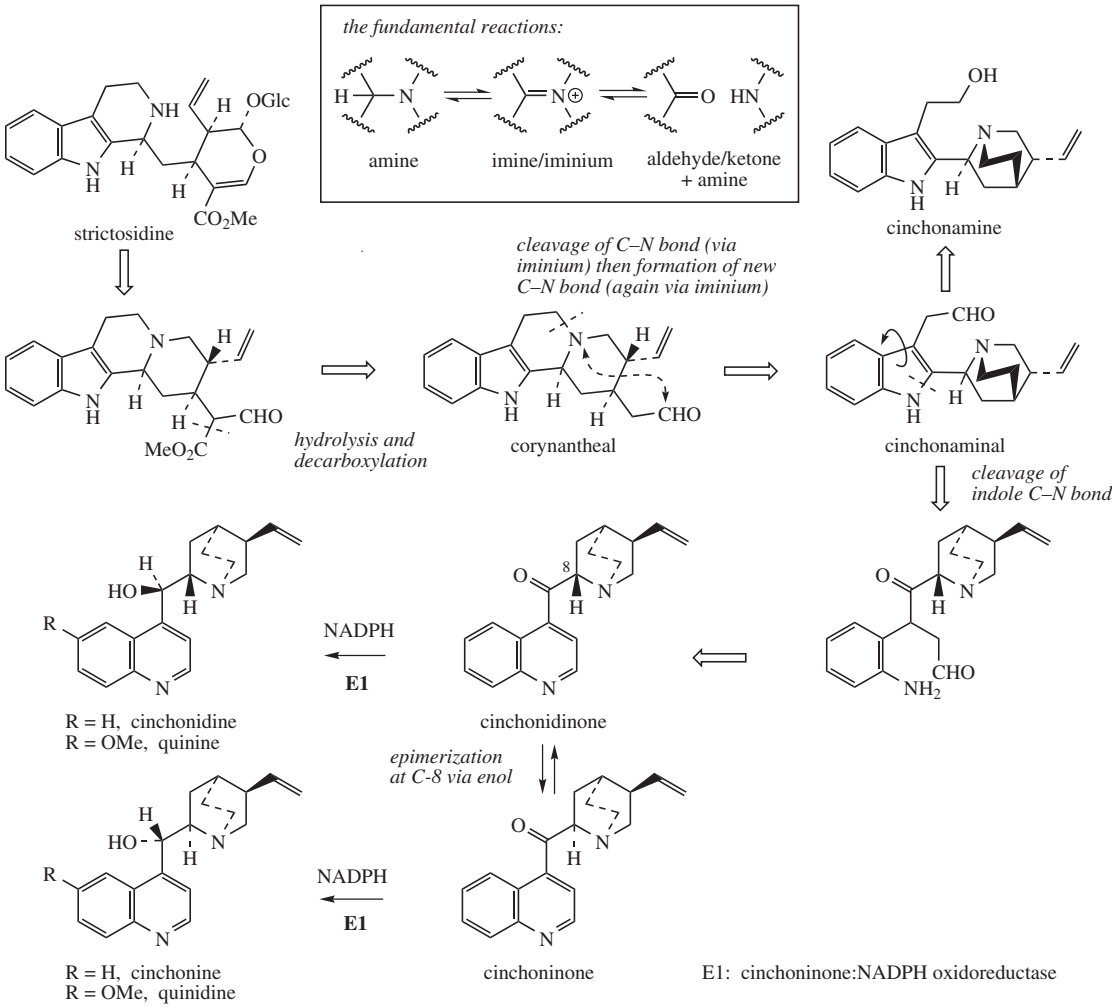


Figure 6.94

Box 6.16 (continued)

content and the range of alkaloids present has favoured cultivation of three main species, together with varieties, hybrids, and grafts. *Cinchona succirubra* provides what is called 'red' bark (alkaloid content 5–7%), *Cinchona ledgeriana* gives 'brown' bark (alkaloid content 5–14%), and *Cinchona calisaya* 'yellow' bark with an alkaloid content of 4–7%. Selected hybrids can yield up to 17% total alkaloids. Bark is stripped from trees which are about 8–12 years old, the trees being totally uprooted by tractor for the process.

A considerable number of alkaloids have been characterized in cinchona bark, four of which account for some 30–60% of the alkaloid content. These are quinine, quinidine, cinchonidine, and cinchonine, quinoline-containing structures representing two pairs of diastereoisomers (Figure 6.92). Quinine and quinidine have opposite configurations at two centres. Cinchonidine and cinchonine are demethoxy analogues of quinine and quinidine respectively; unfortunately, use of the *-id-* syllable in the nomenclature does not reflect a particular stereochemistry. Quinine is usually the major component (half to two-thirds total alkaloid content), but the proportions of the four alkaloids vary according to species or hybrid. The alkaloids are often present in the bark in salt combination with quinic acid (see page 138) or a tannin material called cinchotannic acid. Cinchotannic acid

Box 6.16 (continued)

decomposes due to enzymic oxidation during processing of the bark to yield a red pigment, which is particularly prominent in the 'red' bark.

Cinchona and its alkaloids, particularly **quinine**, have been used for many years in the treatment of malaria, a disease caused by protozoa, of which the most troublesome is *Plasmodium falciparum*. The beneficial effects of cinchona bark were first discovered in South America in the 1630s, and the bark was then brought to Europe by Jesuit missionaries. Religious intolerance initially restricted its universal acceptance, despite the widespread occurrence of malaria in Europe and elsewhere. The name cinchona is a misspelling derived from Chinchon. In an often quoted tale, now historically disproved, the Spanish Countess of Chinchon, wife of the viceroy of Peru, was reputedly cured of malaria by the bark. For many years, the bark was obtained from South America, but cultivation was eventually established by the English in India, and by the Dutch in Java, until just before the Second World War, when almost all the world's supply came from Java. When this source was cut off by Japan in the Second World War, a range of synthetic antimalarial drugs was hastily produced as alternatives to quinine. Many of these compounds were based on the quinine structure. Of the wide range of compounds produced, **chloroquine, primaquine**, and **mefloquine** (Figure 6.95) are important antimalarials. Primaquine is exceptional in having an 8-aminoquinoline structure, whereas chloroquine and mefloquine retain the 4-substituted quinoline as in quinine. The acridine derivative **mepacrine** (Figure 6.95), though not now used for malaria treatment, is of value in other protozoal infections. At one time, synthetic antimalarials had almost entirely superseded natural quinine, but the emergence of *Plasmodium falciparum* strains resistant to the synthetic drugs, especially the widely used inexpensive prophylactic chloroquine, has resulted in reintroduction of quinine. Mefloquine is currently active against chloroquine-resistant strains, but whilst 10 times as active as quinine, it does produce gastrointestinal upsets and dizziness, and it can trigger psychological problems such as depression, panic, or psychosis in some patients. The ability of *Plasmodium falciparum* to develop resistance to modern drugs means malaria still remains a huge health problem, and is probably the major single cause of deaths in the modern world. It is estimated that 200–500 million people are affected by malaria, and some 2–3 million die each year from this disease. **Chloroquine** and its derivative **hydroxychloroquine** (Figure 6.95), although antimalarials, are also used to suppress the disease process in rheumatoid arthritis.

Quinine (Figure 6.92), administered as free base or salts, continues to be used for treatment of multidrug-resistant malaria, though it is not suitable for prophylaxis. The specific mechanism of action is still not thoroughly understood, though it is believed to prevent polymerization of toxic haemoglobin breakdown products formed by the parasite (see artemisinin, page 219). Vastly larger amounts of the alkaloid are consumed in beverages, including vermouth and tonic water. It is amusing to realize that gin was originally added to quinine to make the bitter antimalarial more palatable. Typically, the quinine dosage was up to 600 mg three times a day. Quinine in tonic water is now the mixer added to gin, though the amounts of quinine used (about 80 mg l^{-1}) are well below that providing antimalarial protection. Quinine also has a skeletal muscle relaxant effect with a mild curare-like action. It thus finds use in the prevention and treatment of nocturnal leg cramps, a painful condition affecting many individuals, especially the elderly.

Until recently, **quinidine** (Figure 6.92) was used to treat cardiac arrhymias. It inhibits fibrillation, the uncoordinated contraction of muscle fibres in the heart. However, it is rapidly absorbed by the gastrointestinal tract and overdose can be hazardous, leading to diastolic arrest. This has effectively curtailed its use.

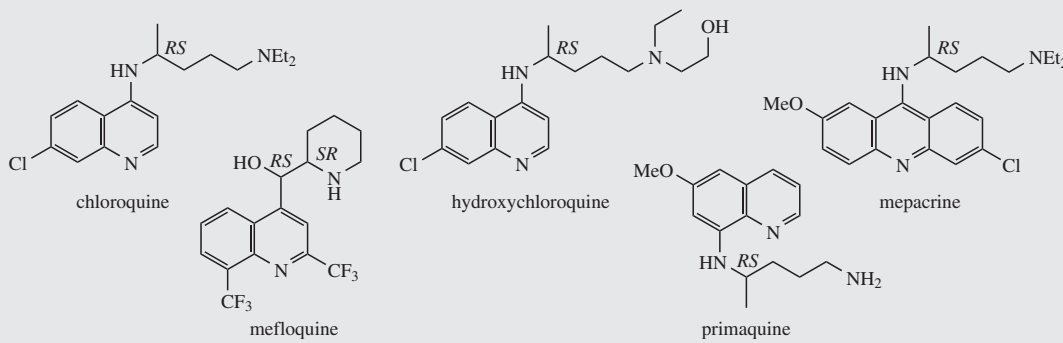


Figure 6.95

Box 6.16 (continued)

Quinidine, cinchonine, and cinchonidine also have antimalarial properties, but these alkaloids are not as effective as quinine. The cardiac effect makes quinidine unsuitable as an antimalarial. However, mixtures of total *Cinchona* alkaloids, even though low in quinine content, are acceptable antimalarial agents. This mixture, termed totaquine, has served as a substitute for quinine during shortages. Quinine-related alkaloids, especially quinidine, are also found in the bark of *Remija pendunculata* (Rubiaceae).
In organic chemistry, quinine and related alkaloids are widely used as chiral ligands for asymmetric synthesis, as resolving agents for acids, and in the elaboration of chiral phases for chromatography.

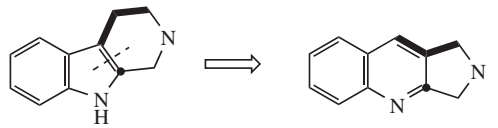


Figure 6.96

Camptothecin (see Figure 6.97) from *Camptotheca acuminata* (Nyssaceae) is a further example of a quinoline-containing structure that is derived in nature by skeletal modification of an indole system. The main rearrangement process is that an original β-carboline 6–5–6 ring system becomes a 6–6–5 pyrroloquinoline by ring expansion of the indole heterocycle (Figure 6.96). In camptothecin, the C_{10} iridoid portion as seen in strictosidine is effectively still intact, and the original ester function is utilized in forming an amide (lactam) linkage to the secondary amine. This occurs relatively early, in that **strictosamide** is an intermediate. An effectively identical reaction is seen in the formation of demethylalangiside in the ipecac alkaloids (see page 362). **Pumiloside** and **deoxypumiloside**, both found in *Ophiorrhiza pumila* (Rubiaceae), another plant producing camptothecin, are potential intermediates. Steps beyond are not yet defined, but involve relatively straightforward oxidation and reduction processes (Figure 6.97). Camptothecin derivatives have provided some useful anticancer drugs [Box 6.17].

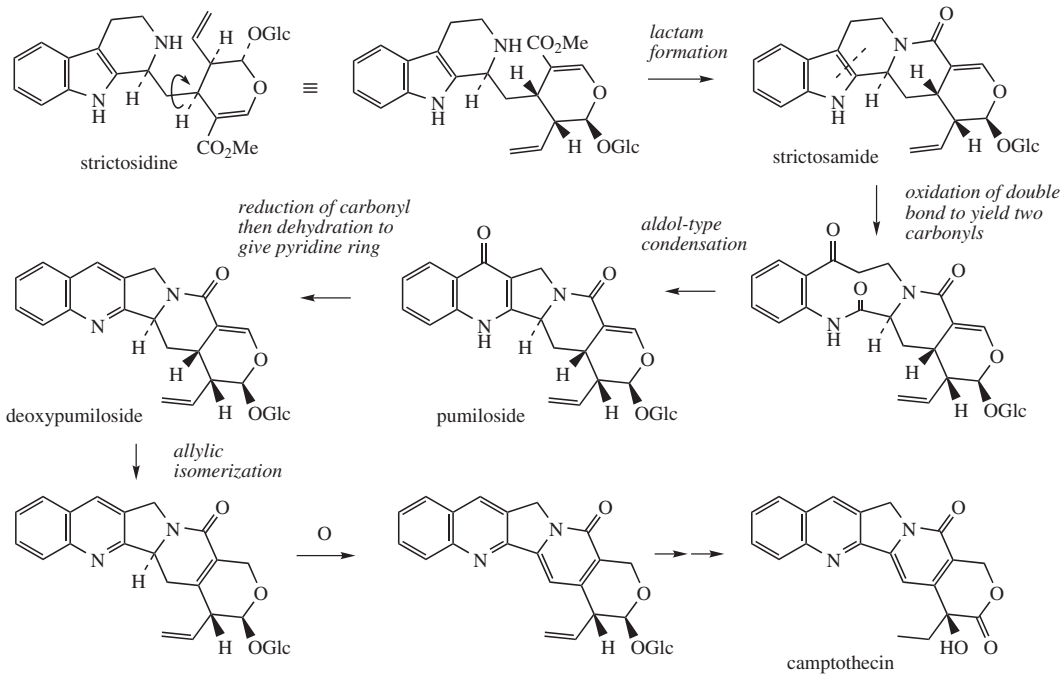


Figure 6.97

Box 6.17

Camptothecin

Camptothecin (Figure 6.97) and derivatives are obtained from the Chinese tree *Camptotheca acuminata* (Nyssaceae). Seeds yield about 0.3% camptothecin, bark about 0.2%, and leaves up to 0.4%. *Camptotheca acuminata* is found only in Tibet and west China, but other sources of camptothecin, such as *Nothapodytes foetida* (formerly *Mappia foetida*) (Icacinaceae), *Merilliodendron megacarpum* (Icacinaceae), *Pyrenacantha klaineana* (Icacinaceae), *Ophiorrhiza pumila* (Rubiaceae), and *Ervatmia heyneana* (Apocynaceae), have been discovered. In limited clinical trials, camptothecin showed broad-spectrum anticancer activity, but toxicity and poor solubility were problems. The natural 10-hydroxycamptothecin (about 0.05% in the bark of *Camptotheca acuminata*) is more active than camptothecin, and is used in China against cancers of the neck and head. Semi-synthetic analogues 9-aminocamptothecin (Figure 6.98) and the water-soluble derivatives **topotecan** and **irinotecan** (Figure 6.98) showed good responses in a number of cancers; topotecan and irinotecan are now available for the treatment of ovarian cancer and colorectal cancer respectively. These drugs are currently made from natural camptothecin, extracted from bark and seeds of *Camptotheca acuminata* and *Nothapodytes foetida*. Irinotecan is a carbamate pro-drug of 10-hydroxy-7-ethylcamptothecin, and is converted into the active drug by liver enzymes. These agents act by inhibition of the enzyme topoisomerase I, which is involved in DNA replication and reassembly, by binding to and stabilizing a covalent DNA–topoisomerase complex (see page 155). Camptothecin has also been shown to have potentially useful activity against pathogenic protozoa such as *Trypanosoma brucei* and *Leishmania donovani*, which cause sleeping sickness and leishmaniasis respectively. Again, this is due to topoisomerase I inhibition.

The camptothecin group of compounds are currently of considerable interest as anticancer agents. Many analogues and pro-drugs have been synthesized; several agents are in clinical trials, and **belotecan** (Figure 6.98) has recently been introduced in Korea. The silicon-containing variant **karenitecin** has shown reduced sensitivity to common tumour-mediated drug resistance mechanisms. The *S*-configuration at C-20 appears essential for activity, but this centre is part of an α-hydroxy-δ-lactone ring that is rapidly hydrolysed under physiological conditions to an open-chain carboxylate form; this is biologically almost inactive. Camptothecin analogues (homocamptothecins) with a seven-membered β-hydroxy-ε-lactone ring retain activity and display enhanced stability towards hydrolysis; **diflomotecan** (Figure 6.94) is a promising drug in this class undergoing clinical trials.

Low concentrations of camptothecin have also been detected in cultures of an unidentified fungus isolated from the inner bark of *Nothapodytes foetida*.

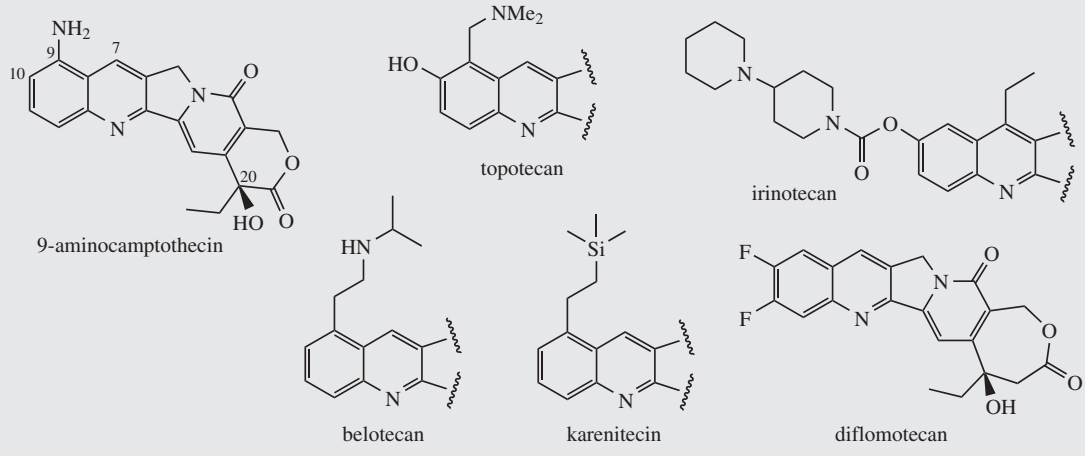


Figure 6.98

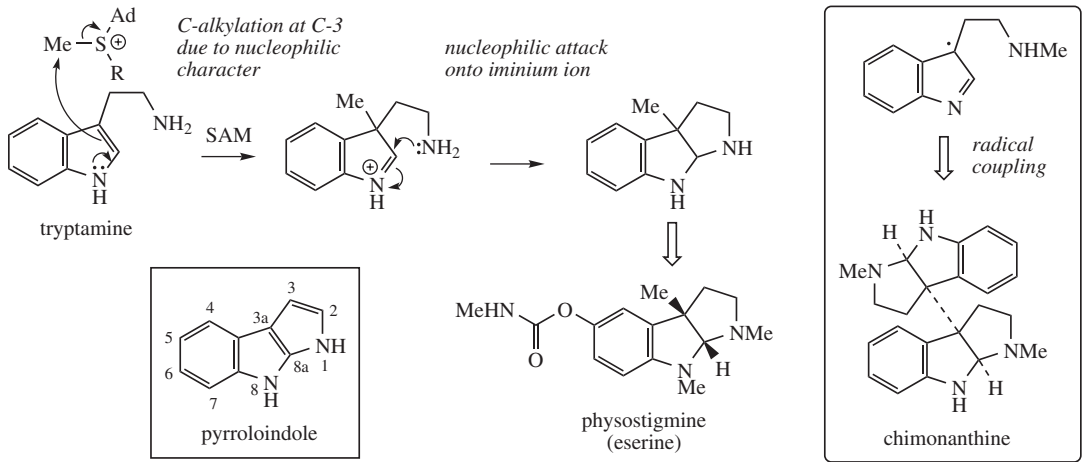


Figure 6.99

Pyrroloindole Alkaloids

Both C-2 and C-3 of the indole ring can be regarded as nucleophilic, but reactions involving C-2 appear to be the most common in alkaloid biosynthesis. There are some examples where the nucleophilic character of C-3 is exploited, however, and the pyrroloindole skeleton typified by **physostigmine** (**eserine**; Figure 6.99) is a likely case. A suggested pathway to physostigmine is by C-3 methylation of tryptamine, followed by ring formation involving attack of the primary amine function onto the iminium ion (Figure 6.95). Further substitution is then necessary. Dimers with this ring system are also known, e.g. **chimonanthine** (Figure 6.99) from *Chimonanthus fragrans* (Calycanthaceae), the point of coupling being C-3 of the indole, and an analogous radical reaction may be proposed. Pyrroloindole alkaloids are quite rare, though examples have been detected in plants, amphibians, and marine algae. Physostigmine is found in seeds of *Physostigma venenosum* (Leguminosae/Fabaceae) and has played an important role in pharmacology because of its anticholinesterase activity [Box 6.18]. The inherent activity is, in fact, derived from the carbamate side-chain rather than the heterocyclic ring system, and this has led to a range of synthetic materials being developed.

Box 6.18

Physostigma

Physostigma venenosum (Leguminosae/Fabaceae) is a perennial woody climbing plant found on the banks of streams in West Africa. The seeds are known as Calabar beans (from Calabar, now part of Nigeria) and have an interesting history in the native culture as an ordeal poison. The accused was forced to swallow a potion of the ground seeds, and if the mixture was subsequently vomited, they were judged innocent and set free. If the poison took effect, the prisoner suffered progressive paralysis and died from cardiac and respiratory failure. It is said that slow consumption allows the poison to take effect, whilst emesis is induced by a rapid ingestion of the dose.

The seeds contain several alkaloids (alkaloid content about 1.5%), the major one (up to 0.3%) being physostigmine (eserine; Figure 6.99). The unusual pyrroloindole ring system is also present in some of the minor alkaloids, e.g. eseramine (Figure 6.100), whilst physovenine (Figure 6.100) contains an undoubtedly related furanoindole system. Another alkaloid, geneserine (Figure 6.101), is an artefact produced by oxidation of physostigmine, probably by formation of an *N*-oxide; in salt form, it retains the pyrroloindole ring system, but under basic conditions it takes up an oxazinoindole form, incorporating the hydroxyl oxygen into the ring system. Interconversion may occur via the indolium cation shown. Solutions of physostigmine are not particularly stable in the presence of air and light, especially under alkaline conditions, oxidizing to a red quinone, rubeserine (Figure 6.101).

Box 6.18 (continued)

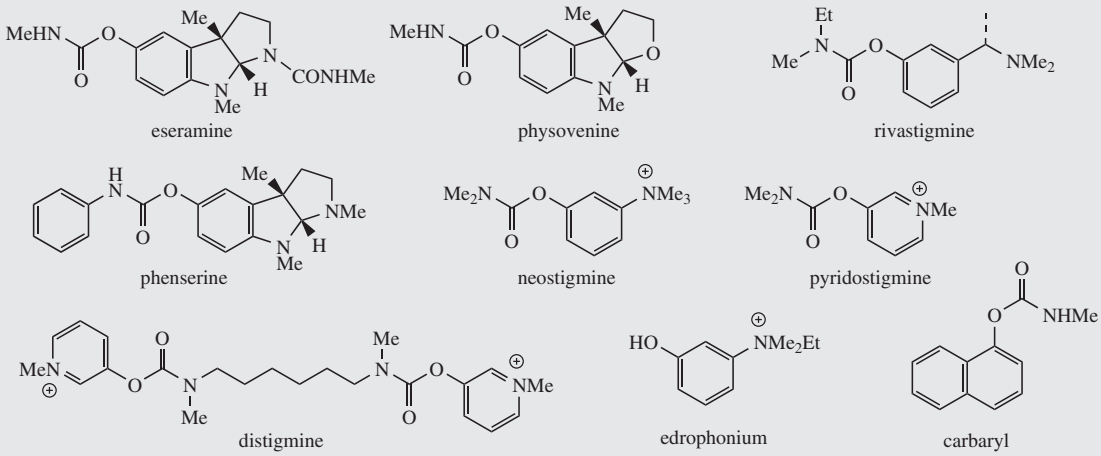


Figure 6.100

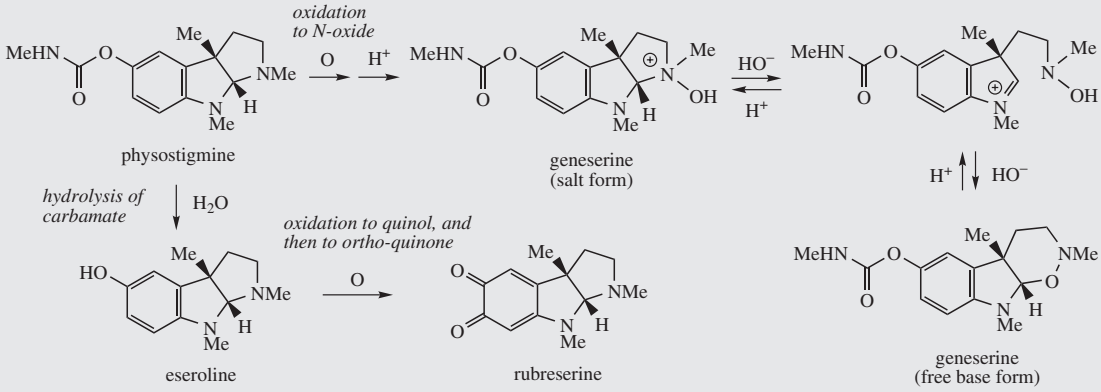


Figure 6.101

Physostigmine (**eserine**) is a reversible inhibitor of acetylcholinesterase, preventing normal destruction of acetylcholine and, thus, enhancing cholinergic activity. Though it is now rarely used as a drug, it has played a pivotal role in investigating the function of acetylcholine as a neurotransmitter. Its major use has been as a miotic, to contract the pupil of the eye, often to combat the effect of mydriatics such as atropine (see page 318). It also reduces intraocular pressure in the eye by increasing outflow of the aqueous humour, and provided a valuable treatment for glaucoma, often in combination with pilocarpine (see page 399). Because it prolongs the effect of endogenous acetylcholine, physostigmine can be used as an antidote to anticholinergic poisons such as hyoscyamine/atropine (see page 318), and it also reverses the effects of competitive muscle relaxants such as curare, tubocurarine, atracurium, etc. (see page 344). Acetylcholinesterase-inhibiting drugs are also of value in the treatment of Alzheimer's disease, which is characterized by a dramatic decrease in functionality of the central cholinergic system. Use of acetylcholinesterase inhibitors can result in significant memory enhancement in patients, and analogues of physostigmine are presently in use (e.g. **rivastigmine**) or have been tested in clinical trials (e.g. **phenserine**; Figure 6.100). These analogues have a longer duration of action, less toxicity, and better bioavailability than physostigmine. Rivastigmine is also used to treat mild to moderate dementia associated with Parkinson's disease.

Box 6.18 (continued)

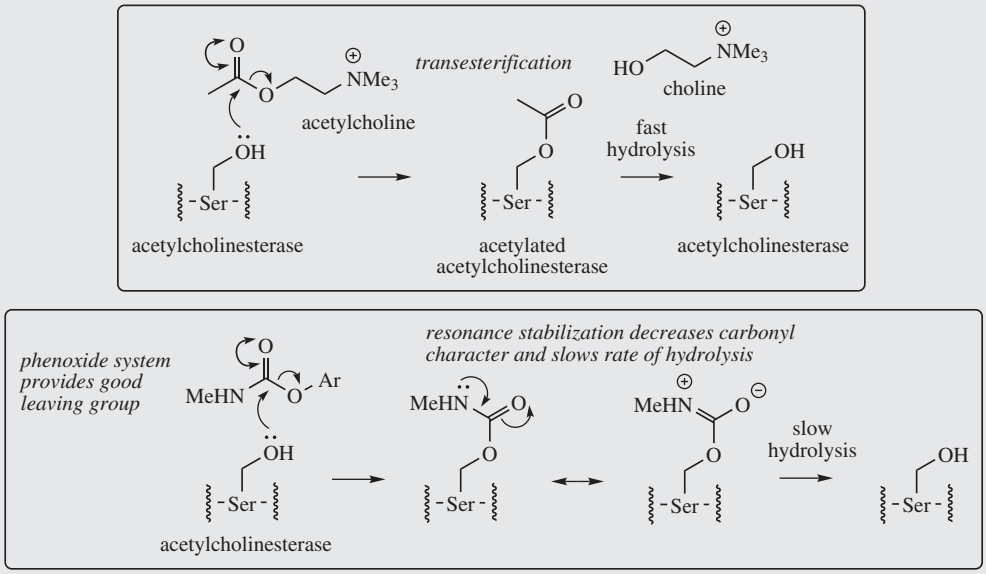


Figure 6.102

The biological activity of physostigmine resides primarily in the carbamate portion, which is transferred to the hydroxyl group of an active site serine in acetylcholinesterase (Figure 6.102). The resultant carbamoyl–enzyme intermediate then hydrolyses only very slowly (minutes rather than microseconds), effectively blocking the active site for most of the time. The slower rate of hydrolysis of the serine carbamate ester is a consequence of decreased carbonyl character resulting from resonance stabilization. Synthetic analogues of physostigmine which have been developed retain the carbamate residue, and an aromatic ring to achieve binding and to provide a good leaving group, whilst ensuring water solubility through possession of a quaternary ammonium system. **Neostigmine, pyridostigmine**, and **distigmine** (Figure 6.100) are examples of synthetic anticholinesterase drugs used primarily for enhancing neuromuscular transmission in the rare autoimmune condition myasthenia gravis, in which muscle weakness is caused by faulty transmission of nerve impulses. **Edrophonium**, though not a carbamate, is a competitive blocker of the acetylcholinesterase active site; it binds to the anionic site of the enzyme, and its action is only very brief. This compound is used mainly for the diagnosis of myasthenia gravis. Neostigmine is also routinely used to reverse the effects of non-depolarizing muscle relaxant drugs, e.g. atracurium and pancuronium (see page 345), after surgery.

A number of carbamate insecticides, e.g. carbaryl (Figure 6.100), also depend on inhibition of acetylcholinesterase for their action, insect acetylcholinesterase being more susceptible to such agents than the mammalian enzyme. Physostigmine displays little insecticidal action because of its poor lipid solubility.

Ergot Alkaloids

Ergot is a fungal disease commonly found on many wild and cultivated grasses that is caused by species of *Claviceps* [Box 6.19]. The disease is eventually characterized by the formation of hard, seed-like 'ergots' instead of normal seeds; these structures, called sclerotia, are the resting stage of the fungus. The poisonous properties of ergots in grain, especially rye, for human or animal consumption have long been recognized, and the causative agents are known to be a group of indole alkaloids, referred to collectively as the ergot alkaloids or ergolines (Figure 6.103). Under natural conditions the alkaloids are elaborated by a combination of fungal and plant metabolisms, but they can be synthesized in cultures of suitable *Claviceps* species. Ergoline alkaloids have also been found in fungi belonging to genera *Aspergillus, Rhizopus*, and *Penicillium*, as well as *Claviceps*, and simple examples are also found in some plants of the Convolvulaceae such as *Ipomoea* and *Rivea* (morning glories),

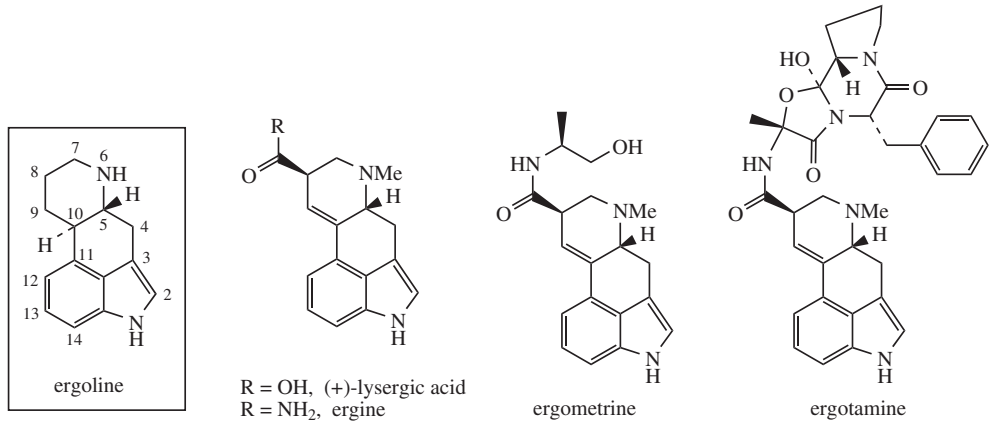


Figure 6.103

though they appear to be of fungal origin [Box 6.19]. Despite their toxicity, some of these alkaloids have valuable pharmacological activities and are used clinically on a routine basis. Medicinally useful alkaloids are derivatives of (+)-**lysergic acid**, which is typically bound as an amide with an amino alcohol as in **ergometrine**, or with a small polypeptide structure as in **ergotamine** (see Figure 6.103).

The building blocks for lysergic acid are tryptophan (less the carboxyl group) and an isoprene unit (Figure 6.104). Alkylation of tryptophan with dimethylallyl diphosphate gives 4-dimethylallyl-L-tryptophan, which then undergoes *N*-methylation (Figure 6.105). Formation of the tetracyclic ring system of lysergic acid is known to proceed through **chanoclavine-I** and **agroclavine**, though the mechanistic details are far from clear, and the enzymes are only partially characterized. Labelling studies have established that the double bond in the dimethylallyl substituent must become a single bond on two separate occasions, allowing rotation to occur as new rings are established. This gives the appearance of *cis–trans* isomerizations as 4-dimethylallyl-L-tryptophan is transformed into

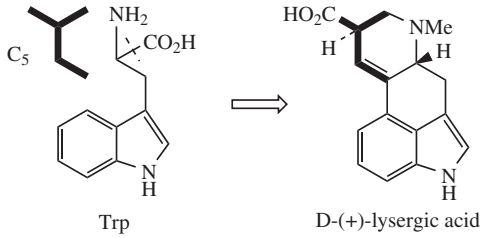
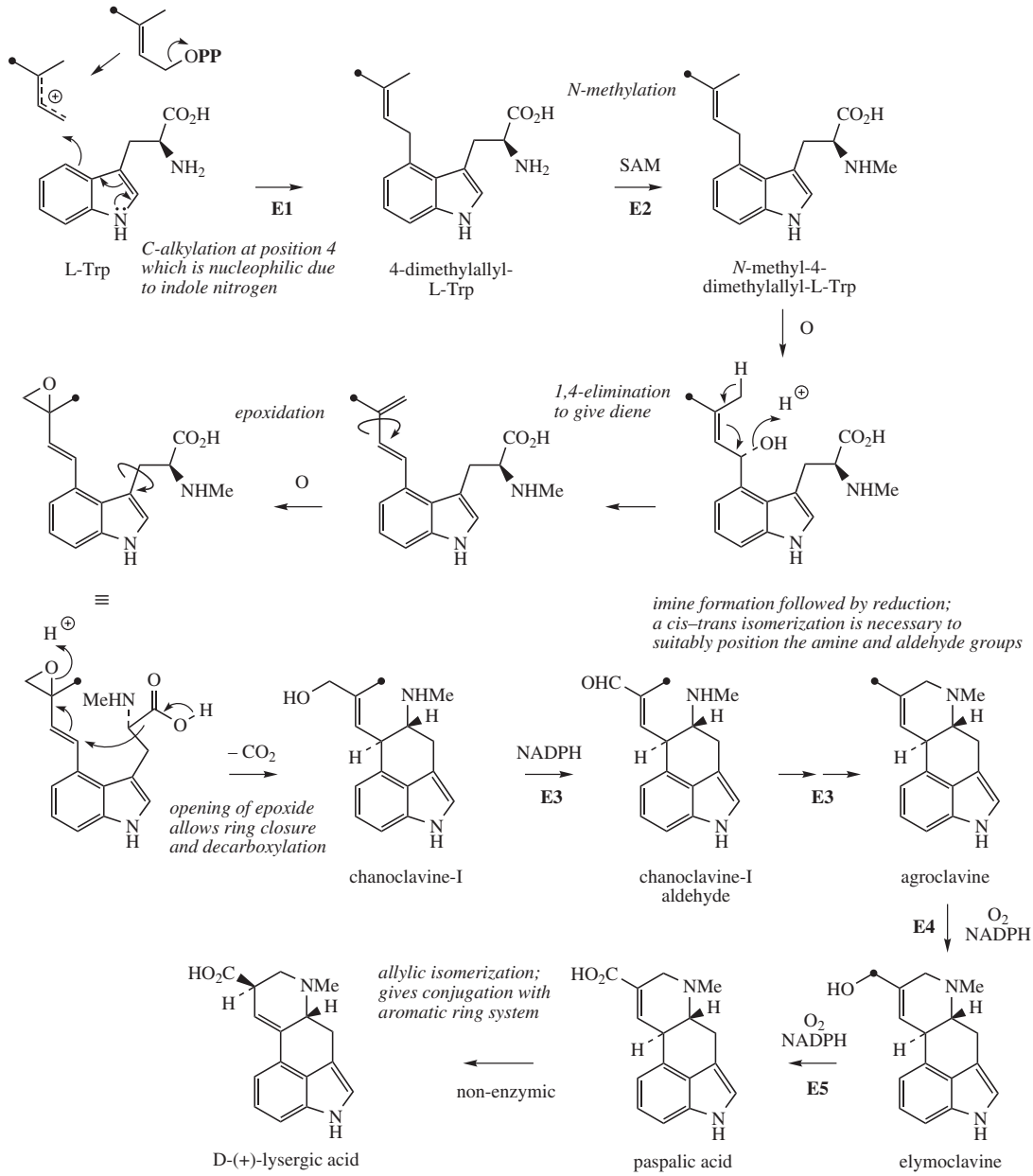


Figure 6.104

chanoclavine-I, and as chanoclavine-I aldehyde cyclizes to agroclavine (Figure 6.105). A suggested sequence to account for the first of these is shown; the second one may involve a substrate–enzyme adduct. In the later stages, agroclavine is hydroxylated to **elymoclavine**, with further oxidation of the primary alcohol to **paspalic acid**, both reactions catalysed by cytochrome P-450 systems. **Lysergic acid** then results from a spontaneous allylic isomerization.

Simple derivatives of lysergic acid require the formation of amides; for example, **ergine** (Figure 6.103) in *Rivea* and *Ipomoea* species is **lysergic acid amide**, whilst **ergometrine** from *Claviceps purpurea* is the amide with 2-aminopropanol. The more complex structures containing peptide fragments, e.g. **ergotamine** (Figure 6.103), are formed by sequentially adding amino acid residues to thioester-bound lysergic acid, giving a linear lysergyl–tripeptide covalently attached to the enzyme complex (Figure 6.106). Peptide formation involves the same processes seen in the non-ribosomal biosynthesis of peptides (see page 438), and is catalysed by a typical non-ribosomal peptide synthase. The enzyme activates lysergic acid and the three amino acids through an ATP-mediated mechanism prior to attachment to the enzyme complex through thioester linkages. A phosphopantetheine arm is used to enable the growing chain to reach the various active sites (compare fatty acid biosynthesis, page 42). The enzyme is known to consist of four modules, each housing domains for adenylation, thiolation, and condensation reactions, and is comprised of two subunits. The smaller subunit is responsible for lysergic acid activation. Cyclization in the tripeptide residue is readily rationalized by the formation of a lactam (amide) which releases the product from the enzyme, followed by hydroxylation of



E1: dimethylallyltryptophan synthase
E2: dimethylallyltryptophan *N*-methyltransferase
E3: chanoclavine-I cyclase
E4: agroclavine 17-monooxygenase
E5: elymocalvine 17-monooxygenase

Figure 6.105

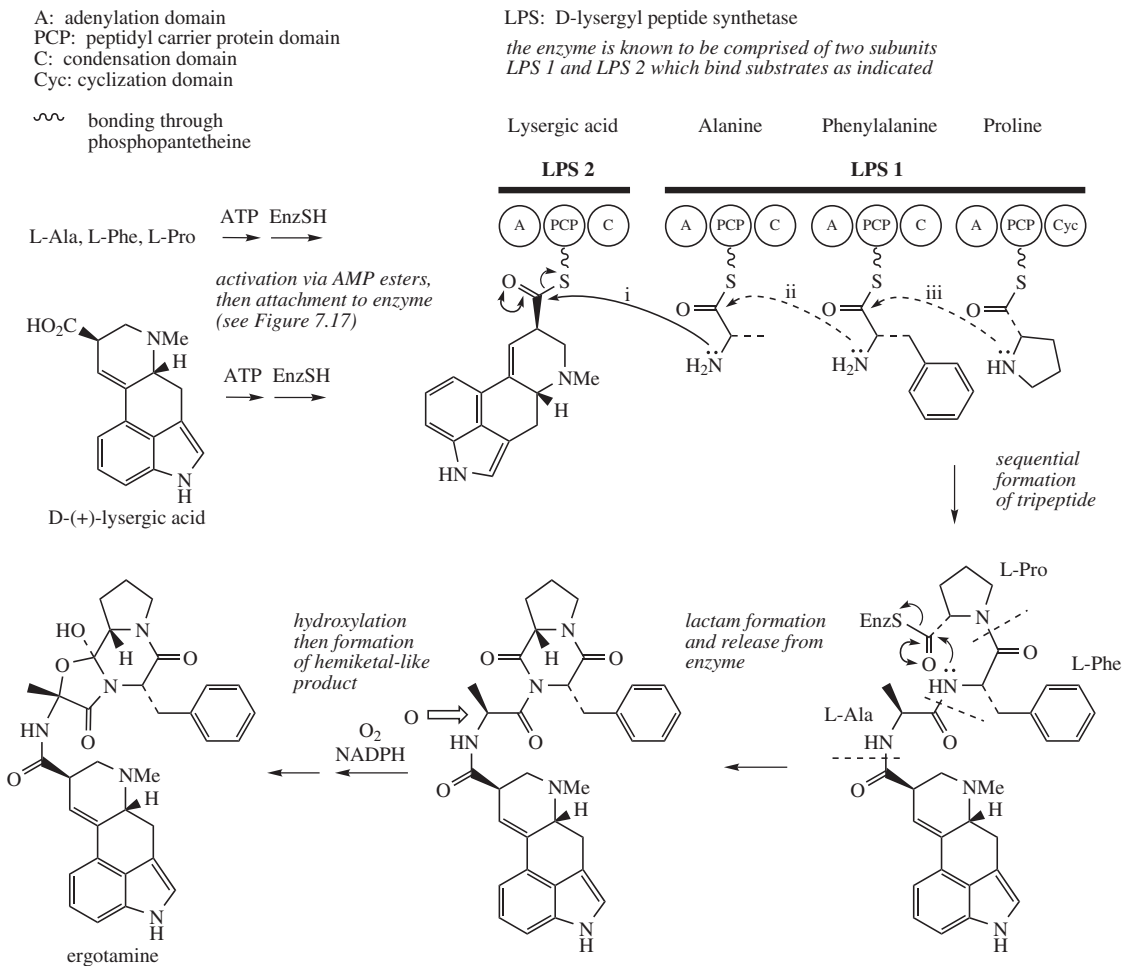


Figure 6.106

the first amino acid of the chain, and generation of a hemiketal-like linkage as shown (Figure 6.106). Precursor studies have shown that the simple amide ergometrine is formed from lysergyl-alanine; this suggests a close similarity with peptide alkaloid biosynthesis, perhaps via a non-ribosomal peptide synthase that adds just a single amino acid.

Box 6.19

Ergot

Medicinal ergot is the dried sclerotium of the fungus *Claviceps purpurea* (Clavicipitaceae) developed on the ovary of rye, *Secale cereale* (Graminae/Poaceae). Ergot is a fungal disease of wild and cultivated grasses, and initially affects the flowers. In due course, a dark sclerotium, the resting stage of the fungus, is developed instead of the normal seed. This protrudes from the seed head, the name ergot deriving from the French word argot (a spur). The sclerotia fall to the ground, germinating in the spring and reinfecting grasses or grain crops by means of spores. Two types of spore are recognized: ascospores, which are formed in

Box 6.19 (continued)

the early stages and are dispersed by the wind, and later on conidiospores are produced, which are insect distributed. The flowers are only susceptible to infection before pollination. Ergots may subsequently be harvested with the grain and contaminate flour or animal feed. The consumption of ergot-infected rye has resulted in the disease ergotism, which has a long, well-documented history.

There are three broad clinical features of ergot poisoning which are due to the alkaloids present and the relative proportions of each component:

- Alimentary upsets, e.g. diarrhoea, abdominal pains, and vomiting.
- Circulatory changes, e.g. coldness of hands and feet due to a vasoconstrictor effect, a decrease in the diameter of blood vessels, especially those supplying the extremeties.
- Neurological symptoms, e.g. headache, vertigo, convulsions, psychotic disturbances, and hallucinations.

These effects usually disappear on removal of the source of poisoning, but much more serious problems develop with continued ingestion, or with doses of heavily contaminated food. The vasoconstrictor effect leads to restricted blood flow in small terminal arteries, death of the tissue, the development of gangrene, and even the shedding of hands, feet, or limbs. Gangrenous ergotism was known as St Anthony's Fire, the Order of St Anthony traditionally caring for sufferers in the Middle Ages. The neurological effects were usually manifested by severe and painful convulsions. Outbreaks of the disease in both humans and animals were relatively frequent in Europe in the Middle Ages, but once the cause had been established, it became relatively simple to avoid contamination. Separation of the ergots from grain, or the use of fungicides during cultivation of the crop, has removed most of the risks, though infection of crops is still common.

The ergot sclerotia contain from 0.15–0.5% alkaloids, and more than 50 have been characterized. The medicinally useful compounds are derivatives of (+)-lysergic acid (Figure 6.107) and can be separated into two groups: the water-soluble amino alcohol derivatives (up to about 20% of the total alkaloids) and water-insoluble peptide derivatives (up to 80% of total alkaloids).

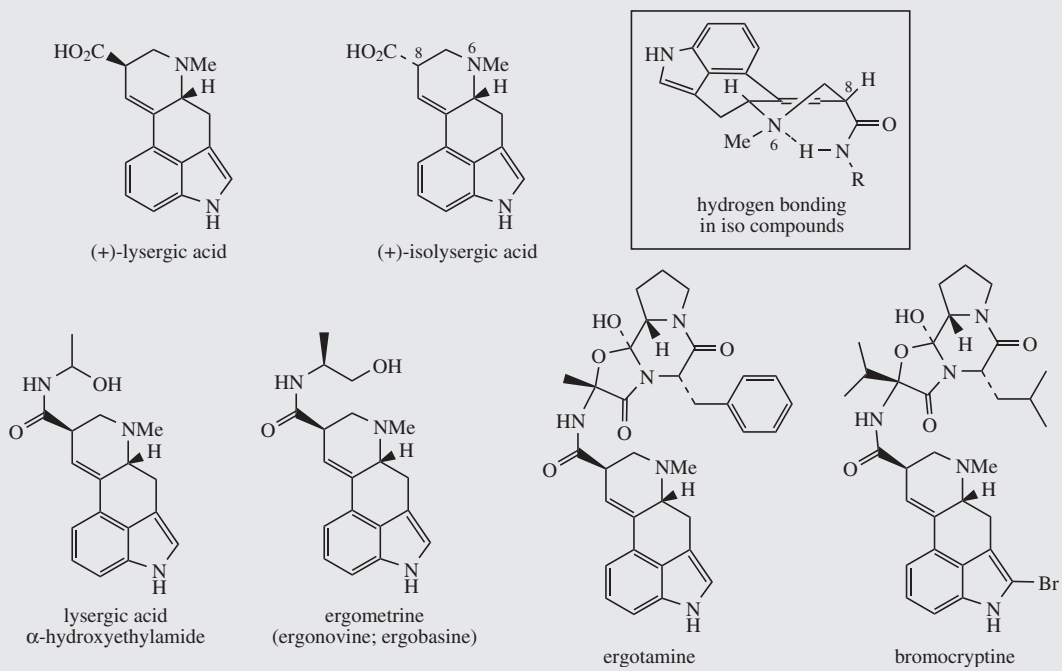


Figure 6.107

Box 6.19 (continued)

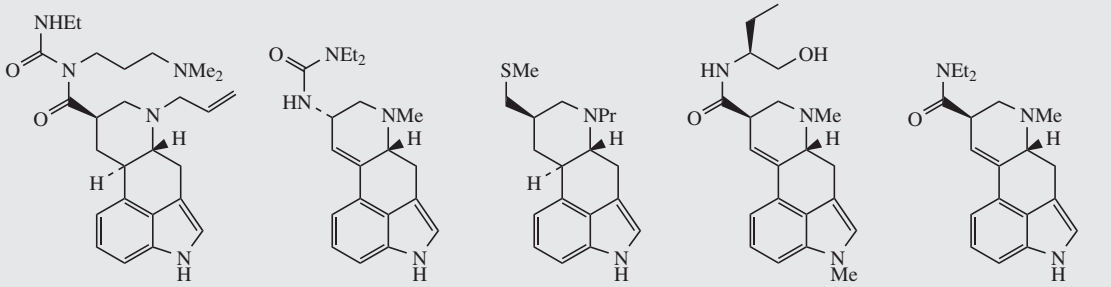
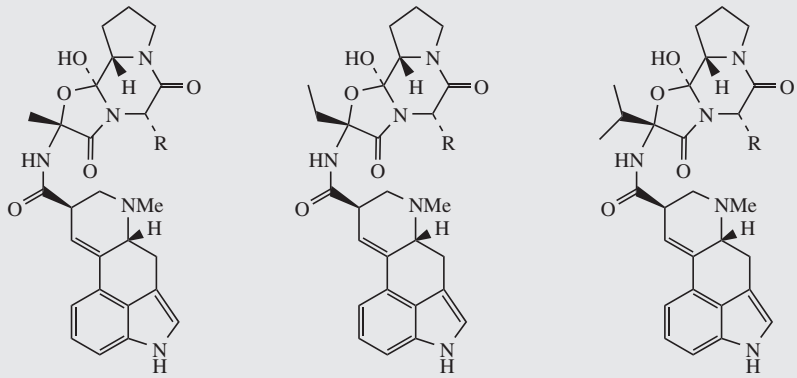


Figure 6.107 (*continued*)

Ergometrine (Figure 6.107), also known as ergonovine in the USA and ergobasine in Switzerland, is an amide of lysergic acid and 2-aminopropanol, and is the only significant member of the first group.

The peptide derivatives contain a cyclized tripeptide fragment bonded to lysergic acid via an amide linkage. Based on the nature of the three amino acids, these structures can be subdivided into three groups: the ergotamine group, the ergoxine group, and the ergotoxine group (Figure 6.108). The amino acids involved are alanine, valine, leucine, isoleucine, phenylalanine, proline,

Peptide alkaloids in Ergot:



	ergotamine group	**ergoxine group**	**ergotoxine group**
R = CH_2Ph	ergotamine	ergostine	ergocristine
R = CH_2CHMe_2	α-ergosine	α-ergoptine	α-ergocryptine
R = *S* CH(Me)Et	[β-ergosine]	[β-ergoptine]	β-ergocryptine
R = $CHMe_2$	ergovaline	ergonine	ergocornine
R = Et	ergobine	ergobutine	ergobutyrine

[ ] not yet known in nature

Figure 6.108

Box 6.19 (continued)

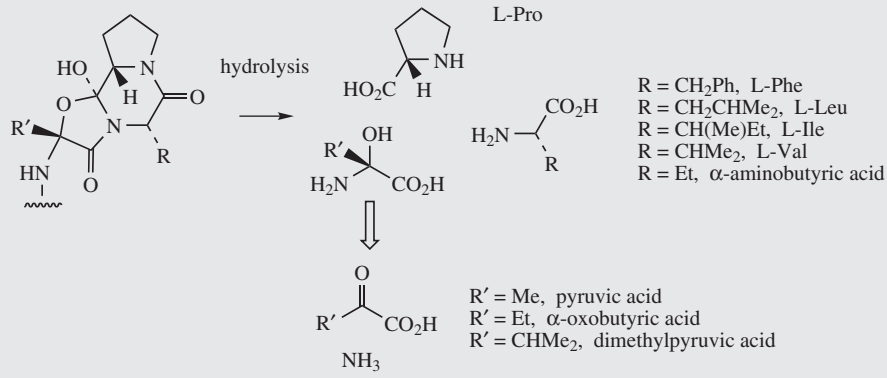


Figure 6.109

and α-aminobutyric acid, in various combinations (see Figure 6.109). All contain proline in the tripeptide, and one of the amino acids is effectively incorporated into the final structure in the form of an α-hydroxy-α-amino acid. Thus, ergotamine incorporates alanine, phenylalanine, and proline residues in its peptide portion. Hydrolysis gives (+)-lysergic acid, proline, and phenylalanine, together with pyruvic acid and ammonia, the latter hydrolysis products a consequence of the additional hydroxylation involving alanine (Figure 6.109). Hydrolysis of the ergotoxine group of alkaloids results in the proximal valine unit being liberated as dimethylpyruvic acid (not systematic nomenclature) and ammonia, and the ergoxine group similarly yields α-oxobutyric acid from the α-aminobutyric acid fragment. The alkaloid 'ergotoxine' was originally thought to be a single compound, but was subsequently shown to be a mixture of alkaloids. The proposed structures β-ergosine and β-ergoptine which complete the combinations shown in Figure 6.108 have not yet been isolated as natural products.

Medicinal ergot is cultivated in the Czech Republic, Germany, Hungary, Switzerland, Austria, and Poland. Fields of rye are infected artificially with spore cultures of *Claviceps purpurea*, either by spraying or by a mechanical process which uses needles dipped in a spore suspension. The ergots are harvested by hand, by machine, or by separation from the ripe grain by flotation in a brine solution. By varying the strain of the fungal cultures, it is possible to maximize alkaloid production (0.4–1.2%), or give alkaloid mixtures in which particular components predominate. Ergots containing principally ergotamine in concentrations of about 0.35% can be cultivated. More recently, ergot of wheat (*Triticum aestivum*) and the wheat–rye hybrid triticale (*Triticosecale*) have been produced commercially; the latter is now the preferred crop for field cultivation.

Alternatively, the ergot alkaloids can be produced by culturing the fungus. Initially, cultures of the rye parasite *Claviceps purpurea* in fermentors did not give the typical alkaloids associated with the sclerotia, e.g. ergometrine and ergotamine. These medicinally useful compounds appear to be produced only in the later stages of development of the fungus. Instead, the cultures produced alkaloids which were not based on lysergic acid, and are now recognized as intermediates in the biosynthesis of lysergic acid, e.g. chanoclavine-I, agroclavine, and elymoclavine (Figure 6.105). Ergot alkaloids which do not yield lysergic acid on hydrolysis have been termed clavine alkaloids. Useful derivatives based on lysergic acid can be obtained by fermentation growth of another fungal species, namely *Claviceps paspali*. Although some strains are available which produce peptide alkaloids in culture, other strains produce high yields of simple lysergic acid derivatives. These include lysergic acid α-hydroxyethylamide (Figure 6.107), lysergic acid amide (ergine; Figure 6.103) which is also an acid-catalysed decomposition product from lysergic acid α-hydroxyethylamide, and the $\Delta^{8,9}$-isomer of lysergic acid, paspalic acid (Figure 6.105). Lysergic acid is obtained from the first two by hydrolysis, or from paspalic acid by allylic isomerization. Other alkaloids, e.g. ergometrine and ergotamine, can then be produced semi-synthetically. High-yielding fermentation methods have also been developed for direct production of ergotamine and the ergotoxine group of peptide alkaloids. About 60% of ergot alkaloid production is now via fermentation.

The pharmacologically active ergot alkaloids are based on (+)-lysergic acid (Figure 6.107), but since one of the chiral centres in this compound (and its amide derivatives) is adjacent to a carbonyl, the configuration at this centre can be changed as a result of enolization brought about by heat or base (compare tropane alkaloids, page 318; again note that enolization is favoured by conjugation with the aromatic ring). The new diastereomeric form of (+)-lysergic acid is (+)-isolysergic acid (Figure 6.107), and alkaloids based on this compound are effectively pharmacologically inactive. They are frequently found along with the (+)-lysergic acid derivatives, in significant amounts if unsuitable isolation techniques are employed or if old ergot samples are

Box 6.19 (continued)

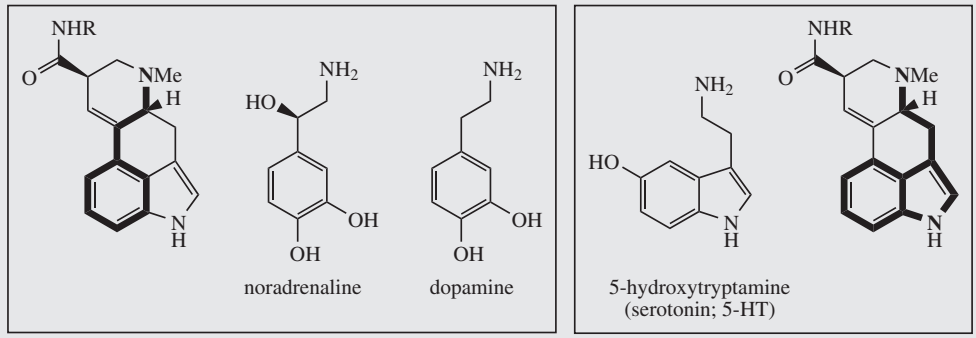


Figure 6.110

processed. In the biologically active lysergic acid derivatives, the amide group occupies an 8-equatorial position, whilst this group is axial in the inactive iso-forms. However, since the tetrahydropyridine ring adopts a half-chair conformation, hydrogen bonding from the amide N–H to the heterocyclic nitrogen at position 6 can occur, and this considerably stabilizes the otherwise unfavourable axial form (see Figure 6.107). Derivatives of (+)-isolysergic acid are named by adding the syllable *–in–* to the corresponding (+)-lysergic acid compound, e.g. ergometr*in*ine, ergotam*in*ine.

The ergot alkaloids owe their pharmacological activity to their ability to act at α-adrenergic, dopaminergic, and serotonergic receptors. The relationship of the general alkaloid structure to those of noradrenaline, dopamine, and 5-HT (serotonin) is shown in Figure 6.110. The pharmacological response may be complex. It depends on the preferred receptor to which the compound binds, though all may be at least partially involved, and on whether the alkaloid is an agonist or antagonist.

Despite the unpleasant effects of ergot as manifested by St Anthony's Fire, whole ergot preparations have been used since the 16th century to induce uterine contractions during childbirth and to reduce haemorrhage following the birth. 'Mothercorn' was a common name for ergot, and is still part of the German language (mutterkorn = ergot). This oxytocic effect (oxytocin is the pituitary hormone that stimulates uterine muscle, see page 430) is still medicinally valuable, but is now achieved through use of the isolated alkaloid ergometrine. The deliberate use of ergot to achieve abortions is dangerous and has led to fatalities.

Ergometrine (**ergonovine**; Figure 6.107) is used as an oxytocic and is injected during the final stages of labour and immediately following childbirth, especially if haemorrhage occurs. Bleeding is reduced because of its vasoconstrictor effects, and it is valuable after Caesarian operations. It is sometimes administered in combination with oxytocin itself (see page 430). Ergometrine is also orally active. It produces faster stimulation of uterine muscle than do the other ergot alkaloids, and probably exerts its effect by acting on α-adrenergic receptors, though it may also stimulate 5-HT receptors.

Ergotamine (Figure 6.107) is a partial agonist of α-adrenoceptors and 5-HT receptors. It is not suitable for obstetric use because it also produces a pronounced peripheral vasoconstrictor action. This property is exploited in the treatment of acute attacks of migraine, where it reverses the dilatation of cranial blood vessels. Ergotamine is effective orally, or by inhalation in aerosol form, and may be combined with caffeine, which is believed to enhance its action.

A number of semi-synthetic lysergic acid derivatives act by stimulation of dopamine receptors in the brain, and are of value in the treatment of neurological disorders such as Parkinson's disease. **Bromocriptine** (2-bromo-α-ergocryptine), **cabergoline, lisuride** (**lysuride**), and **pergolide** (Figure 6.107) are all used in this way. Bromocriptine and cabergoline find wider use, in that they also inhibit release of prolactin by the pituitary and can thus suppress lactation and be used in the treatment of breast tumours. **Methysergide** (Figure 6.107) is a semi-synthetic analogue of ergometrine having a modified amino alcohol side-chain and an *N*-methyl group on the indole ring. It is a potent 5-HT antagonist and, as such, is employed in the prophylaxis of severe migraine headaches, though its administration has to be very closely supervised.

Prolonged treatment with any of the ergot alkaloids is undesirable and it is vital that the clinical features associated with ergot poisoning are recognized. Treatment must be withdrawn immediately if any numbness or tingling develops in the fingers or toes. Side-effects will disappear on withdrawal of the drug, but there have been many cases where misdiagnosis has unfortunately led to foot or toe rot, and the necessity for amputation of the dead tissue.

Undoubtedly the most notorious of the lysergic acid derivatives is lysergide (lysergic acid diethylamide or LSD; Figure 6.107). This widely abused hallucinogen, known as 'acid', is probably the most active and specific psychotomimetic known, and is a mixed agonist–anatagonist at 5-HT receptors, interfering with the normal processes. An effective oral dose is from 30 to 50 μg.

Box 6.19 (continued)

It was synthesized from lysergic acid, and even the trace amounts absorbed during its handling were sufficient to give its creator quite dramatic hallucinations. LSD intensifies perceptions and distorts them. How the mind is affected depends on how the user is feeling at the time, and no two 'trips' are alike. Experiences can vary from beautiful visions to living nightmares, sometimes lasting for days. Although the drug is not addictive, it can lead to schizophrenia and there is danger of serious physical accidents occurring whilst the user is under the influence of the drug.

Morning Glories

Lysergic acid derivatives have also been characterized in the seeds of morning glory (*Ipomoea violacea*), *Turbinia corymbosa* (syn. *Rivea corymbosa*), and other members of the Convolvulaceae. Such seeds formed the ancient hallucinogenic drug Ololiuqui still used by the Mexican Indians in religious and other ceremonies. The active constituent has been identified to be principally ergine (lysergic acid amide; Figure 6.103), and this has an activity about one-twentieth that of LSD, but is more narcotic than hallucinogenic. The alkaloid content of the seeds is usually low, at about 0.05%, but higher levels (0.5–1.3%) have been recorded. Minor ergot-related constituents include ergometrine (Figure 6.107), lysergic acid α-hydroxyethylamide (Figure 6.107), the inactive isolysergic acid amide (erginine), and some clavine alkaloids, e.g. agroclavine and elymoclavine. However, it has now been shown that these alkaloids are not synthesized by the plant itself, but are the product of a plant-associated fungus, transmitted via the seeds of the plant. Plants grown in the presence of systemic fungicides no longer produce ergot alkaloids. *Ipomoea asarifolia, Ipomoea violacea*, and *Turbinia corymbosa* all accumulate ergot alkaloids via associated fungi.

Since morning glories are widely cultivated ornamentals and seeds are readily available, deliberate ingestion by thrill-seekers has been considerable. Although the biological activity is well below that of LSD, the practice is potentially dangerous.

ALKALOIDS DERIVED FROM ANTHRANILIC ACID

Anthranilic acid (Figure 6.111) is a key intermediate in the biosynthesis of L-tryptophan (see page 145) and so contributes to the elaboration of indole alkaloids. During this conversion, the anthranilic acid residue is decarboxylated, so that only the C_6N skeleton is utilized. However, there are also many examples of where anthranilic acid itself functions as an alkaloid precursor, using processes which retain the full skeleton and exploit the carboxyl group (Figure 6.111). It should also be appreciated that, in mammals, L-tryptophan can be degraded back to anthranilic acid (see page 332), but this is not a route of importance in plants.

Quinazoline Alkaloids

Peganine (Figure 6.112) is a quinazoline alkaloid found in *Peganum harmala* (Zygophyllaceae) where it co-occurs with the β-carboline alkaloid harmine (see page 369). It is also responsible for the bronchodilator activity of *Justicia adhatoda* (*Adhatoda vasica*) (Acanthaceae), a plant used in the treatment of respiratory ailments. As a result, the alternative name **vasicine** is also sometimes used for peganine. Studies in *Peganum harmala* have clearly demonstrated peganine to be derived from anthranilic acid, the remaining part of the structure being a pyrrolidine ring supplied by ornithine (see page 313). The peganine skeleton is readily rationalized as a result of nucleophilic attack from the anthranilate nitrogen onto the pyrrolinium cation, followed by amide (lactam) formation (Figure 6.112). Remarkably, this pathway is not operative in *Justicia adhatoda*, and a much less predictable sequence from *N*-acetylanthranilic acid and aspartic acid is observed (Figure 6.112). **Bromhexine** (Figure 6.112) is an expectorant used in veterinary practice developed from the structure of peganine.

Febrifugine (Figure 6.113) is also a quinazoline alkaloid, though no details about its biosynthetic origins are

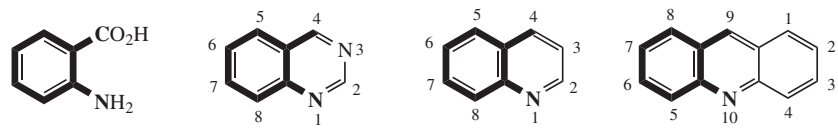


Figure 6.111

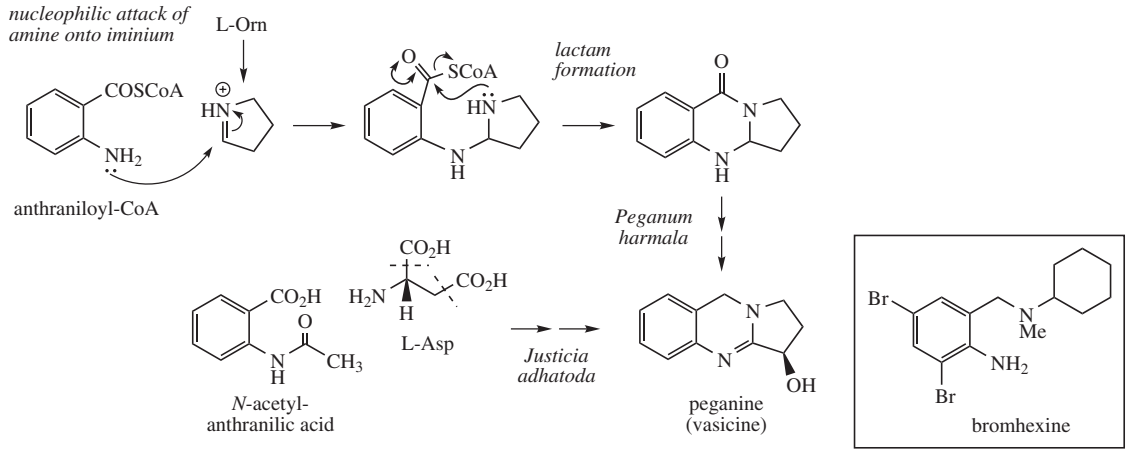


Figure 6.112

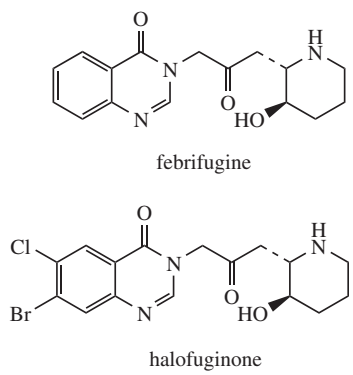


Figure 6.113

available. This alkaloid was isolated from the roots of a Chinese plant *Dichroa febrifuga* (Saxifragaceae), traditionally employed in the treatment of malaria fevers. It is also found in the unrelated *Hydrangea umbellata* (Hydrangeaceae). Febrifugine has powerful antimalarial activity, some 100–200 times greater than that of quinine (see page 382), but unacceptable side-effects, including liver toxicity and strong emetic properties, have precluded its use as a drug. It is currently a template for development of safer synthetic analogues. The halogenated derivative **halofuginone** (Figure 6.113) shows particular promise; it is already widely used as a coccidiostat for poultry, though it has potential for control of diseases involving excessive collagen synthesis, scleroderma, and certain types of cancer.

Quinoline and Acridine Alkaloids

Alkaloids derived from anthranilic acid undoubtedly occur in greatest abundance in plants from the Rutaceae family. Particularly well represented are alkaloids based on quinoline and acridine skeletons (Figure 6.111). Some quinoline alkaloids, such as quinine and camptothecin, have been established to arise by fundamental rearrangement of indole systems and have their origins in tryptophan (see pages 380, 383). A more direct route to the quinoline ring system is by the combination of anthranilic acid and acetate/malonate, and an extension of this process also accounts for the origins of the acridine ring system (see Figure 3.91, page 117). Thus, anthraniloyl-CoA (Figure 6.114) can act as a starter unit for chain extension via one molecule of malonyl-CoA, and amide (lactam) formation generates the heterocyclic system, which will adopt the more stable 4-hydroxy-2-quinolone tautomeric form (Figure 6.114). Position 3 is highly nucleophilic and susceptible to alkylation, especially via dimethylallyl diphosphate in the case of these alkaloids. This allows formation of additional six- and five-membered oxygen heterocyclic rings, as seen with other systems, e.g. coumarins and isoflavonoids (see pages 164 and 176). By an analogous series of reactions, the dimethylallyl derivative can act as a precursor of furoquinoline alkaloids, such as **dictamnine** and **skimmianine** (Figure 6.114). These alkaloids are found in both *Dictamnus albus* and *Skimmia japonica* (Rutaceae). To simplify the mechanistic interpretation of these reactions, it is more convenient to consider the di-enol form of the quinolone system.

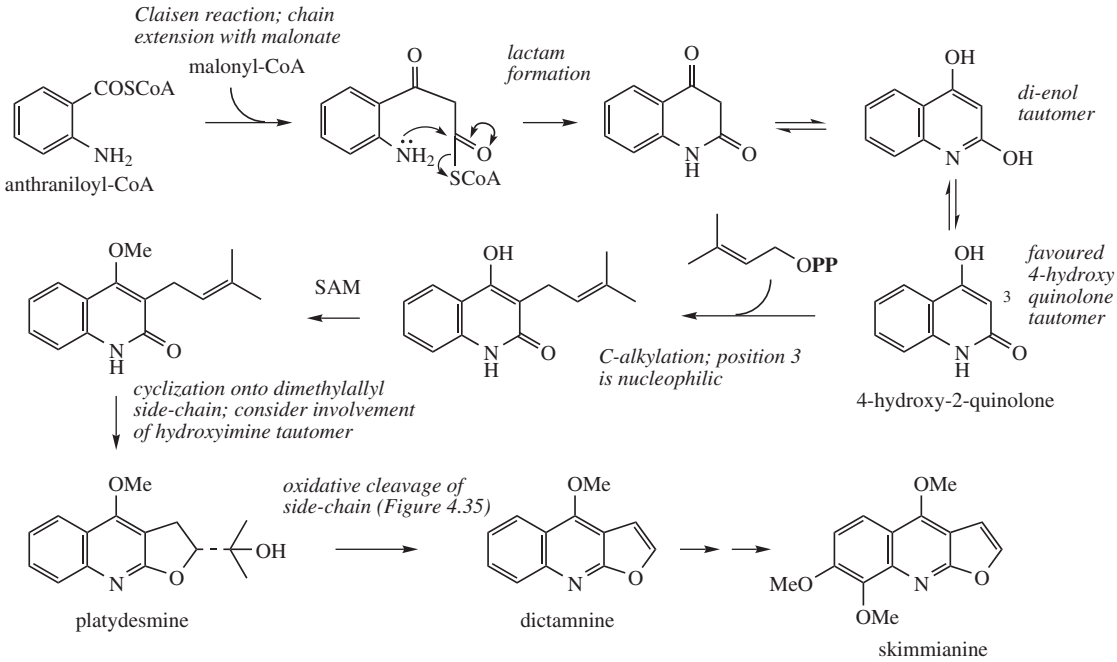


Figure 6.114

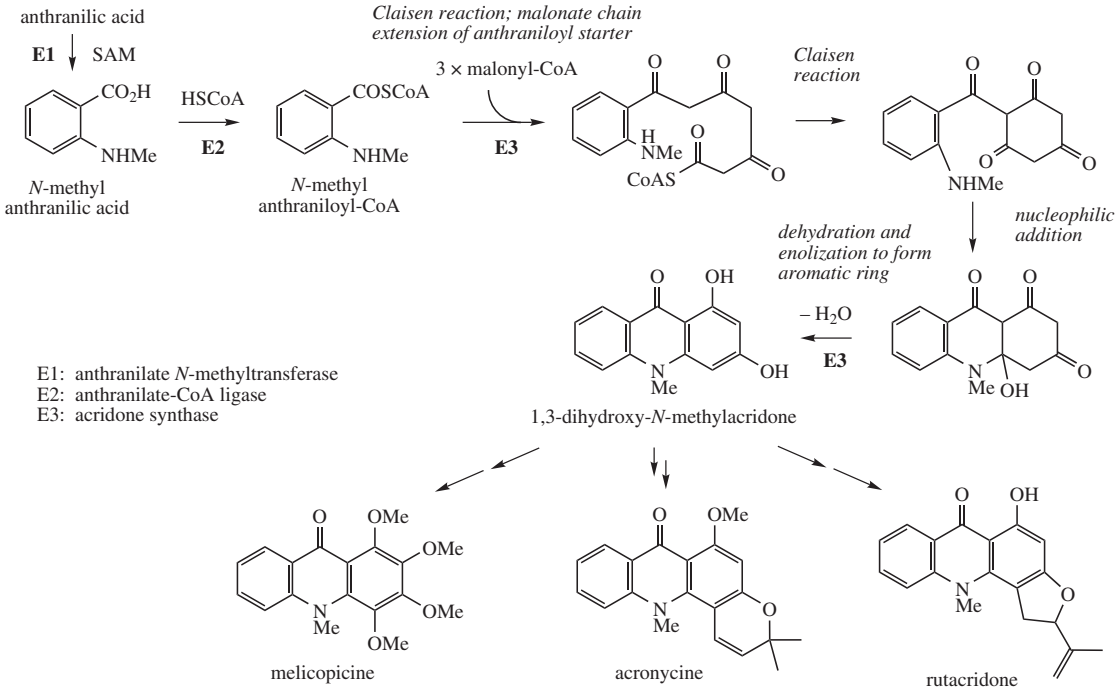


Figure 6.115

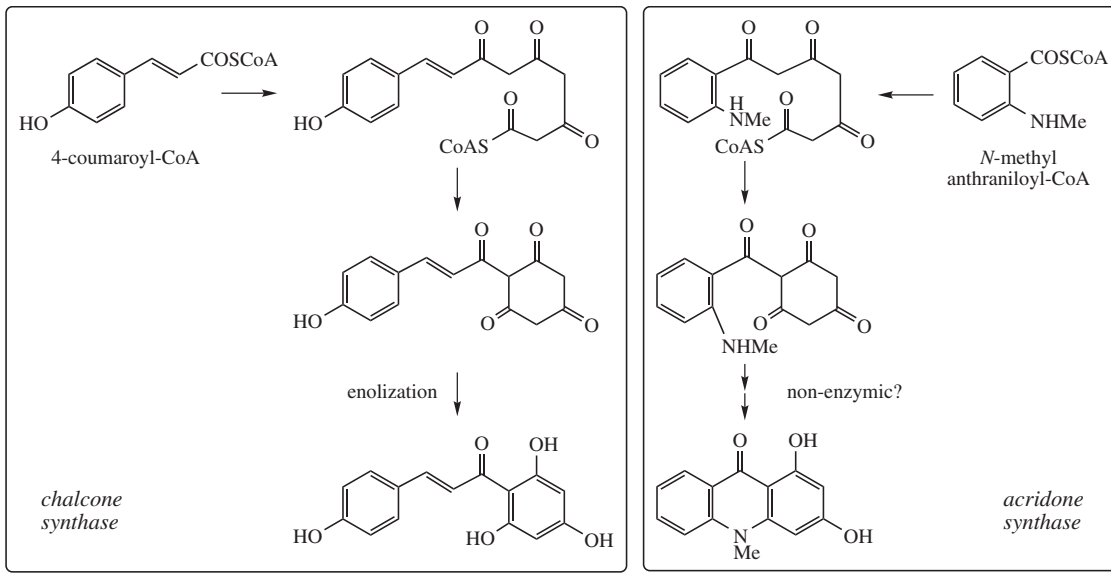


Figure 6.116

Should chain extension of anthraniloyl-CoA (as the *N*-methyl derivative) incorporate three acetate/malonate units, a polyketide would result (Figure 6.115). The acridine skeleton is then produced by sequential Claisen reaction and C–N linkage by an addition reaction, dehydration, and enolization, leading to the stable aromatic tautomer 1,3-dihydroxy-*N*-methylacridone. The enzyme acridone synthase catalyses all these steps and belongs to the family of plant type III polyketide synthases (PKSs). It is closely related structurally to chalcone synthase, the enzyme catalysing chalcone formation from cinnamoyl-CoA and three malonyl-CoA units and involved in flavonoid formation (see page 169) (Figure 6.116). Indeed, acridone synthase also displays a modest chalcone synthase activity, and replacement of amino acids in three critical positions was sufficient to change its activity completely to chalcone synthase, so that it no longer accepted *N*-methylanthraniloyl-CoA as substrate. This may also mean the latter ring formation steps are non-enzymic. In contrast to chalcone synthases, acridone synthase appears to be confined to the Rutaceae family.

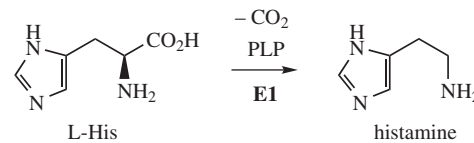
Again, the acetate-derived ring, with its alternate oxygenation, is susceptible to electrophilic attack, and this can lead to alkylation (with dimethylallyl diphosphate) or further hydroxylation. Alkaloids **melicopicine** from *Melicope fareana*, **acronycine** from *Acronychia bauer*i, and **rutacridone** from *Ruta graveolens* (Rutaceae) typify some of the structural variety which may then ensue (Figure 6.115).

ALKALOIDS DERIVED FROM HISTIDINE

Imidazole Alkaloids

The amino acid L-**histidine** (Figure 6.117) contains an imidazole ring, and is thus the likely presursor of alkaloids containing this ring system. There are relatively few examples, however, and definite evidence linking them to histidine is often lacking.

Histamine (Figure 6.117) is the decarboxylation product from histidine and is often involved in human allergic responses, e.g. to insect bites or pollens. Stress stimulates the action of the enzyme histidine decarboxylase and histamine is released from mast cells. It then produces its typical response by interaction with specific histamine receptors, of which there are several types. H_1 receptors are associated with inflammatory and allergic reactions, and H_2 receptors are found in acid-secreting cells in the stomach. The term antihistamine usually relates to H_1 receptor antagonists. Topical antihistamine creams are valuable for



E1: histidine decarboxylase

Figure 6.117

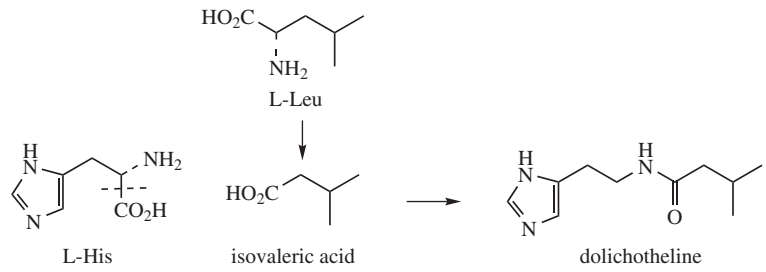


Figure 6.118

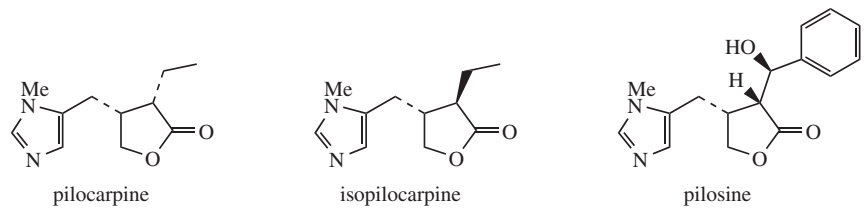


Figure 6.119

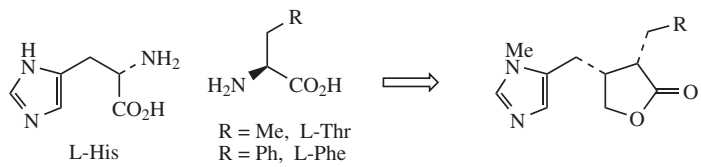


Figure 6.120

pain relief, and oral antihistamines are widely prescribed for nasal allergies such as hay-fever. Major effects of histamine include dilation of blood vessels, inflammation and swelling of tissues, and narrowing of airways. In serious cases, life-threatening anaphylactic shock may occur, caused by a dramatic fall in in blood pressure.

Histidine is a proven precursor of **dolichotheline** (Figure 6.118) in *Dolichothele sphaerica* (Cactaceae), the remaining carbon atoms originating from leucine via isovaleric acid (see page 56). The imidazole alkaloids found in Jaborandi leaves (*Pilocarpus microphyllus* and *Pilocarpus jaborandi*; Rutaceae) are also probably derived from histidine, but experimental data are lacking. Jaborandi leaves contain primarily **pilocarpine** and **pilosine** (Figure 6.119). Pilocarpine is valuable in ophthalmic work as a miotic and as a treatment for glaucoma [Box 6.20]. Additional carbon atoms may originate from acetate or perhaps the amino acid threonine in the case of pilocarpine, whilst pilosine may incorporate a phenylpropane C_6C_3 unit (Figure 6.120).

Box 6.20

Pilocarpus

Pilocarpus or jaborandi consists of the dried leaflets of *Pilocarpus jaborandi, Pilocarpus microphyllus*, or *Pilocarpus pennatifolius* (Rutaceae), small shrubs from Brazil and Paraguay. *Pilocarpus microphyllus* is currently the main source. The alkaloid content (0.5–1.0%) consists principally of the imidazole alkaloid pilocarpine (Figure 6.119), together with small amounts of pilosine

Box 6.20 (continued)

(Figure 6.119) and related structures. Isomers such as isopilocarpine (Figure 6.119) and isopilosine are readily formed if base or heat is applied during extraction of the alkaloids. This is a result of enolization in the lactone ring, followed by adoption of the more favourable *trans* configuration rather than the natural *cis*. However, the iso alkaloids lack biological activity. The alkaloid content of the leaf rapidly deteriorates on storage.

Pilocarpine salts are valuable in ophthalmic practice and are used in eyedrops as miotics and for the treatment of glaucoma. Pilocarpine is a cholinergic agent and stimulates the muscarinic receptors in the eye, causing constriction of the pupil and enhancement of outflow of aqueous humour. The structural resemblance to muscarine and acetylcholine is shown in Figure 6.121. Pilocarpine gives relief for both narrow-angle and wide-angle glaucoma. However, the ocular bioavailability of pilocarpine is low and it is rapidly eliminated, thus resulting in a rather short duration of action. Pilocarpine is antagonistic to atropine (see page 318). Pilocarpine gives relief for dryness of the mouth that is very common in patients undergoing radiotherapy for mouth and throat cancers, and is now prescribed for this purpose.

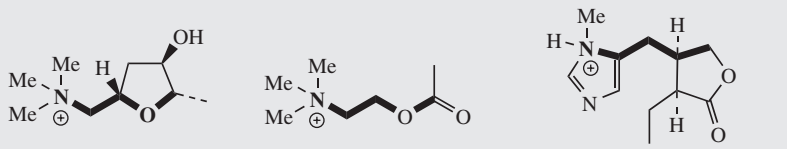
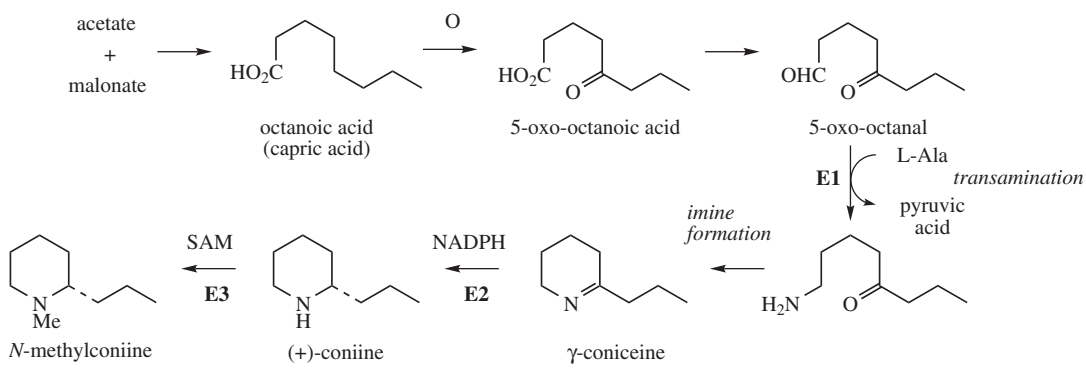


Figure 6.121

ALKALOIDS DERIVED BY AMINATION REACTIONS

The majority of alkaloids are derived from amino acid precursors by processes which incorporate into the final structure the nitrogen atom together with the amino acid carbon skeleton or a large proportion of it. Many alkaloids do not conform to this description, however, and are synthesized primarily from non-amino acid precursors, with the nitrogen atom being inserted into the structure at a relatively late stage. The term 'pseudoalkaloid' is sometimes used to distinguish this group. Such structures are frequently based on terpenoid or steroidal skeletons, though some relatively simple alkaloids also appear to be derived by similar late amination processes. In most of the examples studied, the nitrogen atom is donated from



E1: L-alanine:5-keto-octanal aminotransferase
E2: γ-coniceine reductase
E3: coniine *N*-methyltransferase

Figure 6.122

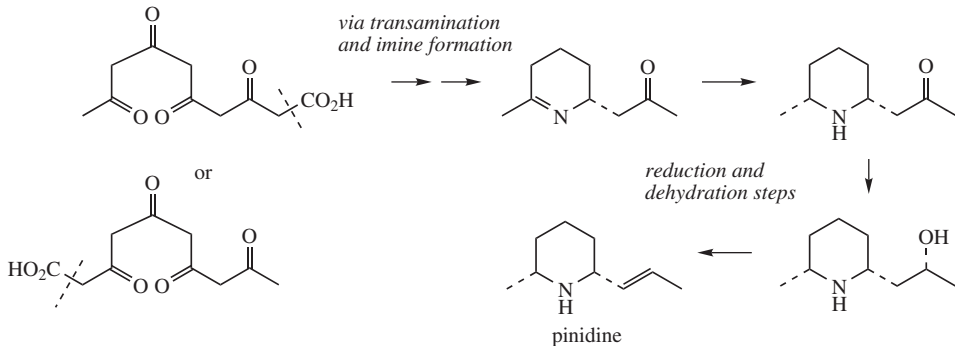


Figure 6.123

an amino acid source through a transamination reaction with a suitable aldehyde or ketone (see page 20).

Acetate-derived Alkaloids

The poison hemlock (*Conium maculatum*; Umbelliferae/Apiaceae) accumulates a range of simple piperidine alkaloids, e.g. **coniine** and **γ-coniceine** (Figure 6.122) [Box 6.21]. These alkaloids would appear to be related to simple lysine-derived compounds such as pelletierine (see page 327); surprisingly, however, a study of their biosynthetic origins excluded lysine as a precursor, and demonstrated instead the sequence shown in Figure 6.122. A fatty acid precursor, capric (octanoic) acid, is utilized, and this is transformed into 5-oxo-octanal by successive oxidation and reduction steps. This ketoaldehyde is then the substrate for a transamination reaction, the amino group originating from L-alanine. Subsequent transformations are imine formation, giving the heterocyclic ring of γ-coniceine, reduction to **coniine**, then methylation to ***N*-methylconiine. Pinidine** (Figure 6.123) from *Pinus* species is found to have a rather similar origin in acetate, and most likely a poly-β-keto acid. During the sequence outlined in Figure 6.123, the carboxyl group is lost. Note that an alternative folding of the poly-β-keto acid and loss of carboxyl might be formulated.

Box 6.21

Conium maculatum

Conium maculatum (Umbelliferae/Apiaceae) or poison hemlock is a large biennial herb indigenous to Europe and naturalized in North and South America. As a common poisonous plant, recognition is important, and this plant can be differentiated from most other members of the Umbelliferae/Apiaceae by its tall, smooth, purple-spotted stems. The dried unripe fruits were formerly used as a pain reliever and sedative, but have no medicinal use now. The ancient Greeks are said to have executed condemned prisoners, including Socrates, using poison hemlock. The poison causes gradual muscular paralysis followed by convulsions and death from respiratory paralysis. All parts of the plant are poisonous due to the alkaloid content, though the highest concentration of alkaloids is found in the green fruit (up to 1.6%). The major alkaloid (about 90%) is the volatile liquid coniine (Figure 6.122), with smaller amounts of structurally related piperidine alkaloids, including *N*-methylconiine and γ-coniceine (Figure 6.122).

In North America, the name hemlock refers to species of *Tsuga* (Pinaceae), a group of coniferous trees, which should not be confused with the poison hemlock.

Phenylalanine-derived Alkaloids

Whilst the aromatic amino acid L-tyrosine is a common and extremely important precursor of alkaloids (see page 336), L-**phenylalanine** is less frequently utilized; usually it contributes only carbon atoms, e.g. C_6C_3, C_6C_2 or C_6C_1 units, without providing a nitrogen atom from its amino group (see colchicine, page 360, lobeline, page 327, etc.). **Ephedrine** (Figure 6.124), the main alkaloid in species of *Ephedra* (Ephedraceae) and a valuable nasal decongestant and bronchial dilator, is a prime example [Box 6.22].

Whilst ephedrine contains the same carbon and nitrogen skeleton as seen in phenylalanine, and L-phenylalanine is a precursor, only seven carbon atoms, a C_6C_1 fragment, are actually incorporated. It is found that phenylalanine is metabolized, probably through cinnamic acid to benzoic acid (see page 157), and this, perhaps as its coenzyme A ester, is acylated with pyruvate, decarboxylation occurring during the addition (Figure 6.124).

The use of pyruvate as a nucleophilic reagent in this way is unusual in secondary metabolism, but occurs in primary metabolism during isoleucine and valine biosynthesis. A thiamine PP-mediated mechanism is suggested (Figure 6.125), i.e. decarboxylation precedes the nucleophilic attack (compare decarboxylation of pyruvate, page 23, and formation of deoxyxylulose phosphate, page 191). This process yields the diketone, and a transamination reaction would then give **cathinone** (Figure 6.124). Reduction of the carbonyl group from either face provides the diastereomeric **norephedrine** or **norpseudoephedrine** (**cathine**). Finally, *N*-methylation would provide **ephedrine** and ***N*-methylephedrine**, or **pseudoephedrine** and ***N*-methylpseudoephedrine** (Figure 6.124). Typically, all of the latter six compounds can be found in *Ephedra* species, the proportions varying according to species. Norpseudoephedrine is also a major constituent of the leaves of khat (*Catha edulis*; Celastraceae), chewed in African and Arab countries as a stimulant [Box 6.22]. Most of the central nervous system stimulant action comes from the more active cathinone, the corresponding carbonyl derivative. These natural compounds are structurally similar to the synthetic amfetamine/dexamfetamine (amphetamine/dexamphetamine) (see Figure 6.127), and have similar properties.

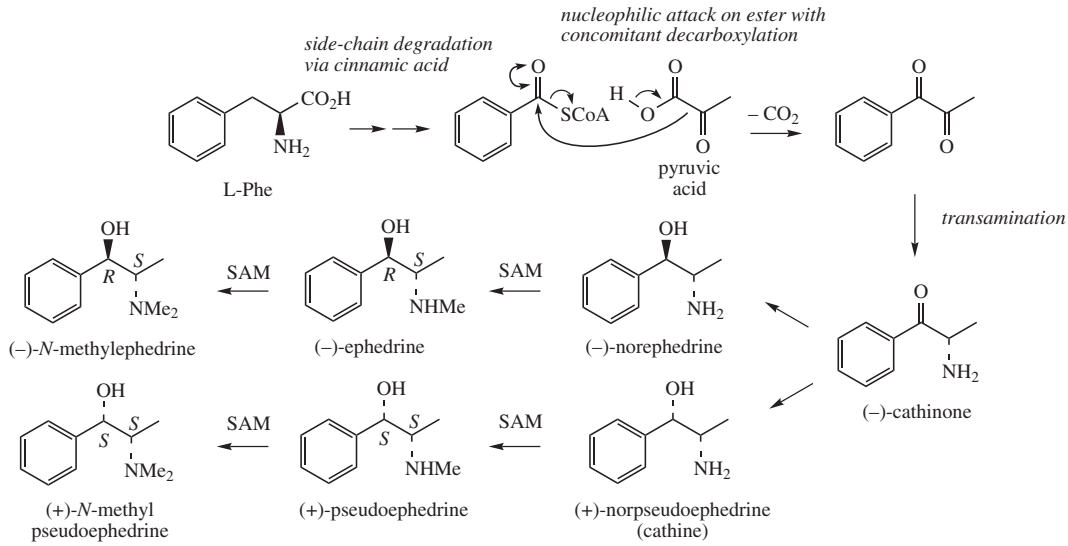


Figure 6.124

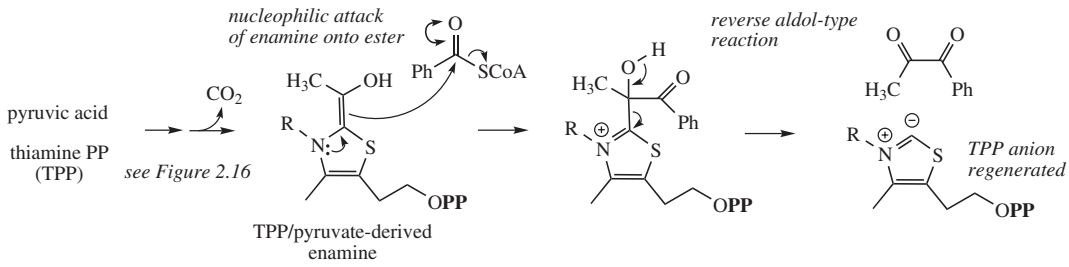


Figure 6.125

Box 6.22

Ephedra

Ephedra or Ma Huang is one of the oldest known drugs, having being used by the Chinese for at least 5000 years. It consists of the entire plant or tops of various *Ephedra* species (Ephedraceae), including *Ephedra sinica* and *Ephedra equisetina* from China, and *Ephedra geriardiana, Ephedra intermedia* and *Ephedra major* from India and Pakistan. The plants are small bushes with slender aerial stems and minute leaves, giving the appearance of being effectively leafless. The plants typically contain 0.5–2.0% of alkaloids, according to species. There are three pairs of optically active diastereomeric alkaloids: (−)-ephedrine and (+)-pseudoephedrine, (−)-methylephedrine and (+)-methylpseudoephedrine, and (−)-norephedrine and (+)-norpseudoephedrine (Figure 6.124). Typically, from 30 to 90% of the total alkaloids is (−)-ephedrine. In *Ephedra intermedia*, the proportion of pseudoephedrine exceeds that of ephedrine.

Ephedrine is an indirectly acting sympathomimetic amine active at both α- and β-adrenergic receptors with effects similar to noradrenaline (see page 336). Lacking the phenolic groups of the catecholamines, it has only weak action on adrenoreceptors, but it is able to displace noradrenaline from storage vesicles in the nerve terminals, which can then act on receptors. It is orally active and has a longer duration of action than noradrenaline. It also has bronchodilator activity, giving relief in asthma, plus a vasoconstrictor action on mucous membranes, making it an effective nasal decongestant. **Pseudoephedrine** is also widely used in compound cough and cold preparations and as a decongestant. The ephedrine and pseudoephedrine used medicinally are usually synthetic. One commercial synthesis of ephedrine involves a fermentation reaction on benzaldehyde using brewer's yeast (*Saccharomyces* sp.), giving initially an alcohol, reductive condensation with methylamine then yields (−)-ephedrine with very high enantioselectivity (Figure 6.126). The fermentation reaction is similar to that shown in Figure 6.125, in that an activated acetaldehyde bound to TPP is produced by the yeast through decarboxylation of pyruvate, and this unit is added stereospecifically to benzaldehyde in an aldol-like reaction.

The herbal drug ephedra/Ma Huang is currently being traded as 'herbal ecstasy'. Consumption gives central nervous system stimulation, but in high amounts it can lead to hallucinations, paranoia, and psychosis. Dietary supplements containing Ma Huang are sold for weight loss and endurance enhancement; but, because of misuse and abuse, these have been regulated or even banned in some countries.

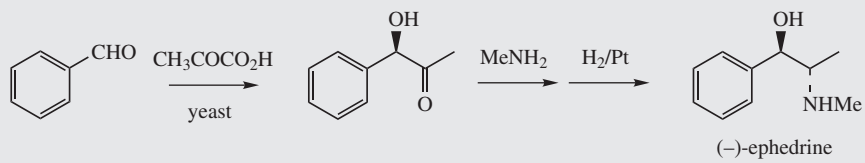


Figure 6.126

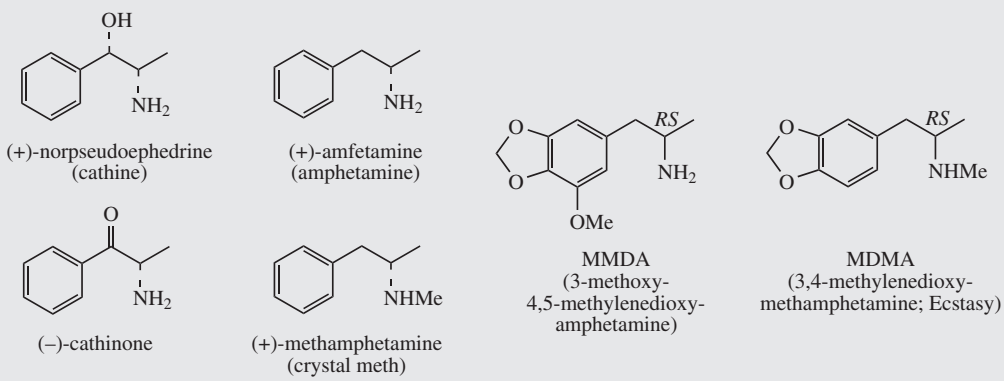


Figure 6.127

Box 6.22 (continued)

Khat

Khat, or Abyssinian tea, consists of the fresh leaves of *Catha edulis* (Celastraceae), a small tree cultivated in Ethiopia, East and South Africa, and in the Yemen. The leaves are widely employed in African and Arabian countries, where they are chewed for a stimulant effect. This traditional use alleviates hunger and fatigue, but also gives a sensation of general well-being (compare coca, page 322). Users become cheerful and talkative, and khat has become a social drug. Prolonged usage can lead to hypertension, insomnia, or even mania. Khat consumption may lead to pyschological dependence, but not normally physical dependence. There is presently little usage outside of Africa and Arabia, although this is increasing due to immigration from these areas. However, for maximum effects, the leaves must be fresh, and this somewhat restricts international trade. Young fresh leaves contain 0.1–0.3% (−)-cathinone (Figure 6.127) as the principal central nervous system stimulant. Cathinone is relatively unstable, decomposing to (+)-norpseudoephedrine (cathine; Figure 6.127) and norephedrine (Figure 6.124) after harvesting or as the leaves are dried. Cathinone has similar pharmacological properties as the synthetic central nervous system stimulant (+)-amfetamine/dexamfetamine (amphetamine/dexamphetamine; Figure 6.127), with a similar potency. Both compounds act by inducing release of catecholamines.

Medicinal use of amfetamine has declined markedly as drug dependence and the severe depression generated on withdrawal have been appreciated. Nevertheless, amfetamine abuse is significant. Amfetamines are taken orally, sniffed, or injected to give a long period of central nervous system stimulation (from hours to days). Users often then take a depressant drug (alcohol, barbiturates, or opioids) to terminate the effects; users rapidly become dependent and develop tolerance. The consumption of khat is not yet restricted in the UK, even though both cathine and cathinone are now controlled drugs. It remains to be seen whether khat will be reclassified and its use restricted in any way. Other amfetamine-like derivatives of note are methamphetamine, methoxymethylenedioxyamphetamine (MMDA) and methylenedioxymethamphetamine (MDMA) (Figure 6.127). Methamphetamine is commonly known as crystal meth, speed, or ice, and is a very addictive and potent psychostimulant. It is usually made illicitly from ephedrine or pseudoephedrine. MMDA is thought to be formed in the body after ingestion of nutmeg (*Myristica fragrans*; Myristicaceae), by an amination process on myristicin (see page 156), and it may be the agent responsible for the euphoric and hallucinogenic effects of nutmeg. MDMA is the illicit drug Ecstasy, a synthetic amfetamine-like stimulant popular among young people. MDMA enhances release of the amine neurotransmitters serotonin, noradrenaline, and dopamine in the brain, and generates multiple short- and long-term neuropsychiatric effects. The use of Ecstasy has resulted in a number of deaths, brought about by subsequent heatstroke and dehydration.

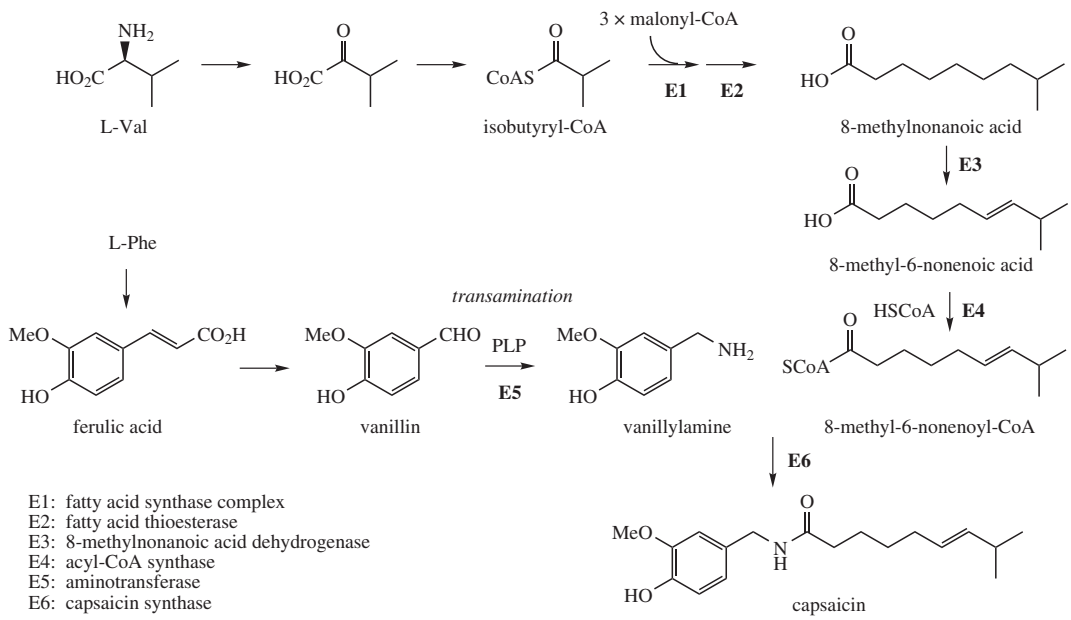


Figure 6.128

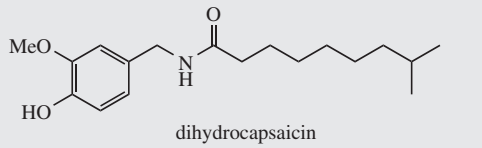
The amide **capsaicin** (Figure 6.128) constitutes the powerfully pungent principal in chilli peppers (*Capsicum annuum*; Solanaceae). Apart from its culinary importance, it is also used medicinally in creams to counter neuralgia caused by herpes infections and in other topical pain-relieving preparations [Box 6.23]. The initial burning effect of capsaicin is found to affect the pain receptors, making them less sensitive. The aromatic portion of capsaicin is derived from phenylalanine through ferulic acid and vanillin (Figure 6.128, compare page 160), this aldehyde being the substrate for transamination to give vanillylamine. The acid portion of the amide structure is of polyketide origin, with a branched-chain fatty acid being produced by chain extension of isobutyryl-CoA. This starter unit is valine derived (see page 56). Some, but not all, of the fatty acid synthase component genes have been characterized.

Box 6.23

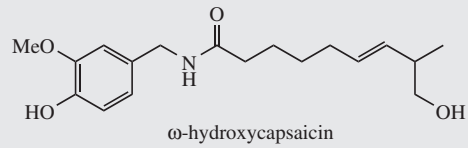
Capsaicin

Capsicum species (Solanaceae), or chilli peppers, are used worldwide in cooking as spices or components of traditional hot sauces. Most chilli peppers are varieties of *Capsicum annuum*, and sometimes *Capsicum frutescens*. The red colour of most pepper fruits is due to carotenoid pigments, e.g. capsanthin (see page 300). Pungency is attributable to the presence of capsaicinoids, amides of branched-chain fatty acids (C_8–C_{13}) with vanillylamine, and varies significantly according to variety. Capsaicinoid content may reach as high as 1%. A range of pungent (and some non-pungent) capsaicinoids has been identified, though capsaicin (Figure 6.128) and dihydrocapsaicin (Figure 6.129) are the major components (80% or more) of most *Capsicum* species. The hydroxy derivative ω-hydroxycapsaicin (Figure 6.129) is non-pungent. Pepper sprays are used for law enforcement and self-defence; they cause intense coughing, lachrymation, temporary blindness, and intense burning sensation. Sweet peppers have negligible capsaicinoid content; pepper fruits are also rich sources of vitamin C (see page 492). Birds are the primary dispersal agent of *Capsicum* seeds, and since they lack a functional receptor (see below), they are oblivious to the presence of capsaicin.

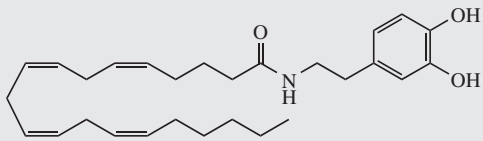
Capsaicin causes a burning sensation in the mouth by binding to an appropriate receptor; since capsaicin contains a vanillyl (4-hydroxy-3-methoxybenzyl) group, the receptor was termed 'vanilloid'. Since then, an array of vanilloid receptor agonists lacking any vanillyl group has been discovered, resulting in a somewhat awkward terminology. Capsaicin binds to the vanilloid receptor subtype 1, recently renamed TRPV1 (transient receptor potential vanilloid type 1), a non-specific cation channel activated not only by capsaicin, but also by heat or acid, both of which cause pain. The non-vanillyl *N*-arachidonoyldopamine (Figure 6.129) and related amides of dopamine with other fatty acids have been identified as endogenous ligands for the vanilloid type 1 receptor (compare cannabinoid receptors, see page 122). Activation of TRPV1 is then followed by a long-lasting desensitization, a particular form of analgesia where only pain sensitivity is lost. This property makes capsaicin valuable as a painkiller, and different from other painkillers. Capsaicin-based creams are used to relieve pain associated with osteoarthritis, neuralgia caused by herpes infections, and diabetic neuropathy. Some synthetic analogues of capsaicin are also being tested as non-narcotic analgesics. It has also been noted that the TRPV1 channel, when activated by capsaicin, is large enough to allow passage of other agents, e.g. lidocaine, allowing a more targeted form of local anasthesia.



dihydrocapsaicin



ω-hydroxycapsaicin



N-arachidonoyldopamine

Figure 6.129

Terpenoid Alkaloids

A variety of alkaloids based on mono-, sesqui-, di-, and tri-terpenoid skeletons have been characterized, but information about their formation in nature is still somewhat sparse. Monoterpene alkaloids are, in the main, structurally related to iridoid materials (see page 206), the oxygen heterocycle being replaced by a nitrogen-containing ring. **β-Skytanthine** from *Skytanthus acutus* (Apocynaceae) and **actinidine** from *Actinidia polygama* (Actinidiaceae) serve as examples (Figure 6.130). The iridoid loganin, so important in the biosynthesis of terpenoid indole alkaloids (see page 370) and the ipecac alkaloids (see page 363), is not a precursor of these structures, and a modified series of reactions starting from geraniol is proposed (Figure 6.130). The formation of the dialdehyde follows closely elaboration of its stereoisomer in loganin biosynthesis (see page 207). This could then act as a substrate for amination via an amino acid, followed by ring formation as seen with coniine (see page 400). Reduction and methylation would yield **β-skytanthine**, whereas further oxidation could provide the pyridine ring of **actinidine**.

Gentianine (Figure 6.130) is probably the most common of the monoterpene alkaloids, but it is frequently formed as an artefact when a plant extract containing suitable iridoid structures is treated with acid and then ammonia, the procedure commonly used during isolation of alkaloids. Thus, the secoiridoid **gentiopicroside** from *Gentiana lutea* (Gentianaceae) is hydrolysed and then reacts with ammonia to give a heterocyclic system that readily dehydrates to a pyridine, generating gentianine (Figure 6.131). Other iridoid structures are known to react with ammonia to produce alkaloid artefacts. In some plants, however, gentianine can be found when no ammonia treatment has been involved, and one may speculate that it is indeed a natural alkaloid.

Perhaps the most dramatic examples of terpenoid alkaloids from a structural and pharmacological point of view are those found in aconite (*Aconitum* species; Ranunculaceae), commonly known as monkshood or wolfsbane, and species of *Delphinium* (Ranunculaceae). Whilst *Aconitum napellus* has had some medicinal use, mainly for external relief of pain, plants of both genera owe their highly toxic nature to diterpenoid alkaloids. Aconite in particular is regarded as extremely toxic, due to the presence of **aconitine** (Figure 6.132) and related C_{19} norditerpenoid alkaloids. Species of *Delphinium* accumulate diterpenoid alkaloids such as **atisine** (Figure 6.132), which tend to be less toxic than aconitine. These alkaloids appear to behave as neurotoxins by acting on sodium channels. Little experimental evidence about their origins is available, though their structural relationship to diterpenes, e.g. ***ent*-kaurene** (see page 229), can be appreciated. This is most apparent in the veatchine-type diterpenoid alkaloids; the other skeletons involve substantial rearrangement reactions.

Steroidal Alkaloids

Many plants in the Solanaceae accumulate steroidal alkaloids based on a C_{27} cholestane skeleton, e.g. **solasodine** and **tomatidine** (Figure 6.133). These are essentially nitrogen analogues of steroidal saponins (see page 263) and have already been briefly considered along with these

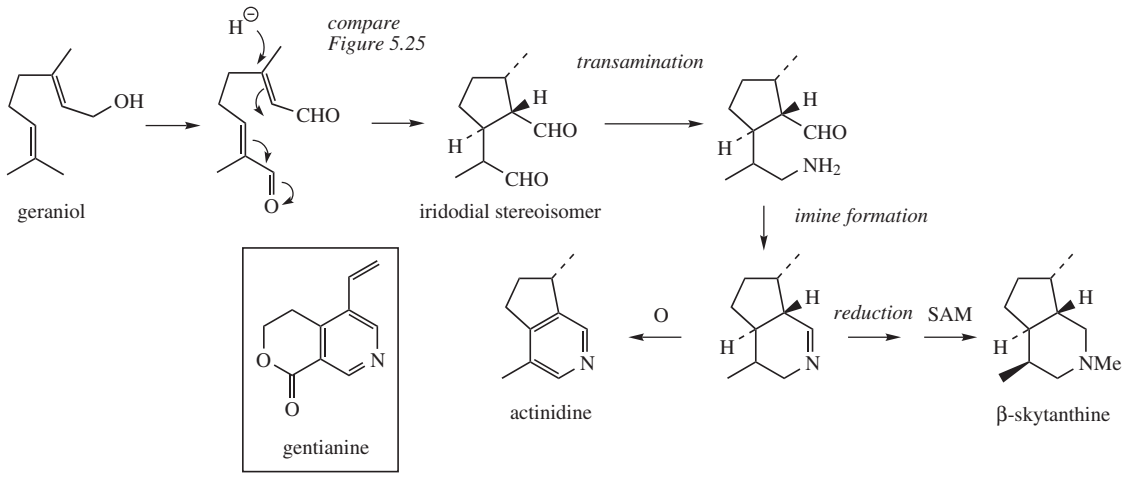


Figure 6.130

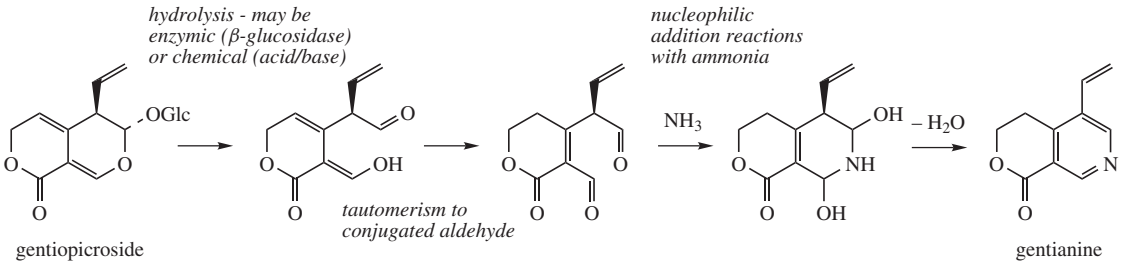


Figure 6.131

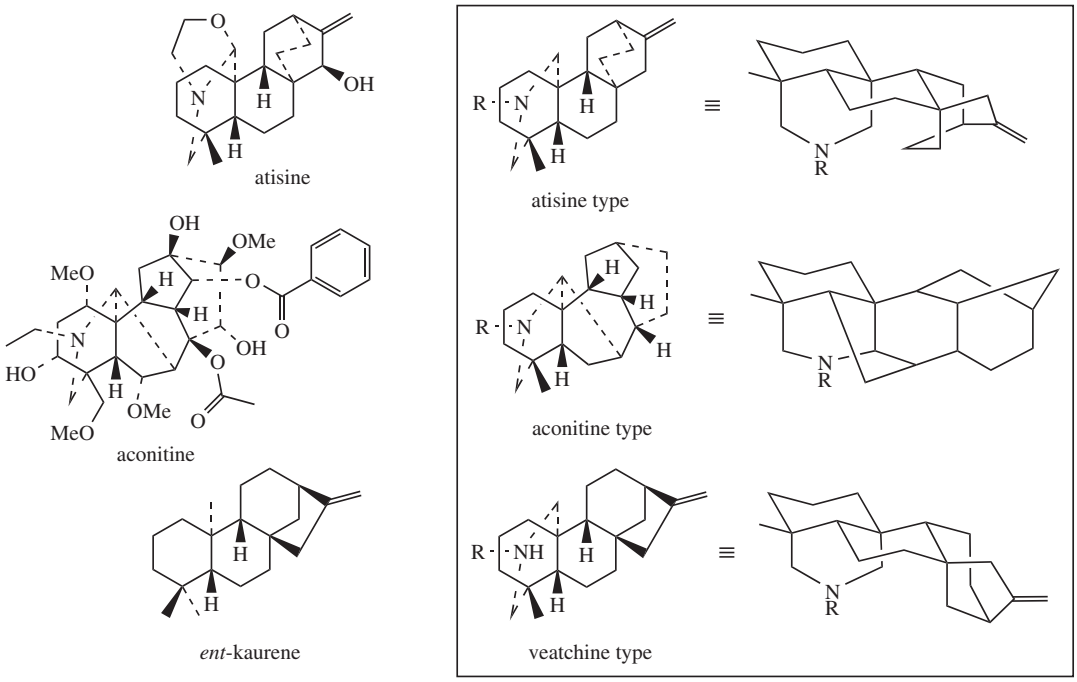


Figure 6.132

compounds. In contrast to the oxygen analogues, these compounds all have the same stereochemistry at C-25 (methyl always equatorial), but C-22 isomers do exist, as solasodine and tomatidine exemplify. They are usually present as glycosides, which have surface activity and haemolytic properties, as do the saponins, but these compounds are also toxic if ingested. **α-Solasonine** from *Solanum* species and **α-tomatine** (Figure 6.133) from tomato (*Lycopersicon esculente*) are typical examples of such glycosides.

As with the sapogenins, this group of steroidal alkaloids is derived from cholesterol, with appropriate side-chain modifications during the sequence (Figure 6.134). Amination appears to employ L-arginine as the nitrogen source, probably via a substitution process on 26-hydroxycholesterol. A second substitution allows 26-amino-22-hydroxycholesterol to cyclize, generating a piperidine ring. After 16β-hydroxylation, the secondary amine is oxidized to an imine, and the spiro-system can be envisaged as the result of a nucleophilic addition of the 16β-hydroxyl onto the imine (or iminium via protonation). Whether the 22*R* (as in **solasodine**) or 22*S* (as in **tomatidine**) configuration is established may depend on this reaction.

A variant on the way the cholesterol side-chain is cyclized can be found in **solanidine** (Figure 6.133),

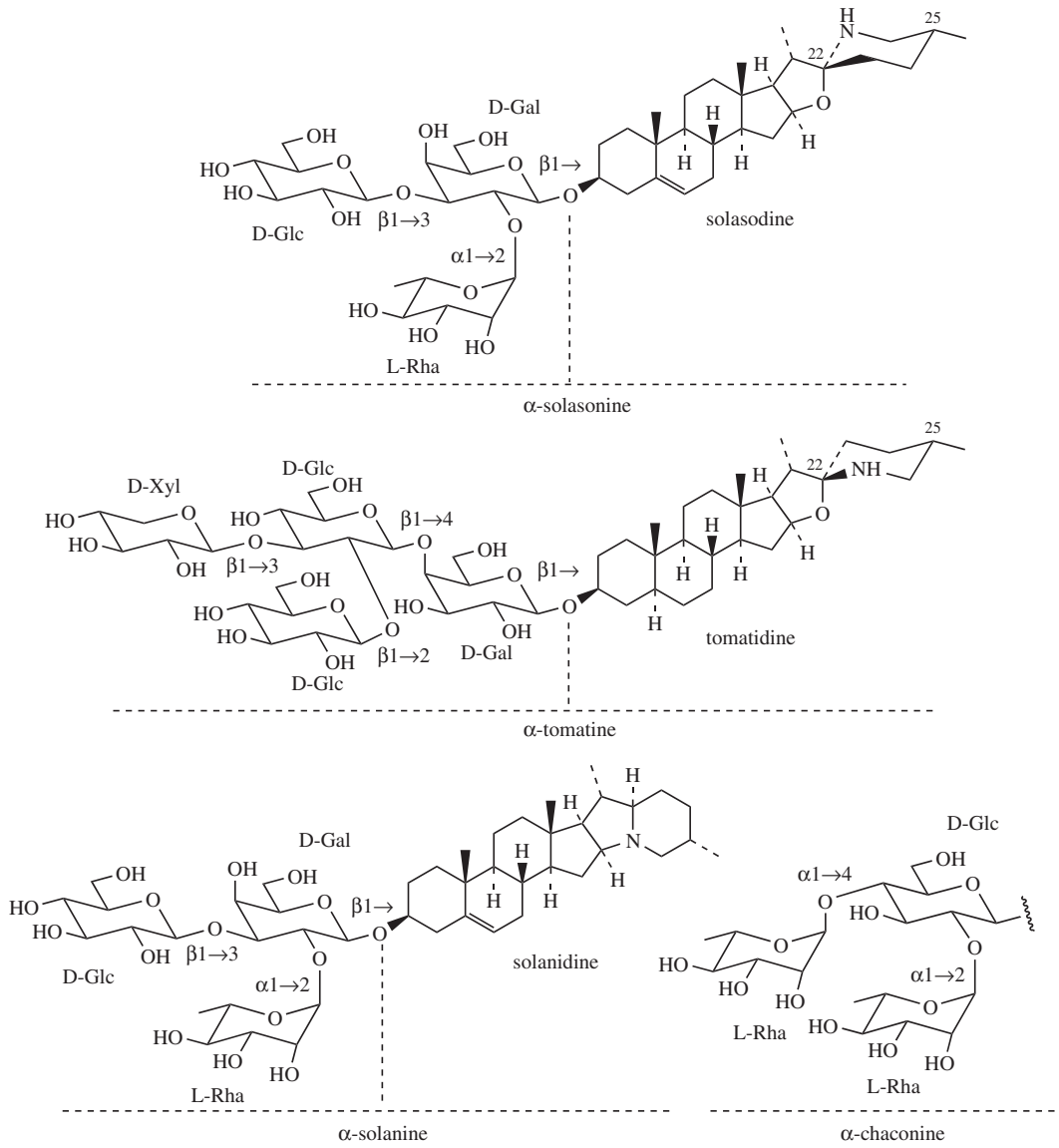


Figure 6.133

which contains a condensed ring system with nitrogen at the bridgehead. Solanidine is found in potatoes (*Solanum tuberosum*) typically as the glycosides **α-solanine** and **α-chaconine** (Figure 6.133). This condensed ring system appears to be produced by a branch from the main pathway to the solasodine/tomatidine structures. Thus, a substitution process will allow generation of the new ring system (Figure 6.135).

Enzymic data relating to the formation of the steroidal alkaloid aglycones are not available, but both enzymic and genetic studies have clarified sequences for elaboration of various glycoside side-chains. Thus, in potato, solanidine is converted into α-solanine by way of γ- and β-solanine. Alternatively, a different sequence of glycosylation reactions leads to α-chaconine (Figure 6.136). Toxicity appears to increase as the glycoside chain is extended.

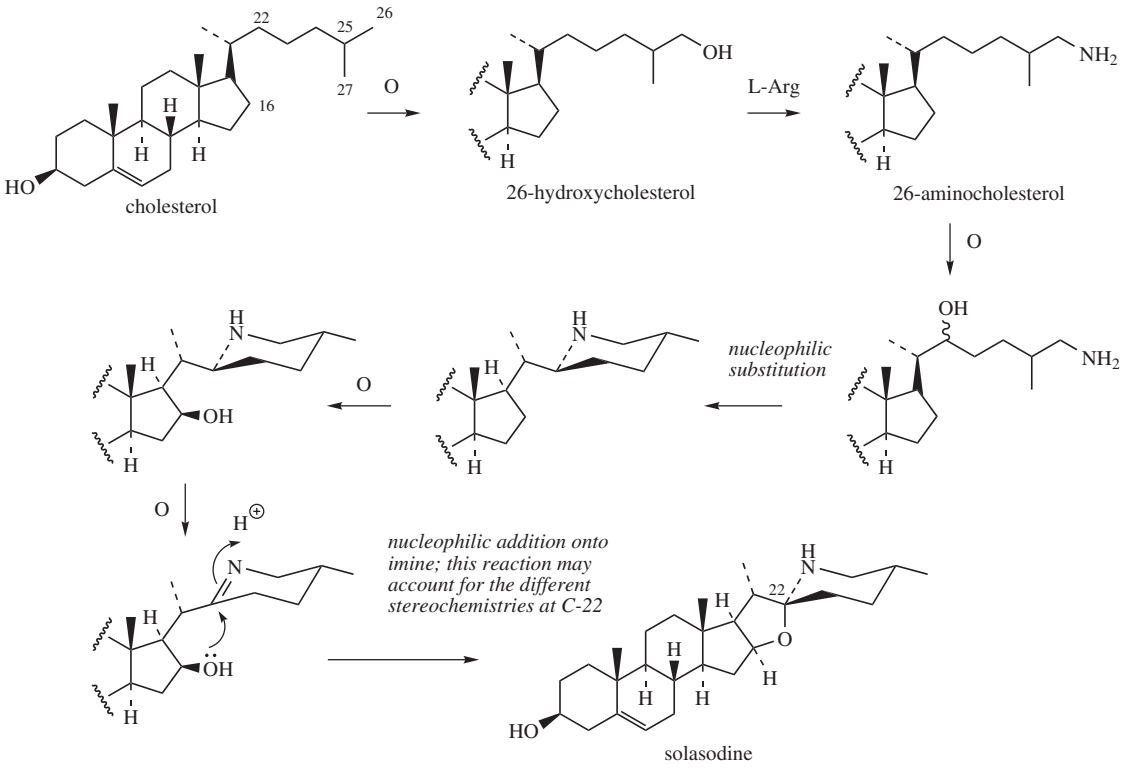


Figure 6.134

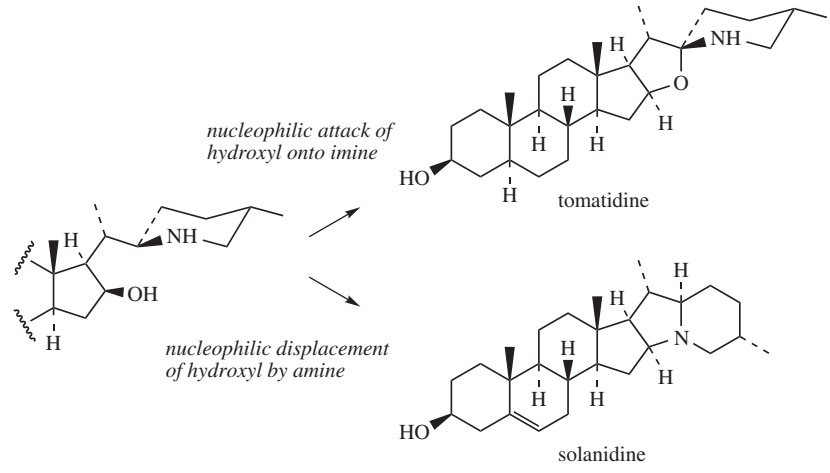
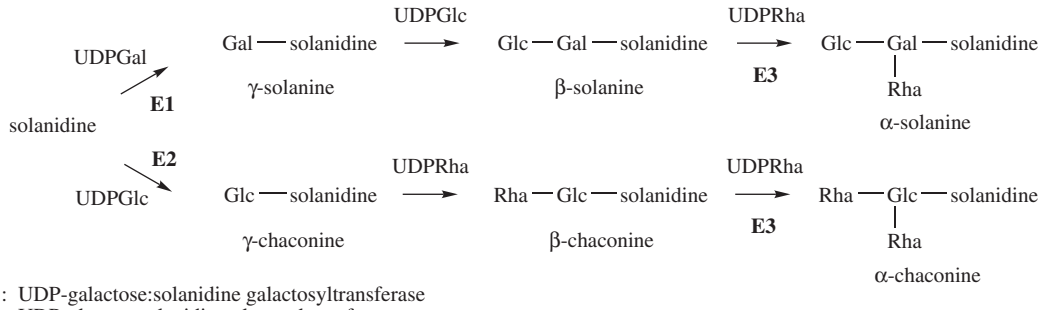


Figure 6.135



E1: UDP-galactose:solanidine galactosyltransferase
E2: UDP-glucose:solanidine glucosyltransferase
E3: UDP-rhamnose:β-steroidal glycoalkaloid rhamnosyltransferase

Figure 6.136

Since the production of medicinal steroids from steroidal saponins (see page 281) requires preliminary degradation to remove the ring systems containing the original cholesterol side-chain, it is immaterial whether these rings contain oxygen or nitrogen. Thus, plants rich in **solasodine** or **tomatidine** could also be employed for commercial steroid production. Similarly, other *Solanum* alkaloids, such as **solanidine**, with nitrogen in a condensed ring system might also be exploited [Box 6.24].

Box 6.24

Solanum Alkaloids

The toxicity of steroidal alkaloid glycosides is of some concern, in that several plant species that can accumulate them are major food crops. Tomatoes, peppers, and especially potatoes fall in this group; the production of glycoalkaloids in potato (*Solanum tuberosum*) has been studied extensively. α-Solanine and α-chaconine (Figure 6.133) account for up to 95% of the glycoalkaloids in potato, and α-chaconine is regarded as the more toxic. The alkaloids inhibit acetylcholinesterase and butyrylcholinesterase, disrupt cell membranes, and may be teratogenic. Mild clinical symptoms of glycoalkaloid poisoning include abdominal pain, vomiting, and diarrhoea. At higher levels, more severe symptoms may occur, including fever, rapid pulse, low blood pressure, rapid respiration, and neurological disorders. The highest glycoalkaloid level in potato plants is found in flowers and sprouts, followed by the leaves, and the lowest amounts are detected in stems and tubers. However, the amount of glycoalkaloids in tubers increases upon wounding and light exposure; green tubers have a significantly higher alkaloid content and are considered unsuitable for human consumption.

The major alkaloidal component in many *Solanum* species is solasodine (Figure 6.133). It is present as glycosides in the leaves, and especially in the unripe fruits. Solasodine may be converted into progesterone by means of the Marker degradation shown in Figure 5.118 (see page 281). Trial cultivations of a number of *Solanum* species, including *Solanum laciniatum* and *Solanum aviculare* (indigenous to New Zealand), *Solanum khasianum* (from India), and *Solanum marginatum* (from Ecuador), have been conducted in various countries. Alkaloid levels of 1–2% have been obtained. These plants are especially suitable for long-term cultivation if the fruits provide suitable quantities, being significantly easier to cultivate than disogenin-producing *Dioscorea* species.

Cultivation of tomato fruits (*Lycopersicon esculentum*) is carried out on a huge scale as a food crop. The aerial parts, currently waste plant material after harvesting, contain about 0.1% tomatidine (Figure 6.133) which may also be processed.

Several plants in the Liliaceae, notably the genus *Veratrum* (Liliaceae/Melanthiaceae), contain a remarkable group of steroidal alkaloids in which a fundamental change to the basic steroid nucleus has taken place. This change expands ring D by one carbon at the expense of ring C, which consequently becomes five-membered. The resulting skeleton is termed a C-nor-D-homosteroid in keeping with these alterations in ring size.

Cholesterol is a precursor of this group of alkaloids, and a possible mechanism accounting for the ring

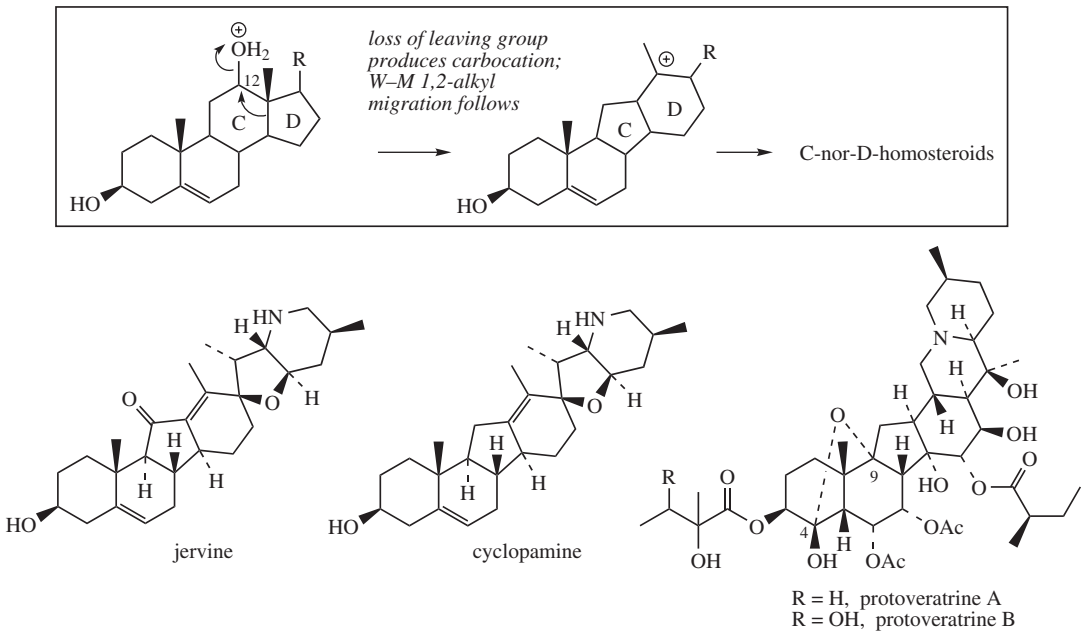


Figure 6.137

modifications is shown in Figure 6.137, where the changes are initiated by loss of a suitable leaving group from C-12. Typical representatives of C-nor-D-homosteroids are **jervine** and **cyclopamine** (Figure 6.137) from *Veratrum californicum*, toxic components in this plant which are responsible for severe teratogenic effects. Animals grazing on *Veratrum californicum* and some other species of *Veratrum* frequently give birth to offspring with cyclopia, a malformation characterized by a single eye in the centre of the forehead. The teratogenic effects of jervine, cyclopamine, and cyclopamine glucoside (cycloposine) on the developing fetus are now well established. Other *Veratrum* alkaloids, especially those found in *Veratrum album* and *Veratrum viride*, have been employed medicinally as

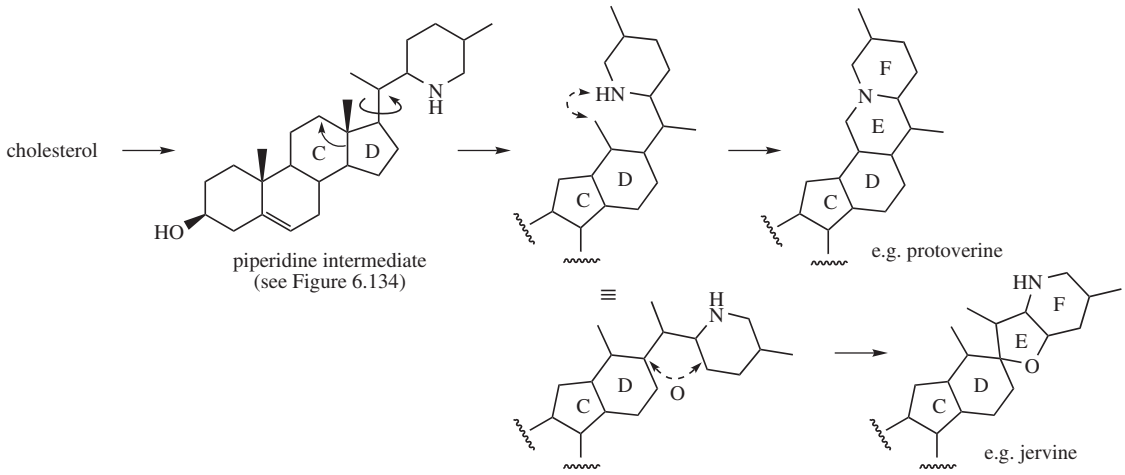


Figure 6.138

hypotensive agents, and used in the same way as *Rauwolfia* alkaloids (see page 372), often in combination with *Rauwolfia*. These medicinal alkaloids, e.g. **protoveratrine A** and **protoveratrine B** (Figure 6.137), which are esters of protoverine, are characterized by fusion of two more six-membered rings onto the C-nor-D-homosteroid skeleton. This hexacyclic system is extensively oxygenated, and a novel hemiketal linkage bridges C-9 with C-4. Both the jervine and protoverine skeletons are readily rationalized through additional cyclization reactions involving a piperidine ring, probably formed by processes analogous to those seen with the *Solanum* alkaloids (Figure 6.134). The skeletal changes are outlined in Figure 6.138, which suggests the participation of the piperidine intermediate from Figure 6.134. Typically, both types of alkaloid are found co-occurring in *Veratrum* species.

Many steroidal derivatives are formed by truncation of the original C_8 side-chain, and C_{21} pregnane derivatives are important animal hormones (see page 287) or intermediates on the way to other natural steroidal derivatives, e.g. cardioactive glycosides (see page 265). Alkaloids based on a pregnane skeleton are found in plants, particularly in the Apocynaceae and Buxaceae, and **pregnenolone** (Figure 6.139) is usually involved in their production. **Holaphyllamine** from *Holarrhena floribunda* (Apocynaceae) is obtained from pregnenolone by replacement of the 3-hydroxyl with an amino group (Figure 6.139). **Conessine** (Figure 6.139) from *Holarrhena antidysenterica* is also derived from pregnenolone; it requires two amination reactions, one at C-3 as for holaphyllamine, plus a further one, originally at C-20, probably via the C-20 alcohol. The new ring system in conessine is then the result of attack of the C-20 amine onto the C-18 methyl, suitably activated, of course. The bark of *Holarrhena antidysenterica* has long been used, especially in India, as a treatment for amoebic dysentery.

The novel steroidal polyamine **squalamine** (Figure 6.140) has been isolated in very small amounts (about 0.001%) from the liver of the dogfish shark (*Squalus acanthias*), and has attracted considerable attention because of its remarkable antimicrobial activity. This compound is a broad-spectrum agent effective at very low concentrations against Gram-positive and Gram-negative bacteria, and also fungi, protozoa, and viruses, including HIV. The sulfated side-chain helps to make squalamine water soluble. The polyamine portion is spermidine (see page 313), a compound widely distributed in both animals and plants. Squalamine has since been found to possess a range of other biological activities, including antiangiogenic properties; synthetic squalamine is in clinical trials as an anticancer agent against solid tumours. Related aminosterol derivatives with similar high antimicrobial activity have also been isolated from the shark liver extracts. These compounds exhibit structural variation in the C_8 side-chain, with spermidine (or in one example, spermine) attached at C-3.

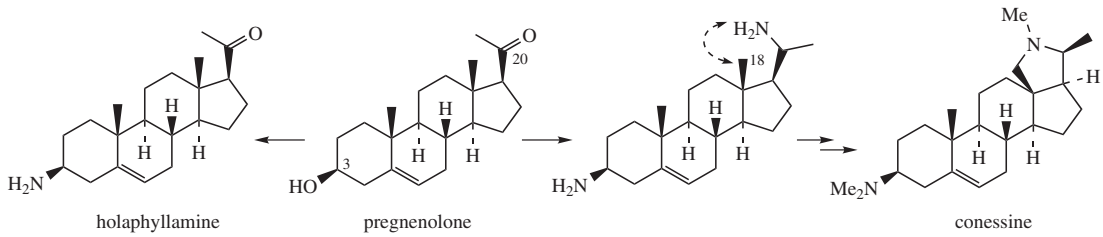


Figure 6.139

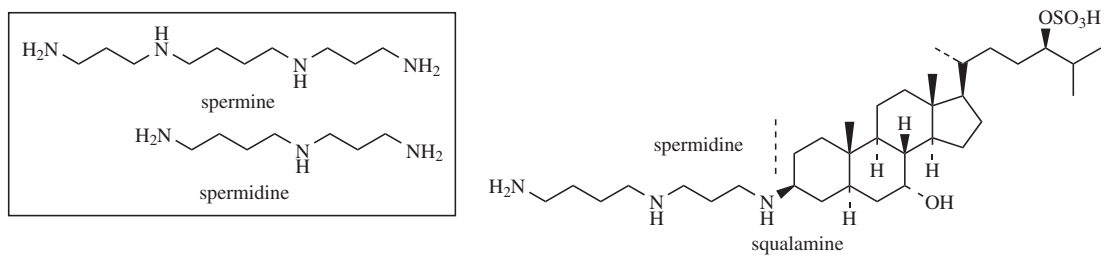


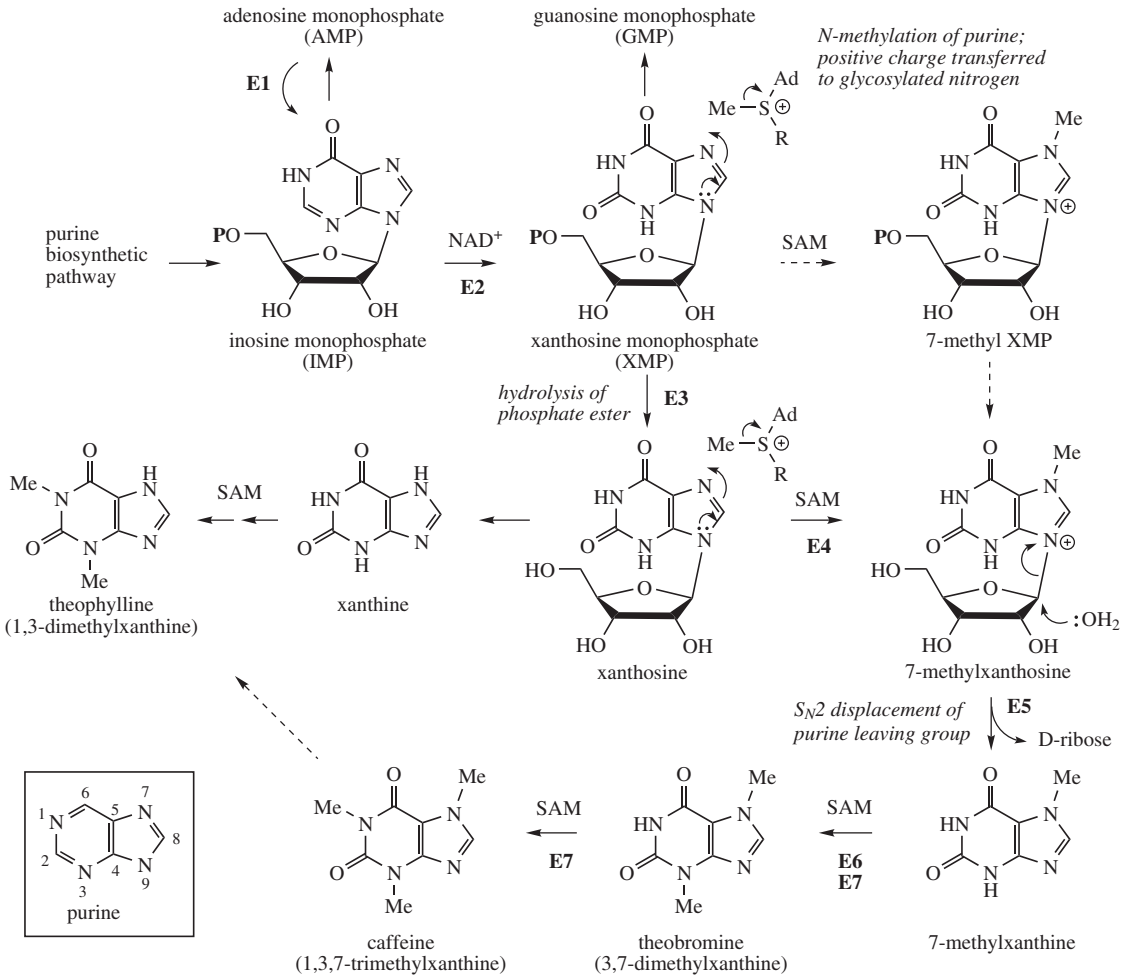
Figure 6.140

PURINE ALKALOIDS

Caffeine

The purine derivatives **caffeine, theobromine**, and **theophylline** (Figure 6.141) are usually referred to as purine alkaloids. They have a rather limited distribution, and their origins are very closely linked with those of the purine bases adenine and guanine, fundamental components of nucleosides, nucleotides, and the nucleic acids. Caffeine, in the form of beverages such as tea, coffee, and cola, is one of the most widely consumed and socially accepted natural stimulants. It is also used medicinally, but theophylline is more important as a drug compound because of its muscle relaxant properties, utilized in the relief of bronchial asthma. Theobromine is a major constituent of cocoa and related chocolate products [Box 6.25].

The purine ring is gradually elaborated by piecing together small components from primary metabolism. The largest component incorporated is glycine, which provides a C_2N unit, whilst the remaining carbon atoms come from formate (by way of N^{10}-formyl-tetrahydrofolate; see page 144) and bicarbonate. Two of



E1: AMP deaminase
E2: IMP dehydrogenase
E3: 5′-nucleotidase
E4: xanthosine 7-*N*-methyltransferase
E5: 7-methylxanthosine nucleosidase
E6: 7-methylxanthine 3-*N*-methyltransferase (theobromine synthase)
E7: theobromine 1-*N*-methyltransferase (caffeine synthase)

Figure 6.141

the four nitrogen atoms are supplied by glutamine and a third by aspartic acid. Synthesis of the nucleotides adenosine 5′-monophosphate (AMP) and guanosine 5′-monophosphate (GMP) is by way of inosine 5′-monophosphate (IMP) and xanthosine 5′-monophosphate (XMP) (Figure 6.141), and the purine alkaloids then branch away through XMP. AMP, if available, can also serve as a source of IMP. Methylation and then loss of phosphate generates the nucleoside **7-methylxanthosine**, which is then released from the sugar. Successive methylations on the nitrogen atoms give **caffeine** by way of **theobromine**, whilst a different methylation sequence can account for the formation of **theophylline**. Theophylline can also be produced by demethylation of caffeine as part of a degradative pathway. Some of the *N*-methyltransferases display rather broad substrate specificity, and this allows minor pathways to operate in certain plants, e.g. the alternative sequence to 7-methylxanthosine via 7-methyl XMP shown in Figure 6.141. In addition, the enzyme caffeine synthase in coffee (*Coffea arabica*; Rubiaceae) has dual functionality, and methylates both theobromine and 7-methylxanthine; a tea (*Camellia sinensis*; Theaceae) enzyme is specific for theobromine.

Box 6.25

Caffeine, Theobromine, and Theophylline

The purine alkaloids caffeine, theobromine, and theophylline (Figure 6.141) are all methyl derivatives of xanthine and they commonly co-occur in a particular plant. The major sources of these compounds are the beverage materials such as tea, coffee, cocoa, and cola, which owe their stimulant properties to these water-soluble alkaloids. They competitively inhibit the phosphodiesterase that degrades cyclic AMP (cAMP). The resultant increase in cAMP levels thus mimics the action of catecholamines and leads to a stimulation of the central nervous system, a relaxation of bronchial smooth muscle, and induction of diuresis, as major effects. These effects vary in the three compounds. **Caffeine** is the best central nervous system stimulant, and has weak diuretic action. **Theobromine** has little stimulant action, but has more diuretic activity and also muscle relaxant properties. **Theophylline** also has low stimulant action and is an effective diuretic, but it relaxes smooth muscle better than caffeine or theobromine.

Caffeine is used medicinally as a central nervous system stimulant, usually combined with another therapeutic agent, as in compound analgesic preparations. **Theobromine** is of value as a diuretic and smooth muscle relaxant, but is not now routinely used. **Theophylline** is an important smooth muscle relaxant for relief of bronchospasm; it is frequently dispensed in slow-release formulations to reduce side-effects. It is also available as **aminophylline**, a more soluble preparation containing theophylline with ethylenediamine in a 2:1 ratio. The alkaloids may be isolated from natural sources, or obtained by total or partial synthesis.

It has been estimated that beverage consumption may provide the following amounts of caffeine per cup or average measure: coffee, 30–150 mg (average 60–80 mg); instant coffee, 20–100 mg (average 40–60 mg); decaffeinated coffee, 2–4 mg; tea, 10–100 mg (average 40 mg); cocoa, 2–50 mg (average 5 mg); cola drink, 25–60 mg. The maximal daily intake should not exceed about 1 g to avoid unpleasant side-effects, e.g. headaches and restlessness. An acute lethal dose is about 5–10 g. The biological effects produced from the caffeine ingested via the different drinks can vary, since its bioavailability is known to be modified by the other constituents present, especially the amount and nature of polyphenolic tannins.

Coffee

Coffee consists of the dried ripe seed of *Coffea arabica, Coffea canephora, Coffea liberica*, or other *Coffea* species (Rubiaceae). The plants are small evergreen trees, widely cultivated in various parts of the world, e.g. Brazil and other South American countries and Kenya. The fruit is deprived of its seed coat, then dried and roasted to develop its characteristic colour, odour, and taste. Coffee seeds contain 1–2% of caffeine and traces of theophylline and theobromine. These are mainly combined in the green seed with chlorogenic acid (see page 150) (5–7%); roasting releases them and also causes some decomposition of chlorogenic acid to quinic acid and caffeic acid. Trigonelline (*N*-methylnicotinic acid) is present in green seeds to the extent of about 0.25–1%; during roasting, this is extensively converted into nicotinic acid (vitamin B_3, see page 31). Volatile oils and tannins provide odour and flavour. A proportion of the caffeine may sublime off during the roasting process, providing some commercial caffeine. Decaffeinated coffee, containing up to 0.08% caffeine, is obtained by removing caffeine, usually by aqueous percolation prior to roasting. This process provides another source of caffeine.

Box 6.25 (continued)

Tea

Tea is the prepared leaves and leaf buds of *Camellia sinensis* (*Thea sinensis*) (Theaceae), an evergreen shrub cultivated in China, India, Japan, and Sri Lanka. For black tea, the leaves are allowed to ferment, allowing enzymic oxidation of the polyphenols, whilst green tea is produced by steaming and drying the leaves to prevent oxidation. During oxidation, colourless catechins (up to 40% in dried leaf; see page 171) are converted into intensely coloured theaflavins and thearubigins. Oolong tea is semi-fermented. Tea contains 1–4% caffeine and small amounts (up to 0.05%) of both theophylline and theobromine. Astringency and flavour come from tannins and volatile oils, the latter containing monoterpene alcohols (geraniol, linalool) and aromatic alcohols (benzyl alcohol, 2-phenylethanol). Theaflavins (see page 172) are believed to act as radical scavengers/antioxidants, and to provide beneficial effects against cardiovascular disease, cancers, and the ageing process generally. Green tea, in particular, contains significant amounts of epigallocatechin gallate (see page 172), a very effective antioxidant regarded as one of the more desirable dietary components. Tea leaf dust and waste is a major source of caffeine.

Cola

Cola, or kola, is the dried cotyledon from seeds of various species of *Cola* (Sterculiaceae), e.g. *Cola nitida* and *Cola acuminata*, trees cultivated principally in West Africa and the West Indies. Seeds are prepared by splitting them open and drying. Cola seeds contain up to 3% caffeine and about 0.1% theobromine, partly bound to tannin materials. Drying allows some oxidation of polyphenols, formation of a red pigment, and liberation of free caffeine. Fresh cola seeds are chewed in tropical countries as a stimulant, and vast quantities of dried seeds are processed for the preparation of cola drinks, e.g. Coca-Cola® and Pepsi-Cola®.

Cocoa

Although cocoa as a drink is now rather unfashionable, it provides the raw material for the manufacture of chocolate and is commercially very important. Cocoa (or cacao) is derived from the roasted seeds of *Theobroma cacao* (Sterculiaceae), a tree widely cultivated in South America and West Africa. The fruits develop on the trunk of the tree; the seeds from them are separated, allowed to ferment, and are then roasted to develop the characteristic chocolate flavour. The kernels are then separated from the husks, ground up, and processed in various ways to give chocolate, cocoa, and cocoa butter.

Cocoa seeds contain 35–50% of oil (cocoa butter or theobroma oil), 1–4% theobromine and 0.2–0.5% caffeine, plus tannins and volatile oils. During fermentation and roasting, most of the theobromine from the kernel passes into the husk, which thus provides a convenient source of the alkaloid. Theobroma oil or cocoa butter is obtained by hot expression from the ground seeds as a whitish solid with a mild chocolate taste. It is a valuable formulation aid in pharmacy, where it is used as a suppository base (see page 48).

Maté Tea

Maté tea is consumed in South America as a stimulant drink. Maté or Paraguay tea consists of the leaves of *Ilex paraguensis* (Aquifoliaceae), South American shrubs of the holly genus. The dried leaf contains 0.8–1.7% caffeine and smaller amounts of theobromine (0.3–0.9%) with little or no theophylline. Considerable amounts (10–16%) of chlorogenic acid (see page 150) are also present.

Guarana

The seeds of the Brazilian plant *Paullinia cupana* (Sapindaceae) are used to make a stimulant drink. Crushed seeds are mixed with water to a paste, which is then sun-dried. Portions of this are then boiled with hot water to provide a refreshing drink. The principal constituent, previously called guaranine, has been shown to be identical to caffeine, and the seeds may contain 3–5%. Small amounts of theophylline (0–0.25%) and theobromine (0.02–0.06%) are also present. Guarana is widely available as tablets and capsules, or as extracts, in health food shops, where it is promoted to relieve mental and physical fatigue. Labels on such products frequently show the active constituent to be guaranine, but may not indicate that this is actually caffeine.

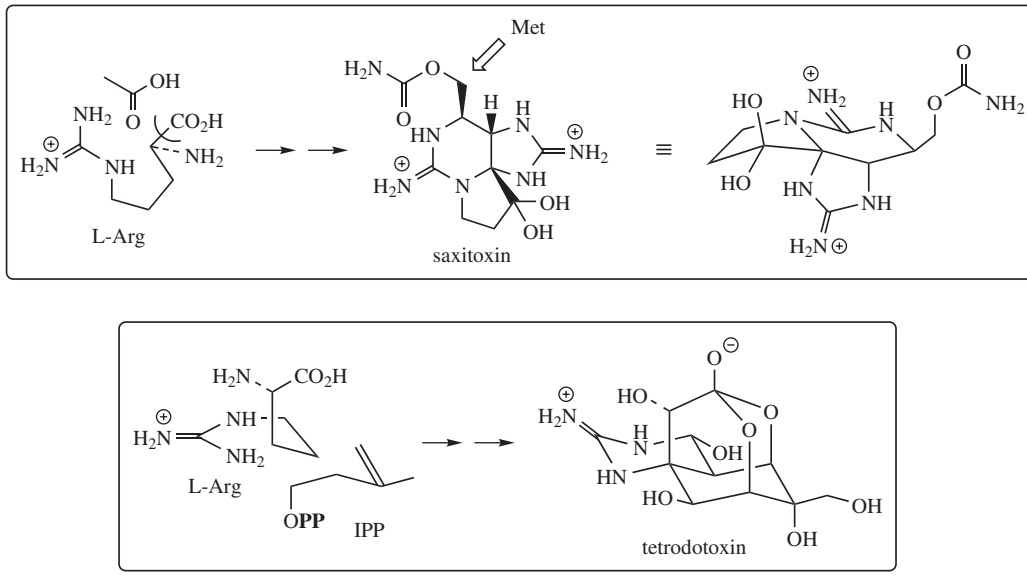


Figure 6.142

Saxitoxin and Tetrodotoxin

The structure of **saxitoxin** (Figure 6.142) contains a reduced purine ring system, but it is not biosynthetically related to the purine alkaloids described above. Not all features of its biosynthetic origin have been established, but the amino acid supplying most of the ring system is known to be L-arginine (Figure 6.142). Acetate and a C_1 unit from methionine are also utilized. Saxitoxin contains two highly polar guanidino functions, one of which is provided by arginine, and is a fast-acting neurotoxin inhibiting nerve conduction by blocking sodium channels [Box 6.26]. It is one of a group of marine toxins referred to as paralytic shellfish poisons, found in a range of shellfish, but ultimately derived from toxic strains of dinoflagellates consumed by the shellfish. Arginine is also a precursor for **tetrodotoxin** (Figure 6.142), another marine neurotoxin containing a polar guanidino group. Tetrodotoxin exists as a zwitterion involving the guanidino group and a hemilactal function. It has been established that the remainder of the carbon skeleton in tetrodotoxin is a C_5 isoprene unit, probably supplied as isopentenyl diphosphate (Figure 6.142). Tetrodotoxin is well known as the toxic principle in the puffer fish (*Tetraodon* species), regarded as delicacy in Japanese cuisine [Box 6.26]. As potent sodium channel blockers, both saxitoxin and tetrodotoxin are valuable pharmacological tools.

Box 6.26

Saxitoxin

Saxitoxin (Figure 6.142) was first isolated from the Alaskan butter clam (*Saxidomus giganteus*) and has since been found in many species of shellfish, especially bivalves such as mussels, scallops, and oysters. These filter feeders consume dinoflagellates (plankton) and can accumulate toxins synthesized by these organisms, particularly during outbreaks known as red tides, when conditions favour formation of huge blooms of the dinoflagellates (see also brevetoxin A, page 93). Species of the dinoflagellate *Gonyaulax* in marine locations or of the cyanobacterium *Aphanizomenon* in freshwater have been identified among the causative organisms, and the problem is encountered widely in temperate and tropical areas (including Europe, North America, and Japan). Commercial production of shellfish is routinely monitored for toxicity, which will slowly diminish as conditions change and the causative organism disappears from the water. About a dozen natural saxitoxin-related structures have been characterized,

Box 6.26 (continued)

and mixtures in various proportions are typically synthesized by a producer, with the possibility that the shellfish may also structurally modify the toxins further. Acute and often fatal poisonings caused by the consumption of contaminated shellfish are termed paralytic shellfish poisoning, which involves paralysis of the neuromuscular system, with death resulting from respiratory failure. Saxitoxin is a cationic molecule which binds to sodium channels to block the influx of sodium ions through excitable nerve membranes, and it is a valuable pharmacological tool for the study of this process. Saxitoxin and tetrodotoxin (below) are some of the most potent non-protein neurotoxins known, and are active at very low concentrations ($\mu g\ kg^{-1}$).

Tetrodotoxin

Tetrodotoxin (Figure 6.142) is traditionally associated with the puffer fish, known in Japan as fugu, a highly prized but risky culinary delicacy; tetrodotoxin is named after the puffer family Tetraodontidae. Preparation of fugu is a skilled operation in which organs containing the highest levels of toxin, e.g. liver, ovaries, testes, are carefully separated from the flesh. Even so, deaths from fugu poisoning are not uncommon, and the element of risk presumably heightens culinary appreciation of the fish. As with saxitoxin, tetrodotoxin appears to be produced by microorganisms, and symbiotic marine bacteria, e.g. *Vibrio* species, have been implicated as the synthesizers. In addition to fugu, several other species of fish, octopus, newts, and frogs have been found to accumulate tetrodotoxin or related structures. The mode of action of tetrodotoxin is exactly the same as that of saxitoxin above, though there are some subtle differences in the mechanism of binding. **Tetrodotoxin** is currently being investigated for use in treatment of cancer pain and management of opiate withdrawal symptoms. It is also being tested as a local and topical anaesthetic in procedures where general anaesthesia is not appropriate.

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