Medicinal Natural Products. Paul M Dewick

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THE MEVALONATE AND DEOXYXYLULOSE PHOSPHATE PATHWAYS: TERPENOIDS AND STEROIDS

The two pathways leading to terpenoids are described: the mevalonate pathway and the recently discovered mevalonate-independent pathway via deoxyxylulose phosphate. Terpenoids may be classified according to the number of isoprenoid units incorporated, and hemiterpenes, monoterpenes and the variants irregular monoterpenes and iridoids, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, tetraterpenes, and higher terpenoids are described in turn, representing groups with increasing numbers of isoprene units. Structures are rationalized through extensive use of carbocation mechanisms and subsequent Wagner–Meerwein rearrangements. Steroids as examples of modified triterpenoids are discussed in detail, including stereochemistry and molecular shape. There follows specific consideration of cholesterol, steroidal saponins, cardioactive glycosides, phytosterols, vitamin D, bile acids, corticosteroids and their semi-synthesis, progestogens, oestrogens, and androgens. Monograph topics giving more detailed information on medicinal agents include volatile oils, pyrethrins, valerian, feverfew, chamomile and matricaria, *Artemisia annua* and artemisinin, gossypol, trichothecenes, *Taxus brevifolia* and taxol, *Ginkgo biloba*, forskolin, liquorice, quillaia, ginseng, vitamin A, cholesterol, dioscorea, fenugreek, sisal, sarsaparilla, yucca, *Digitalis purpurea, Digitalis lanata*, strophanthus, convallaria, squill, soya bean sterols, fusidic acid, vitamin D, bile acids, corticosteroid drugs, progestogen drugs, oestrogen drugs, aromatase inhibitors, oestrogen receptor antagonists, and androgen drugs.

The terpenoids form a large and structurally diverse family of natural products derived from C_5 **isoprene units** (Figure 5.1) joined in a head-to-tail fashion. Typical structures contain carbon skeletons represented by $(C_5)_n$, and are classified as **hemiterpenes** (C_5), **monoterpenes** (C_{10}), **sesquiterpenes** (C_{15}), **diterpenes** (C_{20}), **sesterterpenes** (C_{25}), **triterpenes** (C_{30}) and **tetraterpenes** (C_{40}) (Figure 5.2). Higher polymers are encountered in materials such as rubber. Isoprene itself (Figure 5.1) had been characterized as a decomposition product from various natural cyclic hydrocarbons, and was suggested as the fundamental building block for these compounds, also referred to as 'isoprenoids'. Isoprene is produced naturally but is not involved in the formation of these compounds, and the biochemically active isoprene units were identified as the diphosphate (pyrophosphate) esters **dimethylallyl diphosphate** (**DMAPP**) and **isopentenyl diphosphate** (**IPP**) (Figure 5.2). Relatively few of the natural terpenoids conform exactly to the simple concept of a linear head-to-tail combination of isoprene units as seen with **geraniol** (C_{10}), **farnesol** (C_{15}), and **geranylgeraniol** (C_{20}) (Figure 5.3). **Squalene** (C_{30}) and **phytoene** (C_{40}), although formed entirely of isoprene units, display a tail-to-tail linkage at the centre of the molecules. Most terpenoids are modified further by cyclization reactions, but the head-to-tail arrangement of the units can usually still be recognized, e.g. **menthol**, **bisabolene**, and **taxadiene**. The linear arrangement of

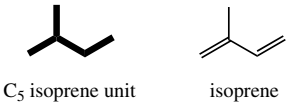

Figure 5.1

isoprene units can be more difficult to appreciate in many other structures when rearrangement reactions have taken place, e.g. steroids, where in addition, several carbons have been lost. Nevertheless, such compounds are formed via regular terpenoid precursors.

Many other natural products contain terpenoid elements in their molecules, in combination with carbon skeletons derived from other sources, such as the acetate and shikimate pathways. Many alkaloids, phenolics, and vitamins discussed in other chapters are examples of this. A particularly common terpenoid fragment in such cases is a single C_5 unit, usually a dimethylallyl substituent, and molecules containing these isolated isoprene units are sometimes referred to as '**meroterpenoids**'. Some examples include furocoumarins (see page 145), rotenoids (see page 155), and ergot alkaloids (see page 368). One should also note that the term '**prenyl**' is in general use to indicate the dimethylallyl substituent. Even macromolecules like proteins can be modified by attaching terpenoid chains. Cysteine residues are alkylated with farnesyl or geranylgeranyl groups, thereby increasing the lipophilicity of the protein and its ability to associate with membranes.

The biochemical isoprene units may be derived by two pathways, by way of intermediates **mevalonic acid** (**MVA**) (Figure 5.4) or 1-deoxy-D-xylulose 5-phosphate (**deoxyxylulose phosphate; DXP**) (Figure 5.6). Mevalonic acid, itself a product of acetate metabolism, had been established as a precursor of the animal sterol cholesterol, and

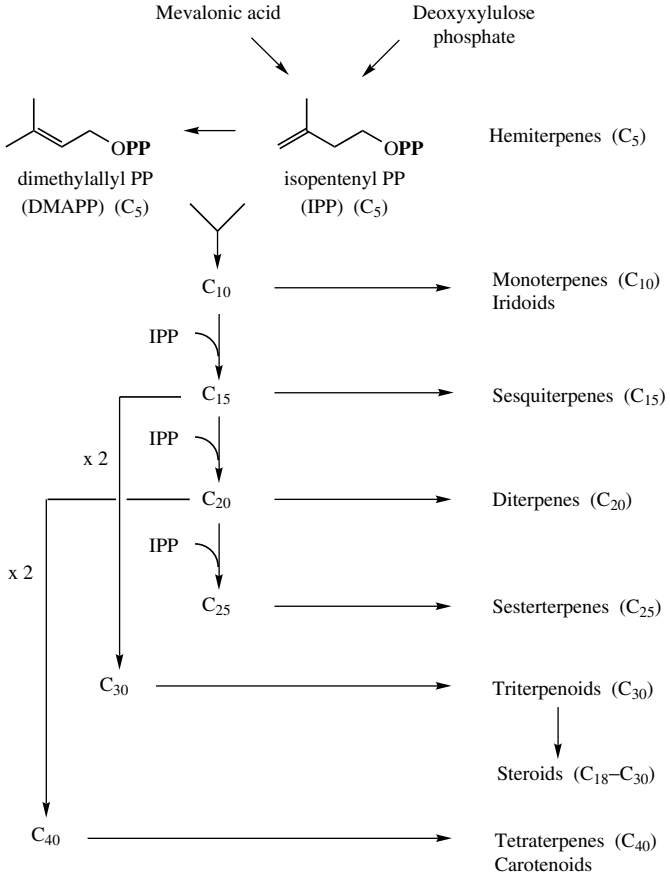

Figure 5.2

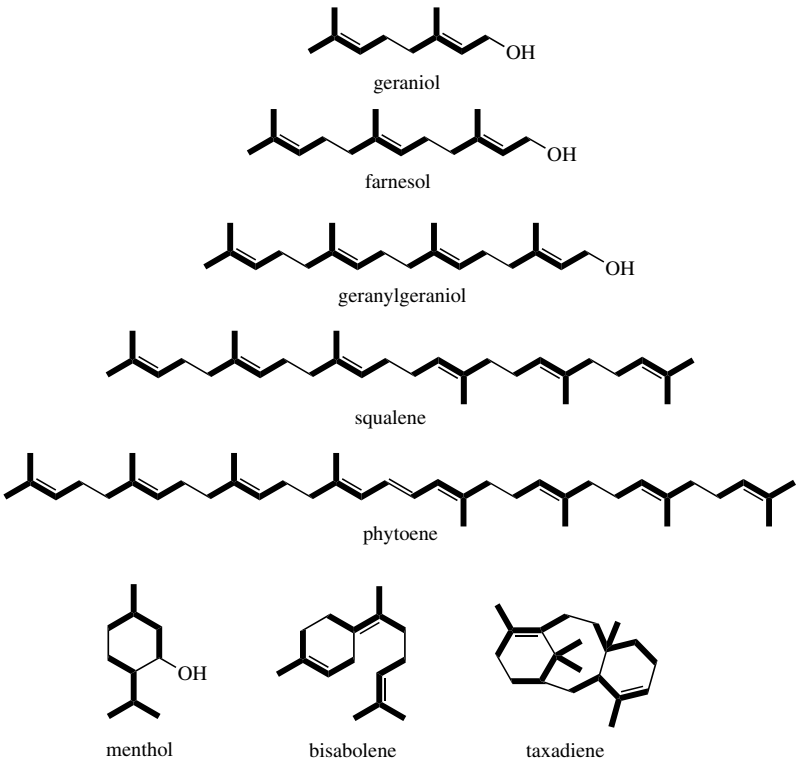

Figure 5.3

the steps leading to and from mevalonic acid were gradually detailed in a series of painstakingly executed experiments. For many years, the early parts of the mevalonate pathway were believed to be common to the whole range of natural terpenoid derivatives, but it has since been discovered that an alternative pathway to IPP and DMAPP exists, via deoxyxylulose phosphate, and that this pathway is probably more widely utilized in nature than is the mevalonate pathway. This pathway is also referred to as the **mevalonate-independent pathway** or the **methylerythritol phosphate pathway**.

Three molecules of acetyl-coenzyme A are used to form **mevalonic acid**. Two molecules combine initially in a Claisen condensation to give acetoacetyl-CoA, and a third is incorporated via a stereospecific aldol addition giving the branched-chain ester **β-hydroxy-β-methylglutaryl-CoA** (**HMG-CoA**) (Figure 5.4). This third acetyl-CoA molecule appears to be bound to the enzyme via a thiol group, and this linkage is subsequently hydrolysed to form the free acid group of HMG-CoA. In the acetate pathway, an acetoacetic acid thioester (bound to the acyl carrier protein) would have been formed using the more nucleophilic thioester of malonic acid. The mevalonate pathway does not use malonyl derivatives and it thus diverges from the acetate pathway at the very first step. In the second step, it should be noted that, on purely chemical grounds, acetoacetyl-CoA is the more acidic substrate, and might be expected to act as the nucleophile rather than the third acetyl-CoA molecule. The enzyme thus achieves what is a less favourable reaction. The conversion of HMG-CoA into (3*R*)-MVA involves a two-step reduction of the thioester group to a primary alcohol, and provides an essentially irreversible and rate-limiting transformation. Drug-mediated inhibition of this enzyme (**HMG-CoA reductase**) can be used to regulate the biosynthesis of mevalonate and ultimately of the steroid cholesterol (see statins, page 112).

The six-carbon compound MVA is transformed into the five-carbon phosphorylated isoprene units in a series of reactions, beginning with

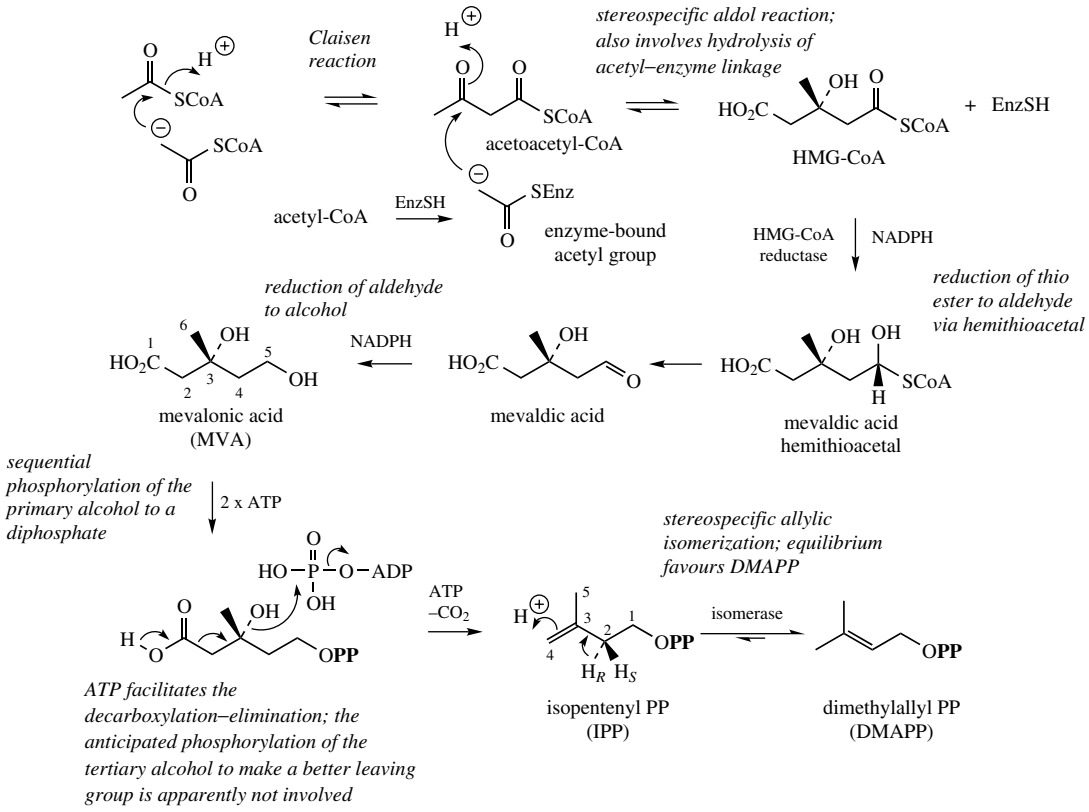

Figure 5.4

phosphorylation of the primary alcohol group. Two different ATP-dependent enzymes are involved, resulting in mevalonic acid diphosphate, and decarboxylation/dehydration then follow to give **IPP**. Whilst a third molecule of ATP is required for this last transformation, there is no evidence for phosphorylation of the tertiary hydroxyl, though this would convert the hydroxyl into a better leaving group. Perhaps ATP assists the loss of the hydroxyl as shown in Figure 5.4. IPP is isomerized to the other isoprene unit, **DMAPP**, by an isomerase enzyme which stereospecifically removes the *pro-R* proton (H_R) from C-2, and incorporates a proton from water on to C-4. Whilst the isomerization is reversible, the equilibrium lies heavily on the side of DMAPP. This conversion generates a reactive electrophile and therefore a good alkylating agent. DMAPP possesses a good leaving group, the diphosphate, and can yield via an S_N1 process an allylic carbocation which is stabilized by charge delocalization (Figure 5.5). In contrast, IPP with its terminal double bond is more likely to act as a nucleophile, especially towards the electrophilic DMAPP. These differing reactivities are the basis of terpenoid biosynthesis, and carbocations feature strongly in mechanistic rationalizations of the pathways.

1-Deoxy-D-xylulose 5-phosphate is formed from the glycolytic pathway intermediates pyruvic acid and glyceraldehyde 3-phosphate with the loss of the pyruvate carboxyl (Figure 5.6). Thiamine diphosphate-mediated decarboxylation of pyruvate

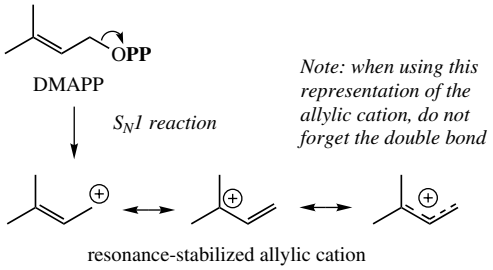

Figure 5.5

(compare page 21) produces an acetaldehyde equivalent bound in the form of an enamine, which reacts as a nucleophile in an addition reaction with the glyceraldehyde 3-phosphate. Subsequent release from the TPP carrier generates **deoxyxylulose phosphate**, which is transformed into **2-*C*-methyl-D-erythritol 4-phosphate** by a rearrangement reaction, conveniently rationalized as a pinacol-like rearrangement (Figure 5.6), coupled with a reduction. The expected aldehyde product from the rearrangement step is not detectable, and the single enzyme catalyses the rearrangement and reduction reactions without release of any intermediate. Analogous rearrangements are seen in the biosynthesis of the amino acids valine, leucine, and isoleucine. The methylerythritol phosphate contains the branched-chain system equivalent to the isoprene unit, but the complete sequence of steps

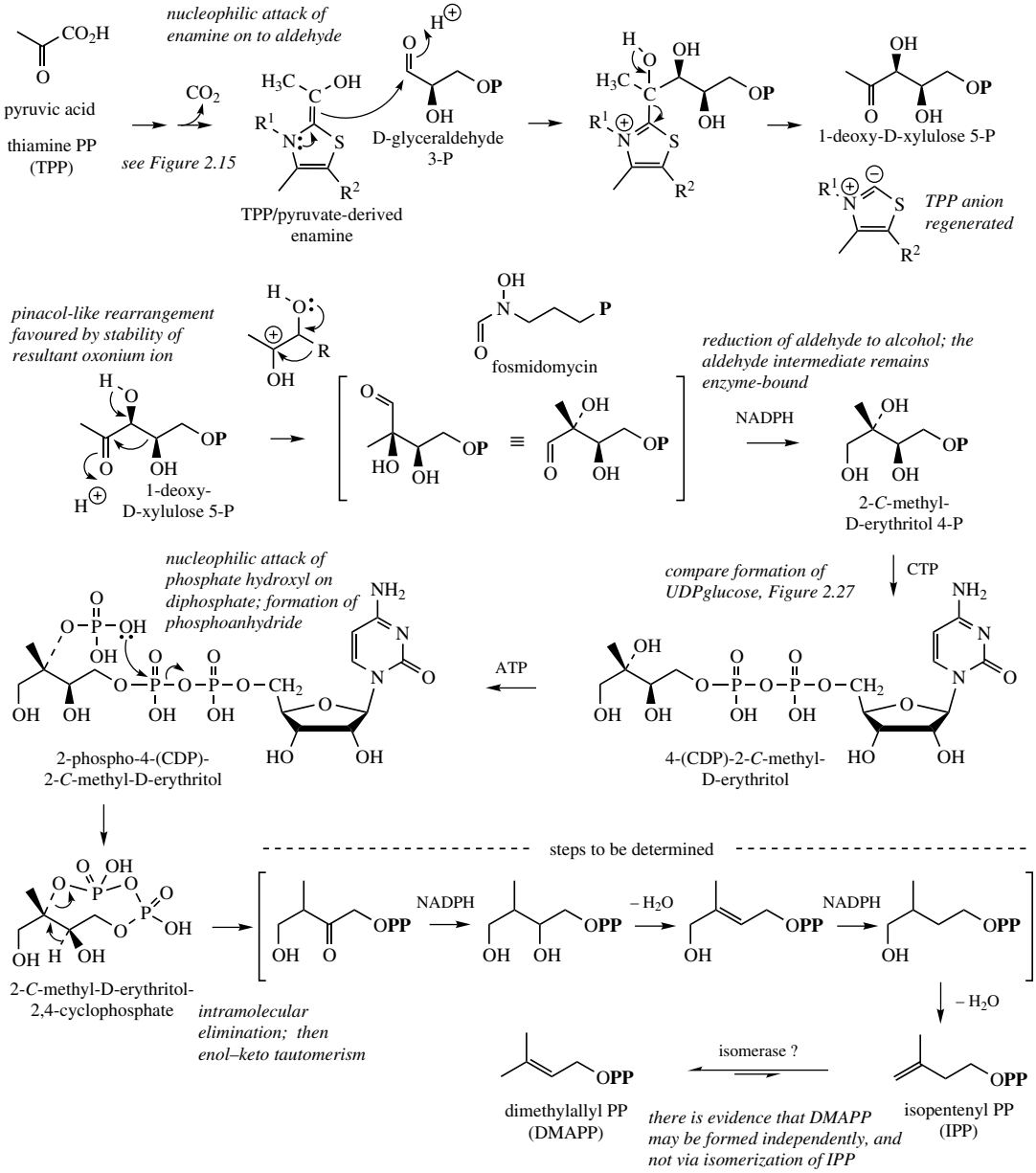

Figure 5.6

leading to the intermediate **isopentenyl phosphate** has yet to be elucidated. Reaction of methylerythritol phosphate with cytidine triphosphate (CTP) produces a cytidine diphospho derivative (compare uridine diphosphoglucose in glucosylation, page 29), which is then phosphorylated via ATP. The resultant 2-phosphate is converted into a cyclic phosphoanhydride with loss of cytidine phosphate. This cyclophosphate, by steps not yet known (a possible sequence is proposed in Figure 5.6), leads to **IPP**, and links the deoxyxylulose pathway with the mevalonate pathway. **DMAPP** may then be derived by isomerism of IPP, or may be produced independently; this also remains to be clarified. Deoxyxylulose phosphate also plays an important role as a precursor of thiamine (vitamin B_1, page 30) and pyridoxol phosphate (vitamin B_6, page 33).

Whether the mevalonate pathway or the deoxyxylulose phosphate pathway supplies isoprene units for the biosynthesis of a particular terpenoid has to established experimentally. Animals appear to lack the deoxyxylulose phosphate pathway, so utilize the mevalonate pathway exclusively. Many other organisms, including plants, are equipped to employ both pathways, often concurrently. In plants, the two pathways appear to be compartmentalized, so that the mevalonate pathway enzymes are localized in the cytosol, whereas the deoxyxylulose phosphate pathway enzymes are found in chloroplasts. Accordingly, triterpenoids and steroids (cytosolic products) are formed by the mevalonate pathway, whilst most other terpenoids are formed in the chloroplasts and are deoxyxylulose phosphate derived. Of course there are exceptions. There are also examples where the two pathways can supply different portions of a molecule, or where there is exchange of late-stage common intermediates between the two pathways resulting in a contribution of isoprene units from each pathway. In the following part of this chapter, these complications will not be considered further, and in most cases there is no need to consider the precise source of the isoprene units. The only area of special pharmacological interest where the early pathway is of particular concern is steroid biosynthesis, which appears to be from mevalonate in the vast majority of organisms. Thus, inhibitors of the mevalonate pathway enzyme HMG-CoA reductase will reduce steroid production, but will not affect the formation of terpenoids derived via deoxyxylulose phosphate. Equally, it is possible to inhibit terpenoid production without affecting steroid formation by the use of deoxyxyxlulose phosphate pathway inhibitors, such as the antibiotic **fosmidomycin** from *Streptomyces lavendulae*. This acts as an analogue of the rearrangement intermediate (Figure 5.6). Regulation of cholesterol production in humans is an important health concern (see page 236).

HEMITERPENES (C_5)

IPP and DMAPP are reactive hemiterpene intermediates in the pathways leading to more complex terpenoid structures. They are also used as alkylating agents in the formation of meroterpenoids as indicated above, but examples of these structures are discussed under the section appropriate to the major substructure, e.g. alkaloids, shikimate, acetate. Relatively few true hemiterpenes are produced in nature, with **isoprene**, a volatile compound which is released by many species of plants, especially trees, being the notable example. Isoprene is formed by loss of a proton from the allylic cation (Figure 5.7).

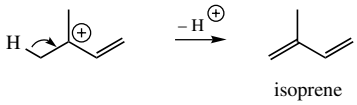

Figure 5.7

MONOTERPENES (C_{10})

Combination of DMAPP and IPP via the enzyme prenyl transferase yields **geranyl diphosphate** (**GPP**) (Figure 5.8). This is believed to involve ionization of DMAPP to the allylic cation, addition to the double bond of IPP, followed by loss of a proton. Stereochemically, the proton lost (H_R) is analogous to that lost on the isomerization of IPP to DMAPP. This produces a monoterpene diphosphate, geranyl PP, in which the new double bond is *trans* (*E*). **Linalyl PP** and **neryl PP** are isomers of geranyl PP, and are likely to be formed from geranyl PP by ionization to the allylic cation, which can thus allow a change in attachment of the diphosphate group (to the tertiary carbon in linalyl

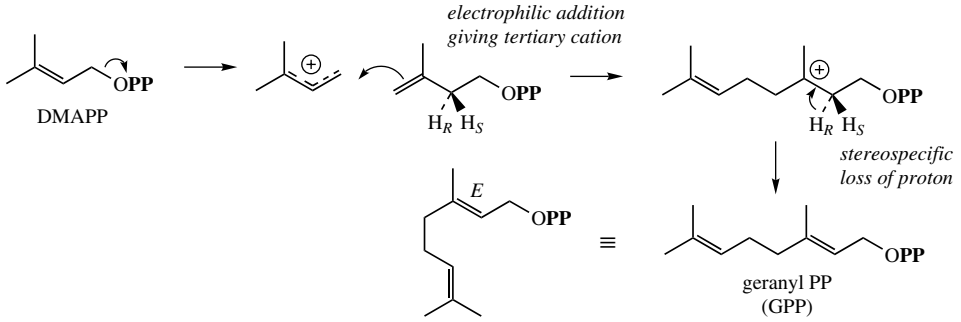

Figure 5.8

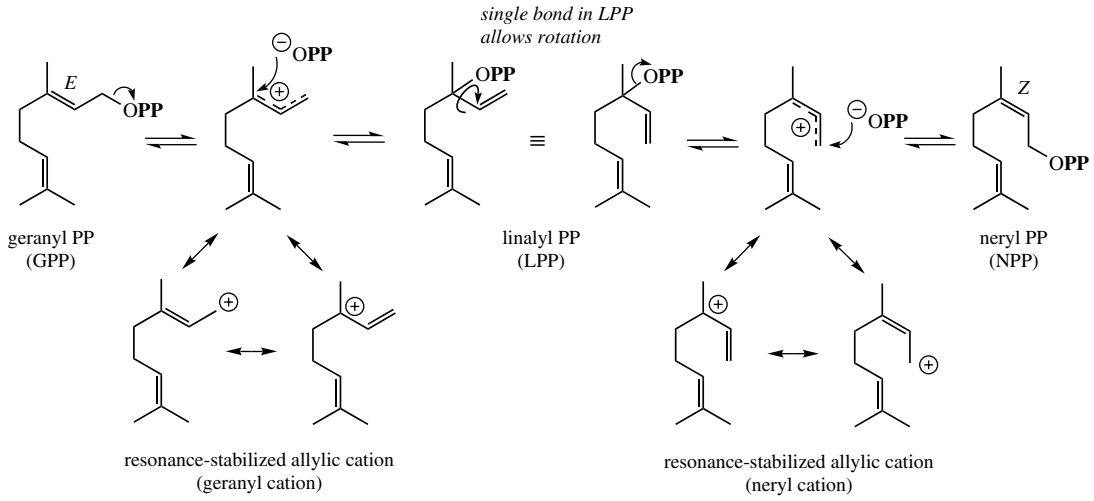

Figure 5.9

PP) or a change in stereochemistry at the double bond (to *Z* in neryl PP) (Figure 5.9). These three compounds, by relatively modest changes, can give rise to a range of linear monoterpenes found as components of volatile oils used in flavouring and perfumery (Figure 5.10). The resulting compounds may be hydrocarbons, alcohols, aldehydes, or perhaps esters, especially acetates.

The range of monoterpenes encountered is extended considerably by cyclization reactions, and monocyclic or bicyclic systems can be created. Some of the more important examples of these ring systems are shown in Figure 5.11. Such cyclizations would not be expected to occur with the precursor geranyl diphosphate, the *E* stereochemistry of the double bond being unfavourable for ring formation (Figure 5.9). Neryl PP or linalyl PP, however, do have favourable stereochemistry, and either or both of these would seem more immediate precursors of the monocyclic menthane system, formation of which could be represented as shown in Figure 5.12, generating a carbocation (termed menthyl or α-terpinyl) having the menthane skeleton. It has been found that monoterpene cyclase enzymes are able to accept all three diphosphates, with linalyl PP being the best substrate, and it appears they have the ability to isomerize the substrates initially as well as to cyclize them. It is convenient therefore to consider the species involved in the cyclization as the delocalized allylic cation tightly bound to the diphosphate anion, and bond formation follows due to the proximity of the π-electrons of the double bond (Figure 5.12).

In Chapter 2, the possible fates of carbocations were discussed. These include quenching with nucleophiles (especially water), loss of a proton,

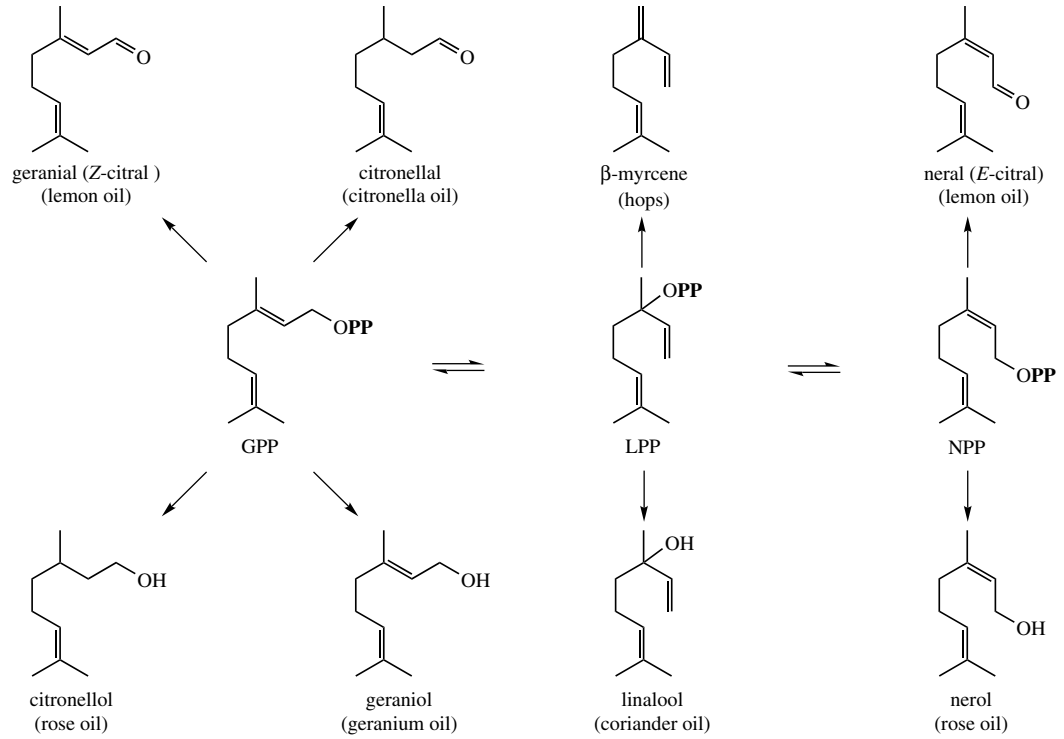

Figure 5.10

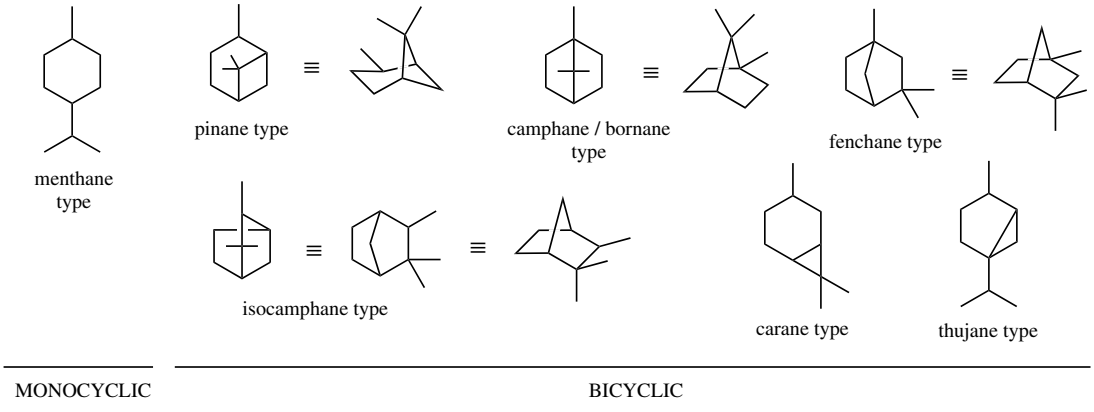

Figure 5.11

cyclization, and the possibility that Wagner–Meerwein rearrangements might occur (see page 15). All feature strongly in terpenoid biosynthesis. The newly generated menthyl cation could be quenched by attack of water, in which case the alcohol **α-terpineol** would be formed, or it could lose a proton to give **limonene** (Figure 5.13). Alternatively, folding the cationic side-chain towards the double bond (via the surface characteristics of the enzyme) would allow a repeat of the cyclization mechanism, and produce bicyclic bornyl and pinyl cations, according to which end of the double bond was involved in forming the new bonds (Figure 5.14). **Borneol** would result from quenching of the bornyl cation with water, and then oxidation of the secondary

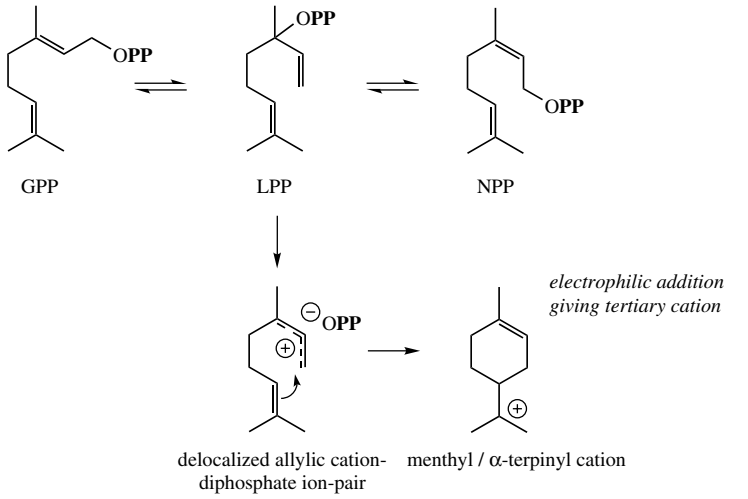

Figure 5.12

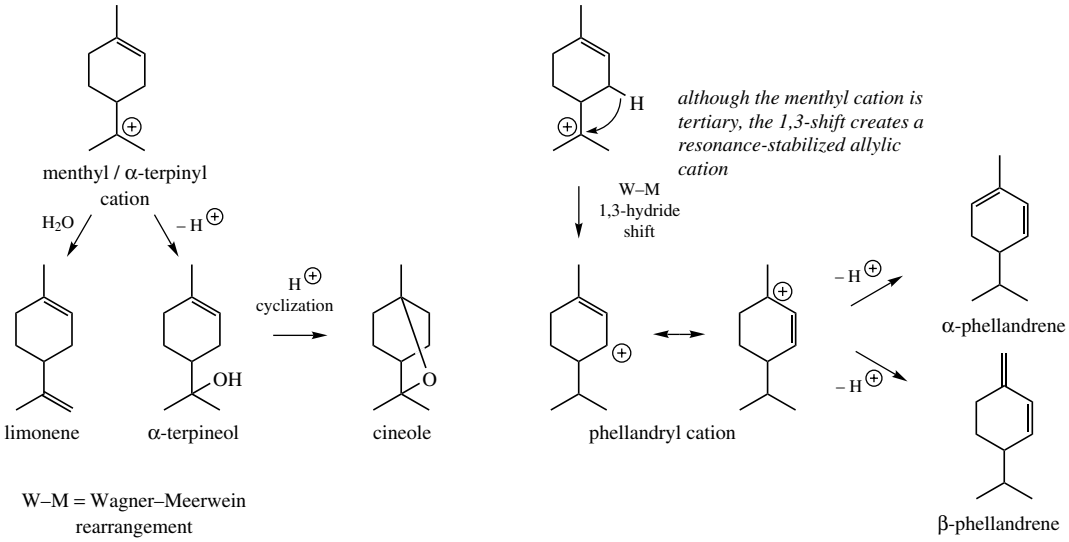

Figure 5.13

alcohol could generate the ketone **camphor**. As an alternative to discharging the positive charge by adding a nucleophile, loss of a proton would generate an alkene. Thus **α-pinene** and **β-pinene** arise by loss of different protons from the pinyl cation, producing the double bonds as cyclic or exocyclic respectively. A less common termination step involving loss of a proton is the formation of a cyclopropane ring as exemplified by **3-carene** and generation of the carane skeleton.

The chemistry of terpenoid formation is essentially based on the reactivity of carbocations, even though, in nature, these cations may not exist as such discrete species, but rather as tightly bound ion pairs with a counter-anion, e.g. diphosphate. The analogy with carbocation chemistry is justified, however, since a high proportion of natural terpenoids have skeletons which have suffered rearrangement processes. Rearrangements of the Wagner–Meerwein type (see page 15), in which carbons or hydride migrate to achieve enhanced stability for the cation via tertiary against secondary character, or by reduction of ring strain, give a mechanistic rationalization for

the biosynthetic pathway. The menthyl cation, although it is a tertiary, may be converted by a 1,3-hydride shift into a favourable resonance-stabilized allylic cation (Figure 5.13). This allows the formation of **α-** and **β-phellandrenes** by loss of a proton from the phellandryl carbocation. The bicyclic pinyl cation, with a strained four-membered ring, rearranges to the less strained five-membered fenchyl cation (Figure 5.14), a change which presumably more than makes up for the unfavourable tertiary to secondary carbocation transformation. This produces the fenchane skeleton, exemplified by **fenchol** and **fenchone**. The isocamphyl tertiary carbocation is formed from the bornyl secondary carbocation by a Wagner–Meerwein rearrangement, and so leads to **camphene**. A hydride shift converting the menthyl cation into the terpinen-4-yl cation only changes one tertiary carbocation system for another, but allows formation of **α-terpinene**, **γ-terpinene**, and the α-terpineol isomer, **terpinen-4-ol**. A further cyclization reaction on the terpinen-4-yl cation generates the thujane skeleton, e.g. **sabinene** and **thujone**. Terpinen-4-ol is the primary antibacterial component of tea tree oil from *Melaleuca alternifolia* (Myrtaceae); thujone has achieved notoriety as the neurotoxic agent in wormwood oil from *Artemisia absinthium* (Compositae/Asteraceae) used in preparation of the drink absinthe, now banned in most countries.

So far, little attention has been given to the stereochemical features of the resultant monoterpene. Individual enzyme systems present in a particular organism will, of course, control the folding of the substrate molecule and thus define the stereochemistry of the final product. Most

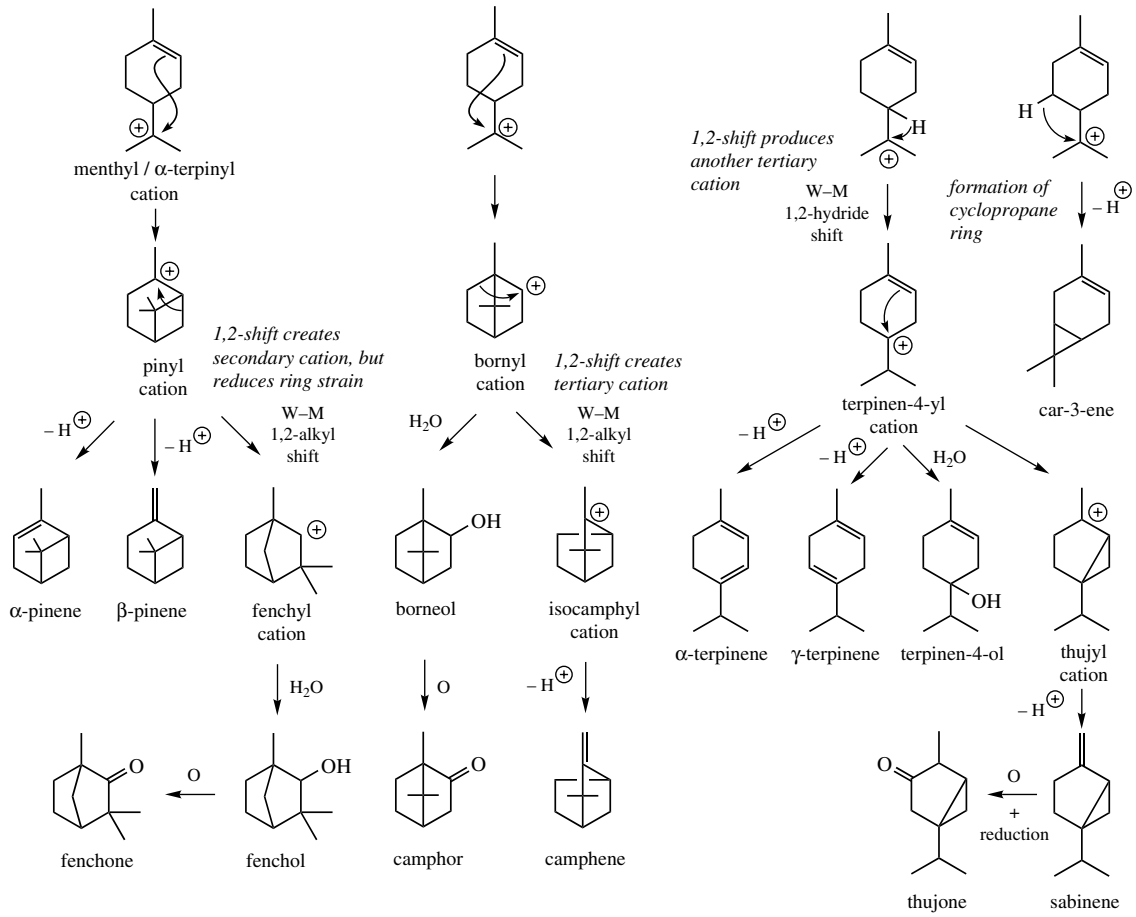

Figure 5.14

monoterpenes are optically active, and there are many examples known where enantiomeric forms of the same compound can be isolated from different sources, e.g. (+)-**camphor** in sage (*Salvia officinalis*; Labiatae/Lamiaceae) and (−)-camphor in tansy (*Tanacetum vulgare*; Compositae/Asteraceae), or (+)-**carvone** in caraway (*Carum carvi*; Umbelliferae/Apiaceae) and (−)-carvone in spearmint (*Mentha spicata*; Labiatae/Lamiaceae). There are also examples of compounds found in both enantiomeric forms in the same organism, examples being (+)- and (−)-**limonene** in peppermint (*Mentha* x *piperita*; Labiatae/Lamiaceae) and (+)- and (−)-**α-pinene** in pine (*Pinus* species; Pinaceae). The individual enantiomers can produce different biological responses, especially towards olfactory receptors in the nose. Thus the characteristic caraway odour is due to (+)-carvone whereas (−)-carvone smells of spearmint. (+)-Limonene smells of oranges whilst (−)-limonene resembles the smell of lemons. The origins of the different enantiomeric forms of limonene and α-pinene are illustrated in Figure 5.15. This shows the precursor geranyl PP being folded in two mirror image conformations, leading to formation of the separate enantiomers of linalyl PP. Analogous carbocation reactions will then explain production of the optically active monoterpenes. Where a single plant produces both enantiomers, it appears to contain two separate enzyme systems each capable of elaborating a single enantiomer. Furthermore, a single enzyme typically accepts geranyl PP as substrate, catalyses the isomerization to linalyl PP, and converts this into a final product without the release of free intermediates. Sometimes, multiple products in varying

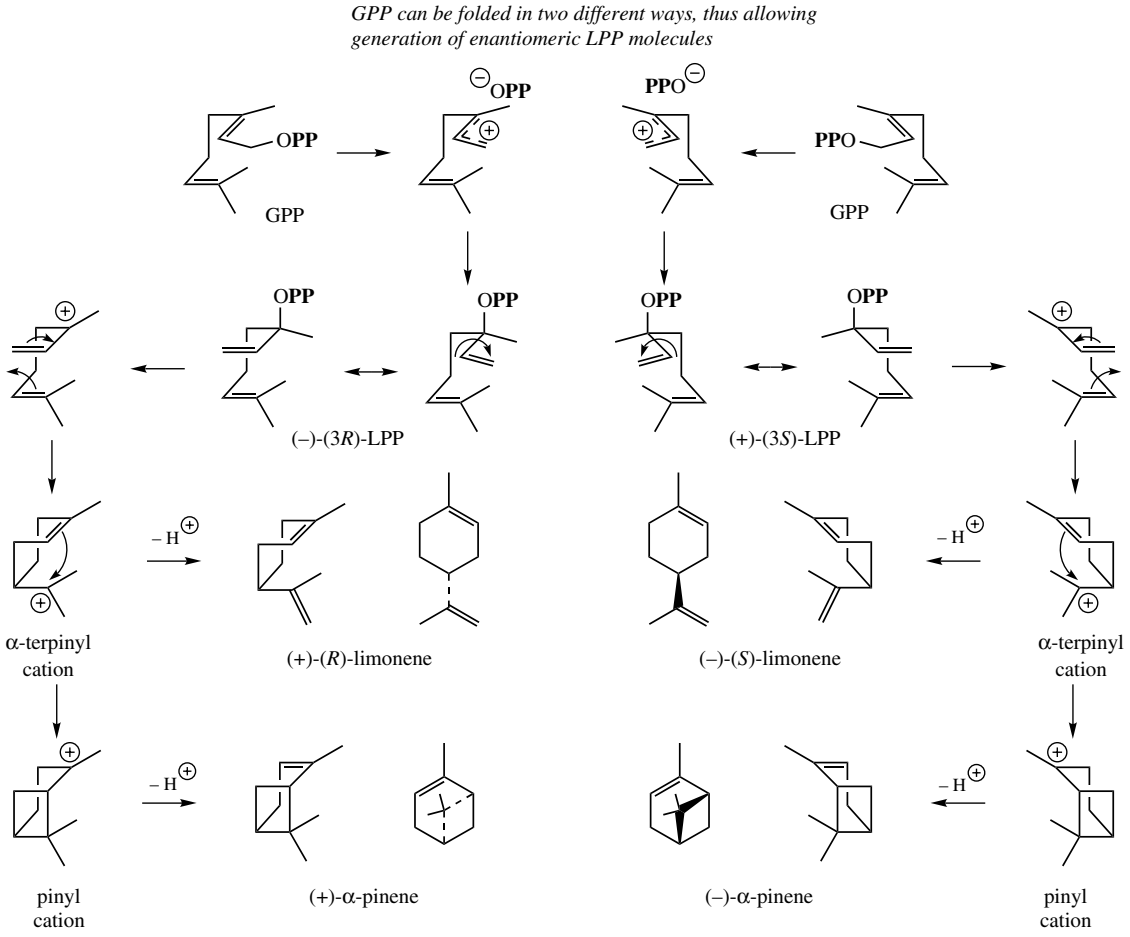

Figure 5.15

amounts, e.g. limonene, myrcene, α-pinene, and β-pinene, are synthesized by a single enzyme, reflecting the common carbocation chemistry involved in these biosyntheses, and suggesting the enzyme is predominantly providing a suitable environment for the folding and cyclization of the substrate. Subsequent reactions such as oxidation of an alcohol to a ketone, e.g. borneol to **camphor** (Figure 5.14), or heterocyclic ring formation in the conversion of α-terpineol into **cineole** (Figure 5.13), require additional enzyme systems.

In other systems, a particular structure may be found as a mixture of diastereoisomers. Peppermint (*Mentha* x *piperita*; Labiatae/Lamiaceae) typically produces (−)-**menthol**, with smaller amounts of the stereoisomers (+)-**neomenthol**, (+)-**isomenthol**, and (+)-**neoisomenthol**, covering four of the possible eight stereoisomers (Figure 5.16). Oils from various *Mentha* species also contain significant amounts of ketones, e.g. (−)-**menthone**, (+)-**isomenthone**, (−)-**piperitone**, or (+)-**pulegone**. The metabolic relationship of

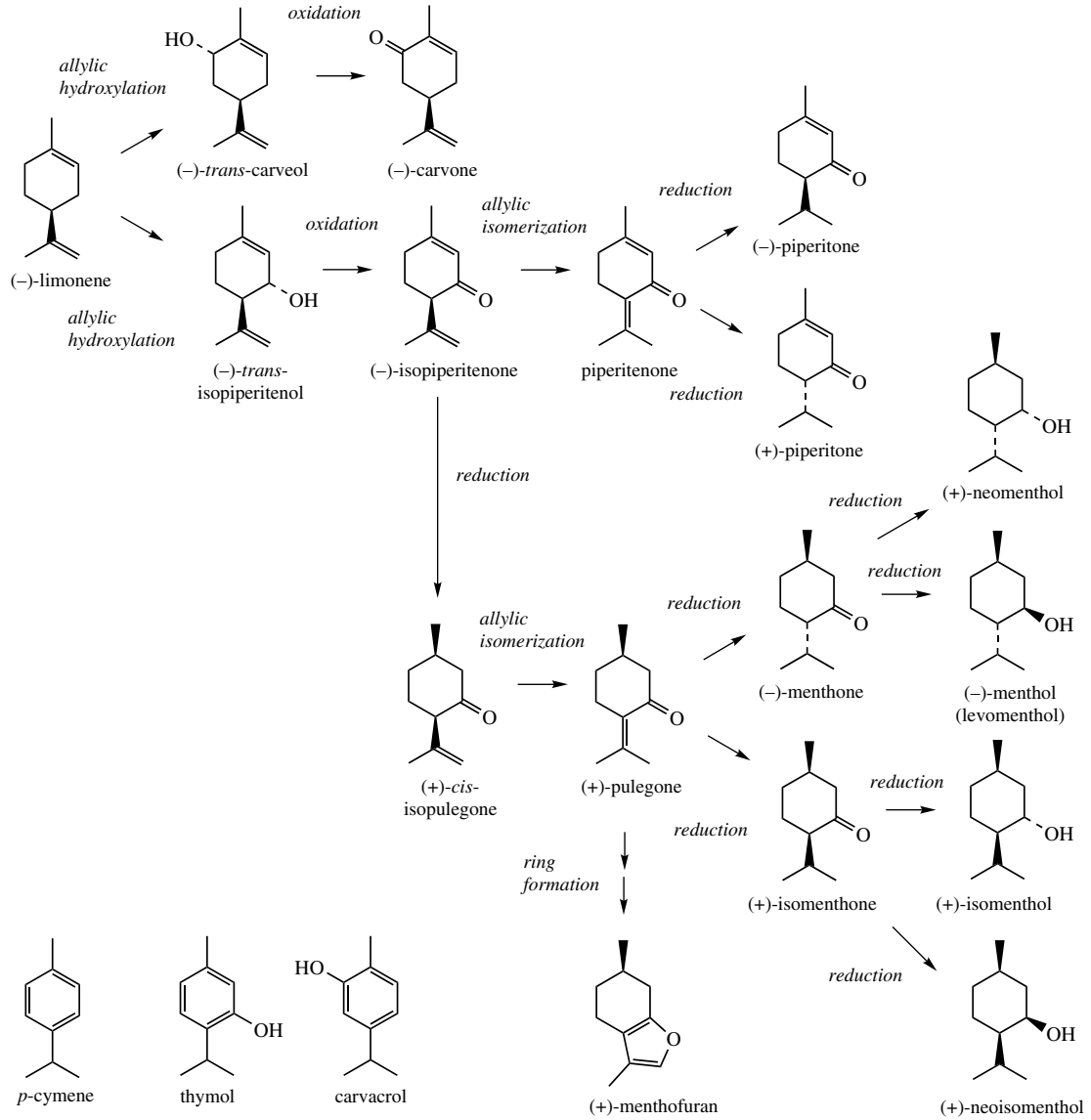

Figure 5.16

Table 5.1 Volatile oils containing principally terpenoid compounds

Major volatile oils have been divided into two groups. Those oils containing principally chemicals which are terpenoid in nature and which are derived by the deoxyxylulose phosphate pathway are given in Table 5.1 below. Oils which are composed predominantly of aromatic compounds which are derived via the shikimate pathway are listed in Table 4.1 on page 139. The introductory remarks to Table 4.1 are also applicable to Table 5.1.

Oils	Plant source	Plant part used	Oil content (%)	Major constituents with typical (%) composition	Uses, notes
Bergamot	*Citrus aurantium* ssp. *bergamia* (Rutaceae)	fresh fruit peel (expression)	0.5	limonene (42) linalyl acetate (27) γ-terpinene (8) linalool (7)	flavouring, aromatherapy, perfumery also contains the furocoumarin bergapten (up to 5%) and may cause severe photosensitization (see page 146)
Camphor oil	*Cinnamomum camphora* (Lauraceae)	wood	1–3	camphor (27–45) cineole (4–21) safrole (1–18)	soaps
Caraway	*Carum carvi* (Umbelliferae/Apiaceae)	ripe fruit	3–7	(+)-carvone (50–70) limonene (47)	flavour, carminative, aromatherapy
Cardamom	*Elettaria cardamomum* (Zingiberaceae)	ripe fruit	3–7	α-terpinyl acetate (25–35) cineole (25–45) linalool (5)	flavour, carminative, ingredient of curries, pickles

(*Continued overleaf*)

Table 5.1 (*Continued*)

Oils	Plant source	Plant part used	Oil content (%)	Major constituents with typical (%) composition	Uses, notes
Chamomile (Roman chamomile)	*Chamaemelum nobile* (*Anthemis nobilis*) (Compositae/Asteraceae)	dried flowers	0.4–1.5	aliphatic esters of angelic, tiglic, isovaleric, and isobutyric acids (75–85) small amounts of monoterpenes	flavouring, aromatherapy blue colour of oil is due to chamazulene (see page 196)
Citronella	*Cymbopogon winterianus* *C. nardus* (Graminae/Poaceae)	fresh leaves	0.5–1.2	(+)-citronellal (25–55) geraniol (+)-citronellol (10–15) geranyl acetate (8) (20–40)	perfumery, aromatherapy, insect repellent
Coriander	*Coriandrum sativum* (Umbelliferae/Apiaceae)	ripe fruit	0.3–1.8	(+)-linalool (60–75) γ-terpinene (5) α-pinene (5) camphor (5)	flavour, carminative
Dill	*Anethum graveolens* (Umbelliferae/Apiaceae)	ripe fruit	3–4	(+)-carvone (40–65)	flavour, carminative
Eucalyptus	*Eucalyptus globulus* *E. smithii* *E. polybractea* (Myrtaceae)	fresh leaves	1–3	cineole (= eucalyptol) (70–85) α-pinene (14)	flavour, antiseptic, aromatherapy
Eucalyptus (lemon-scented)	*Eucalyptus citriodora* (Myrtaceae)	fresh leaves	0.8	citronellal (65–85)	perfumery

Ginger	*Zingiber officinale* (Zingiberaceae)	dried rhizome	1.5–3	zingiberene (34) β-sesquiphellandrene (12) β-phellandrene (8) β-bisabolene (6)	flavouring the main pungent principles in ginger (gingerols) are not volatile
Juniper	*Juniperus communis* (Cupressaceae)	dried ripe berries	0.5–2	α-pinene (45–80) myrcene (10–25) limonene (1–10) sabinene (0–15)	flavouring, antiseptic, diuretic, aromatherapy juniper berries provide the flavouring for gin
Lavender	*Lavandula angustifolia L. officinalis* (Labiatae/Lamiaceae)	fresh flowering tops	0.3–1	linalyl acetate (25–45) linalool (25–38)	perfumery, aromatherapy inhalation produces mild sedation and facilitates sleep
Lemon	*Citrus limon* (Rutaceae)	dried peel from fruit (expression)	0.1–3	(+)-limonene (60–80) β-pinene (8–12) γ-terpinene (8–10) citral (= geranial + neral) (2–3)	flavouring, perfumery, aromatherapy terpeneless lemon oil is obtained by removing much of the terpenes under reduced pressure; this oil is more stable and contains 40–50% citral

(*Continued overleaf*)

Table 5.1 (*Continued*)

Oils	Plant source	Plant part used	Oil content (%)	Major constituents with typical (%) composition	Uses, notes
Lemon-grass	*Cymbopogon citratus* (Graminae/Poaceae)	fresh leaves	0.1–0.3	citral (= geranial + neral) (50–85)	perfumery, aromatherapy
Matricaria (German chamomile)	*Matricaria chamomilla (Chamomilla recutica)* (Compositae/Asteraceae)	dried flowers	0.3–1.5	(−)-α-bisabolol (10–25%) bisabolol oxides A and B (10–25%) chamazulene (1–15%)	flavouring dark blue colour of oil is due to chamazulene
Orange (bitter)	*Citrus aurantium* ssp. *amara* (Rutaceae)	dried peel from fruit (expression)	0.5–2.5	(+)-limonene (92–94) myrcene (2)	flavouring, aromatherapy the main flavour and odour comes from the minor oxygenated components terpeneless orange oil is obtained by removing much of the terpenes under reduced pressure; this oil contains about 20% aldehydes, mainly decanal
Orange (sweet)	*Citrus sinensis* (Rutaceae)	dried peel from fruit (expression)	0.3	(+)-limonene (90–95) myrcene (2)	flavouring, aromatherapy

					the main flavour and odour comes from the minor oxygenated components terpeneless orange oil is obtained by removing much of the terpenes under reduced pressure; this oil contains about 20% aldehydes, mainly octanal and decanal.
Orange flower (Neroli)	*Citrus aurantium* ssp. *amara* (Rutaceae)	fresh flowers	0.1	linalool (36) β-pinene (16) limonene (12) linalyl acetate (6)	flavour, perfumery, aromatherapy
Peppermint	*Mentha* x *piperita* (Labiatae/Lamiaceae)	fresh leaf	1–3	menthol (30–50) menthone (15–32) menthyl acetate (2–10), menthofuran (1–9)	flavouring, carminative, aromatherapy
Pine	*Pinus palustris* or other *Pinus* species (Pinaceae)	needles, twigs		α-terpineol (65)	antiseptic, disinfectant, aromatherapy
Pumilio pine	*Pinus mugo* ssp. *pumilio* (Pinaceae)	needles	0.3–0.4	α- and β-phellandrene (60) α- and β-pinene (10–20) bornyl acetate (3–10)	inhalant the minor components bornyl acetate and borneol are mainly responsible for the aroma

(*Continued overleaf*)

Table 5.1 (*Continued*)

Oils	Plant source	Plant part used	Oil content (%)	Major constituents with typical (%) composition	Uses, notes
Rose (attar of rose, otto of rose)	*Rosa damascena*, *gallica, R. alba*, and *R. R. centifolia* (Rosaceae)	fresh flowers	0.02–0.03	citronellol (36) geraniol (17) 2-phenylethanol (3) $C_{14}-C_{23}$ straight chain hydrocarbons (25)	perfumery, aromatherapy
Rosemary	*Rosmarinus officinalis* (Labiatae/Lamiaceae)	fresh flowering tops	1–2	cineole (15–45) α-pinene (10–25) camphor (10–25) β-pinene (8)	perfumery, aromatherapy
Sage	*Salvia officinalis* (Labiatae/Lamiaceae)	fresh flowering tops	0.7–2.5	thujone (40–60) camphor (5–22) cineole (5–14) β-caryophyllene (10) limonene (6)	aromatherapy, food flavouring
Sandalwood	*Santalum album* (Santalaceae)	heartwood	4.5–6.3	sesquiterpenes: α-santalol (50) β-santalol (21)	perfumery, aromatherapy

Spearmint	*Mentha spicata* (Labiatae/Lamiaceae)	fresh leaf	1–2	(−)-carvone (50–70) (−)-limonene (2–25)	flavouring, carminative, aromatherapy
Tea tree	*Melaleuca alternifolia* (Myrtaceae)	fresh leaf	1.8	terpinen-4-ol (30–45) γ-terpinene (10–28) α-terpinene (5–13) *p*-cymene (0.5–12) cineole (0.5–10) α-terpineol (1.5–8)	antiseptic, aromatherapy an effective broad spectrum antiseptic widely used in creams, cosmetics, toiletries
Thyme	*Thymus vulgaris* (Labiatae/Lamiaceae)	fresh flowering tops	0.5–2.5	thymol (40) *p*-cymene (30) linalool (7) carvacrol (1)	antiseptic, aromatherapy, food flavouring
Turpentine oil	*Pinus palustris* and other *Pinus* species (Pinaceae)	distillation of the resin (turpentine) secreted from bark		(+)- and (−)-α-pinene (35:65) (60–70) β-pinene (20–25)	counter-irritant, important source of industrial chemicals residue from distillation is colophony (rosin), composed chiefly of diterpene acids (abietic acids, see page 209)

these various compounds has been established as in Figure 5.16, which illustrates how the stereochemistry at each centre can be established by stereospecific reduction processes on double bonds or carbonyl groups. The pathway also exemplifies that oxygen functions can be introduced into the molecule at positions activated by adjacent double bonds (allylic oxidation), as well as being introduced by quenching of carbocations with water. Thus limonene is a precursor of **carvone** (the main constituent of spearmint oil from *Mentha spicata*) as well as menthone and piperitone, initial hydroxylation occurring at an alternative allylic site on the ring. **Menthofuran** exemplifies a further oxidative modification generating a heterocyclic ring. Both pulegone and menthofuran are considered hepatotoxic. Pulegone is a major constituent of oil of pennyroyal from *Mentha pulegium*, which has a folklore history as an abortifacient. Pulegone is metabolized in humans first to menthofuran, and then to electrophilic metabolites that form adducts with cellular proteins (compare pyrrolizidine alkaloids, page 305).

***p*-Cymene**, and the phenol derivatives **thymol** and **carvacrol** (Figure 5.16) found in thyme (*Thymus vulgaris*; Labiatae/Lamiaceae), are representatives of a small group of aromatic compounds that are produced in nature from isoprene units, rather than by the much more common routes to aromatics involving acetate or shikimate (see also cannabinol, page 85, and gossypol, page 200). These compounds all possess the carbon skeleton typical of monocyclic monoterpenes, and their structural relationship to limonene and the more common oxygenated monoterpenes such as menthone or carvone suggests pathways in which additional dehydrogenation reactions are involved.

Data on volatile oils containing terpenoid constituents isolated from these and other plant materials are given in Table 5.1. Volatile oils in which the main components are aromatic and derived from the shikimate pathway are listed in Table 4.1, page 139.

IRREGULAR MONOTERPENES

A number of natural monoterpene structures contain carbon skeletons which, although obviously derived from isoprene C_5 units, do not seem to fit the regular head-to-tail coupling mechanism, e.g. those in Figure 5.17. These structures are termed irregular monoterpenes and seem to be limited almost exclusively to members of the plant family the Compositae/Asteraceae. Allowing for possible rearrangements, the two isoprene units appear to have coupled in another manner and this is borne out by information available on their biosynthesis, though this is far from fully understood. Thus, although DMAPP and IPP are utilized in their biosynthesis, geranyl PP and neryl PP do not appear to be involved. Pre-eminent amongst these structures are **chrysanthemic acid** and **pyrethric acid** (Figure 5.18), found in ester form as the **pyrethrins*** (pyrethrins, cinerins, and jasmolins, Figure 5.18), which are valuable insecticidal components in pyrethrum flowers, the flower heads of *Chrysanthemum cinerariaefolium* (Compositae/Asteraceae). These cyclopropane structures are readily recognizable as derived from two isoprene units, and a mechanism for the derivation of chrysanthemic acid is given in Figure 5.19 (compare this mechanism with that involved in the formation of presqualene PP during steroid biosynthesis, page 214). This invokes two DMAPP units joining by a modification of the standard mechanism, with termination achieved by cyclopropane ring formation. Little is known about the origins of **pyrethrolone**, **cinerolone**, and **jasmolone** (Figure 5.18), the alcohol portions of the pyrethrins, though it is possible that these are cyclized and modified fatty acid derivatives, the cyclization resembling the biosynthetic pathway to prostaglandins (see page 53). Thus, α-linolenic acid via 12-oxophytodienoic acid could be the precursor of jasmolone, with β-oxidation and then decarboxylation accounting for the chain shortening (Figure 5.20). Certainly, this type of

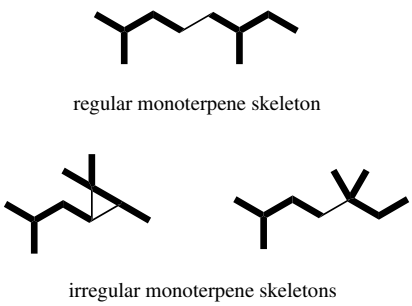

Figure 5.17

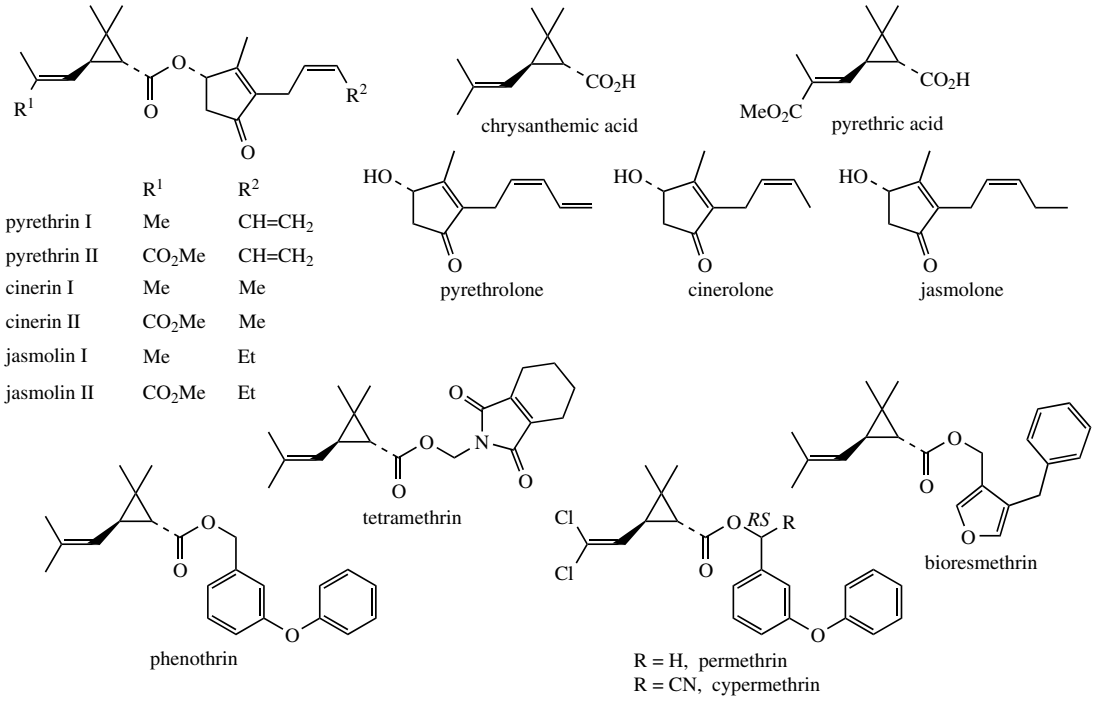

Figure 5.18

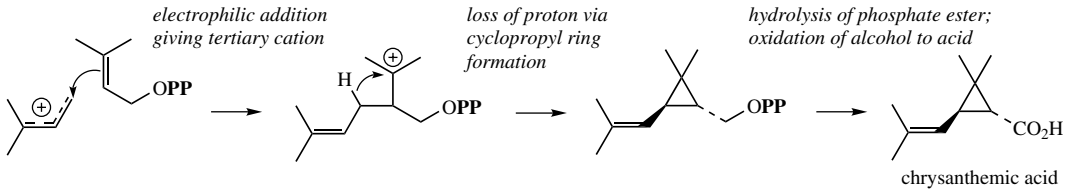

Figure 5.19

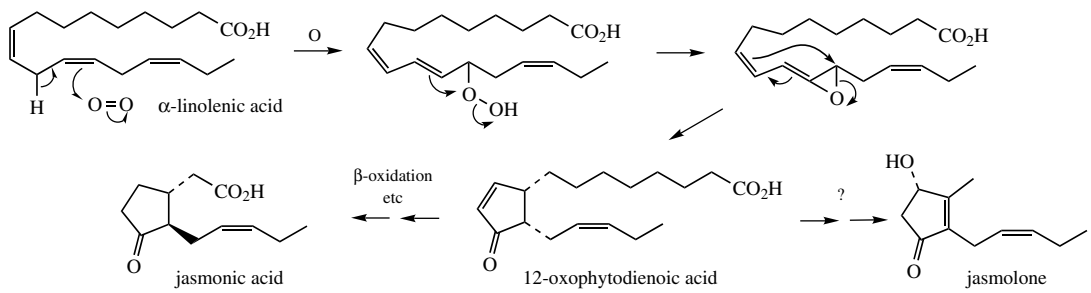

Figure 5.20

pathway operates in the formation of **jasmonic acid** (Figure 5.20), which forms part of a general signalling system in plants, particularly the synthesis of secondary metabolites in response to wounding or microbial infection.

IRIDOIDS (C_{10})

The **iridane** skeleton (Figure 5.21) found in **iridoids** is monoterpenoid in origin and contains a cyclopentane ring which is usually fused

Pyrethrins

The **pyrethrins** are valuable insecticidal components of pyrethrum flowers, *Chrysanthemum cinerariaefolium* (= *Tanacetum cinerariifolium*) (Compositae/Asteraceae). The flowers are harvested just before they are fully expanded, and usually processed to an extract. Pyrethrum cultivation is conducted in East Africa, especially Kenya, and more recently in Ecuador and Australia. The natural pyrethrins are used as a constituent of insect sprays for household use and as post-harvest insecticides, having a rapid action on the nervous system of insects, whilst being biodegradable and non-toxic to mammals, though they are toxic to fish and amphibians. This biodegradation, initiated by air and light, means few insects develop resistance to the pyrethrins, but it does limit the lifetime of the insecticide under normal conditions to just a few hours.

The flowers may contain 0.7–2% of pyrethrins, representing about 25–50% of the extract. A typical pyrethrin extract contains pyrethrin I (35%), pyrethrin II (32%), cinerin I (10%), cinerin II (14%), jasmolin I (5%), and jasmolin II (4%), which structures represent esters of chrysanthemic acid or pyrethric acid with the alcohols pyrethrolone, cinerolone, and jasmolone (Figure 5.18). Pyrethrin I is the most insecticidal component, with pyrethrin II providing much of the rapid knock-down (paralysing) effect. A wide range of synthetic pyrethroid analogues, e.g. **bioresmethrin**, **tetramethrin**, **phenothrin**, **permethrin**, and **cypermethrin** (Figure 5.18), have been developed, which have increased lifetimes up to several days and greater toxicity towards insects. These materials have become widely used household and agricultural insecticides. Tetramethrin, bioresmethrin, and phenothrin are all esters of chrysanthemic acid but with a modified alcohol portion, providing improvements in knock-down effect and in insecticidal activity. Replacement of the terminal methyls of chrysanthemic acid with chlorine atoms, e.g. permethrin, conferred greater stability towards air and light, and opened up the use of pyrethroids in agriculture. Inclusion of a cyano group in the alcohol portion as in cypermethrin improved insecticidal activity several-fold. Modern pyrethroids now have insecticidal activities over a thousand times that of pyrethrin I, whilst maintaining extremely low mammalian toxicity. Permethrin and phenothrin are employed against skin parasites such as head lice.

to a six-membered oxygen heterocycle, e.g. **nepetalactone** from catmint *Nepeta cataria* (Labiatae/Lamiaceae), a powerful attractant and stimulant for cats. The iridoid system arises from geraniol by a type of folding (Figure 5.22) which is different from that already encountered with monoterpenoids, and also different is the lack of phosphorylated intermediates and subsequent carbocation mechanism in its formation. The fundamental cyclization to **iridodial** is formulated as attack of hydride on the dialdehyde, produced by a series of hydroxylation and oxidation reactions on geraniol. Further oxidation gives **iridotrial**, in which hemiacetal formation then leads to production of the heterocyclic ring. In iridotrial, there is an equal chance that the original methyls from the head of geraniol end up as

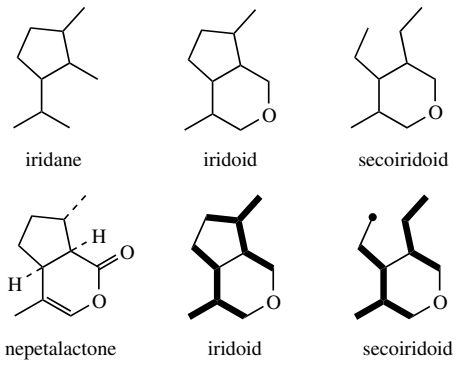

Figure 5.21

the aldehyde or in the heterocyclic ring. A large number of iridoids are found as glycosides, e.g. **loganin**, glycosylation effectively transforming the

hemiacetal linkage into an acetal. The pathway to loganin involves, in addition, a sequence of reactions in which the remaining aldehyde group is oxidized to the acid and methylated, giving **deoxyloganin**, and the final step is a hydroxylation reaction. Loganin is a key intermediate in the biosynthesis of many other iridoid structures, and also features in the pathway to a range of complex terpenoid indole alkaloids (see page 350) and tetrahydroisoquinoline alkaloids (see page 343). Fundamental in this further metabolism is cleavage of the simple monoterpene skeleton still recognizable in loganin to give **secologanin**, representative of the **secoiridoids** (Figure 5.21). This is catalysed by a cytochrome P-450-dependent mono-oxygenase, and a free radical mechanism is proposed in Figure 5.22. Secologanin now contains a free aldehyde group, together with further aldehyde and enol groups, these latter two fixed as an acetal by the presence of the glucose. As we shall see with some of the complex alkaloids, these functionalities can be released again by hydrolysing off the glucose and reopening the hemiacetal linkage. **Gentiopicroside**

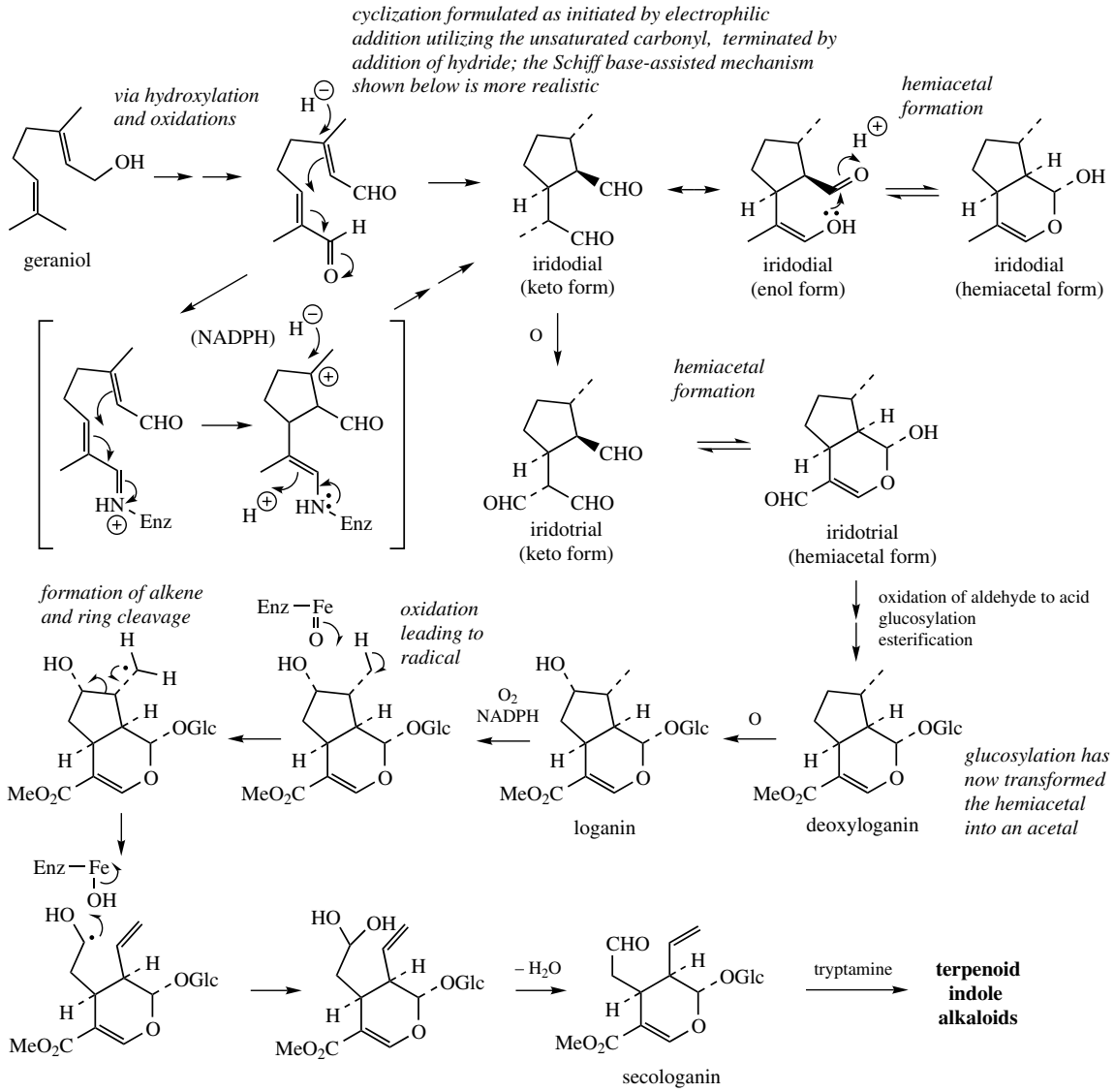

Figure 5.22

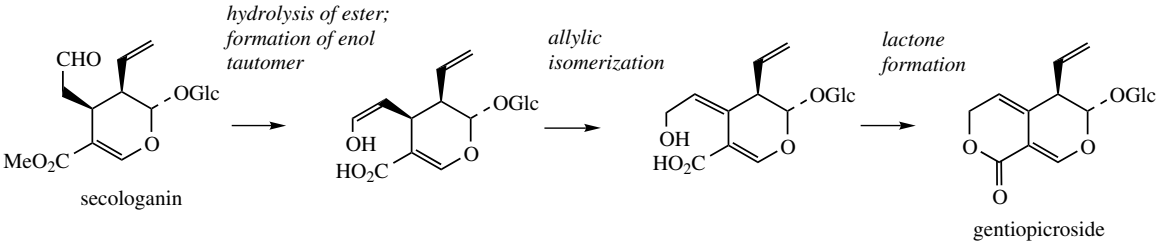

Figure 5.23

(Figure 5.23) is another example of a secoiridoid, and is found in Gentian root (*Gentiana lutea*; Gentianaceae), contributing to the bitter taste of this herbal drug. Its relationship to secologanin is suggested in Figure 5.23. The alkaloid gentianine (see page 386) is also found in Gentian root, and represents a nitrogen analogue of the secoiridoids, in which the pyran oxygen has been replaced by nitrogen.

A range of epoxyiridoid esters has been identified in the drug valerian* (*Valeriana officinalis*; Valerianaceae). These materials, responsible for the sedative activity of the crude drug, are termed **valepotriates**. **Valtrate** (Figure 5.24) is a typical example, and illustrates the structural relationship to loganin, though these compounds contain additional ester functions, frequently isovaleryl. The hemiacetal is now fixed as an ester, rather than as a glycoside.

Valerian

Valerian root consists of the dried underground parts of *Valeriana officinalis* (Valerianaceae), a perennial herb found throughout Europe. Drug material comes from wild and cultivated plants, and is carefully dried at low temperature (less than 40 °C) to minimize decomposition of constituents. Valerian preparations are widely used as herbal tranquillizers to relieve nervous tension, anxiety, and insomnia. Valerian was especially popular during the First World War, when it was used to treat shell-shock. The drug does possess mild sedative and tranquillizing properties, but for maximum activity the roots need to be freshly harvested and carefully dried. The major active principles are generally held to be a number of epoxyiridoid esters called valepotriates (0.5–1.6%), the principal component of which is valtrate (about 80%) (Figure 5.24). Minor valepotriates have the same parent iridoid alcohol as valtrate, but differ with respect to esterifying acids, e.g. isovaltrate (Figure 5.24), or are based on the reduced iridoid seen in didrovaltrate, again with various ester functionalities. Acid entities characterized in this group of compounds are mainly isovaleric (3-methylbutyric) and acetic (as in valtrate/isovaltrate/didrovaltrate), though more complex diester groups involving 3-acetoxyisovaleric and isovaleroxyisovaleric acids are encountered. During drying and storage, some of the valepotriate content may decompose by hydrolysis to liberate quantities of isovaleric acid, giving a characteristic odour, and structures such as baldrinal (Figure 5.24) (from valtrate) and homobaldrinal (from isovaltrate). Samples of old or poorly prepared valerian may contain negligible amounts of valepotriates. Standardized mixtures of valepotriates, containing didrovaltrate (80%), valtrate (15%), and acevaltrate (Figure 5.24) (5%), are available in some countries. These materials are usually extracted from the roots of other species of *Valeriana*, which produce higher amounts of valepotriates than *V. officinalis*, e.g. *V. mexicana* contains up to about 8%. Some other species of *Valeriana* that contain similar valepotriate constituents are used medicinally, including *V. wallichi* (Indian valerian) and *V. edulis* (Mexican valerian).

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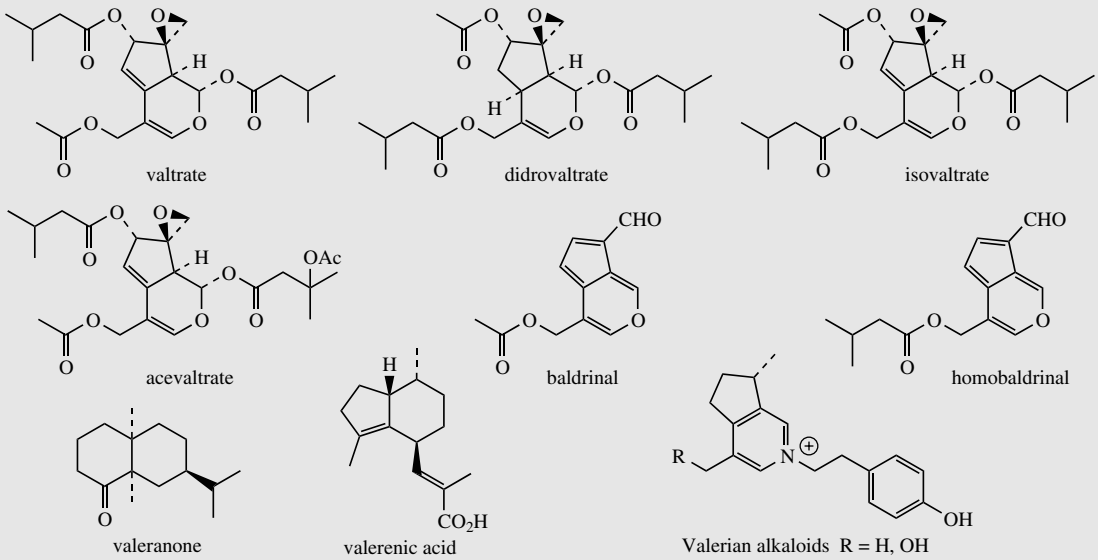

Figure 5.24

Despite the information given above, many workers believe the sedative activity of valerian cannot be due to the valepotriates, which are very unstable and not water soluble. Some of the sedative activity is said to arise from sesquiterpene derivatives such as valerenic acid (about 0.3%) and those constituting the volatile oil content (0.5–1.3%), e.g. valeranone (Figure 5.24), which have been shown to be physiologically active. GABA (γ-aminobutyric acid) and glutamine have also been identified in aqueous extracts of valerian, and these have been suggested to contribute to the sedative properties. Small amounts of some iridoid-related alkaloids (Figure 5.24) have been isolated from valerian root (see page 386). The valepotriates valtrate and didrovaltrate are reported to be cytotoxic *in vitro*, and this may restrict future use of valerian. The reactive epoxide group is likely to be responsible for these cytotoxic properties.

SESQUITERPENES (C_{15})

Addition of a further C_5 IPP unit to geranyl diphosphate in an extension of the prenyl transferase reaction leads to the fundamental sesquiterpene precursor, **farnesyl diphosphate** (**FPP**) (Figure 5.25). Again, an initial ionization of GPP seems likely, and the proton lost from C-2 of IPP is stereochemically analogous to that lost in the previous isoprenylation step. FPP can then give rise to linear and cyclic sesquiterpenes. Because of the increased chain length and additional double bond, the number of possible cyclization modes is also increased, and a huge range of mono-, bi-, and tri-cyclic structures can result. The stereochemistry of the double bond nearest the diphosphate can adopt an *E* configuration (as in FPP), or a *Z* configuration via ionization, as found with geranyl/neryl PP (Figure 5.26). In some systems, the tertiary diphosphate **nerolidyl PP** (compare linalyl PP, page 172) has been implicated as a more immediate precursor than farnesyl PP (Figure 5.26). This allows different possibilities for folding the carbon chain, dictated of course by the enzyme involved, and cyclization by electrophilic attack on to an appropriate double bond. As with the monoterpenes, standard reactions of carbocations rationally explain most of the common structural skeletons encountered, and a representative selection of these is given in

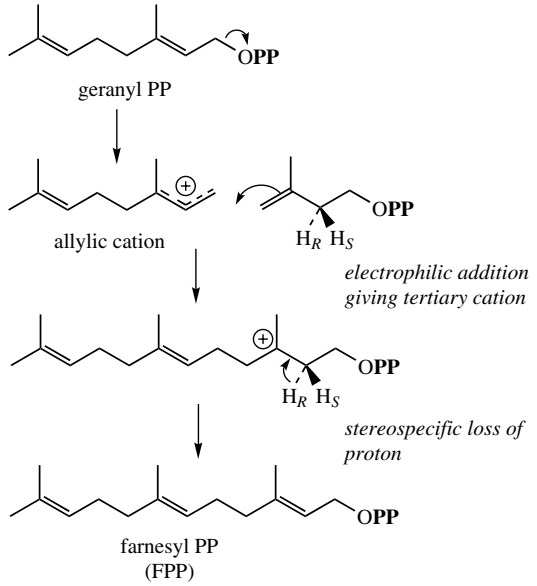

Figure 5.25

Figure 5.27. One of these cyclized systems, the bisabolyl cation, is analogous to the monoterpene menthane system, and further modifications in the six-membered ring can take place to give essentially monoterpene variants with an extended hydrocarbon substituent, e.g. **γ-bisabolene** (Figure 5.28), which contributes to the aroma of ginger (*Zingiber officinale*; Zingiberaceae) along with the related structures such as **zingiberene** and **β-sesquiphellandrene** (Figure 5.29). Sesquiterpenes will in general be less volatile than monoterpenes. Simple quenching of the bisabolyl cation with water leads to **α-bisabolol** (Figure 5.28), a major component of matricaria (German chamomile)* flowers (*Matricaria chamomilla*; Compositae/Asteraceae). So-called **bisabolol oxides** A and B are also present, compounds probably derived from bisabolol by cyclization reactions (Figure 5.28) on an intermediate epoxide (compare Figure 4.34, page 146).

Other cyclizations in Figure 5.27 lead to ring systems larger than six carbons, and seven-, ten-, and 11-membered rings can be formed as shown. The two ten-membered ring systems (germacryl and *cis*-germacryl cations), or the two 11-membered systems (humulyl and *cis*-humulyl cations), differ only in the stereochemistry associated with the double bonds. However, this affects further cyclization processes and is responsible for extending the variety of natural sesquiterpene derivatives. The germacryl cation, without further cyclization, is a precursor of the germacrane class of sesquiterpenes, as exemplified by **parthenolide** (Figure 5.30), the antimigraine agent in feverfew* (*Tanacetum parthenium*; Compositae/Asteraceae). Parthenolide is actually classified as a germacranolide, the suffix 'olide' referring to the lactone group. Whilst the details of the pathway are not known, a series of simple oxidative transformations (Figure 5.30) can produce the α,β-unsaturated lactone and epoxide groupings.

The α,β-unsaturated lactone functionality is a common feature of many of the biologically

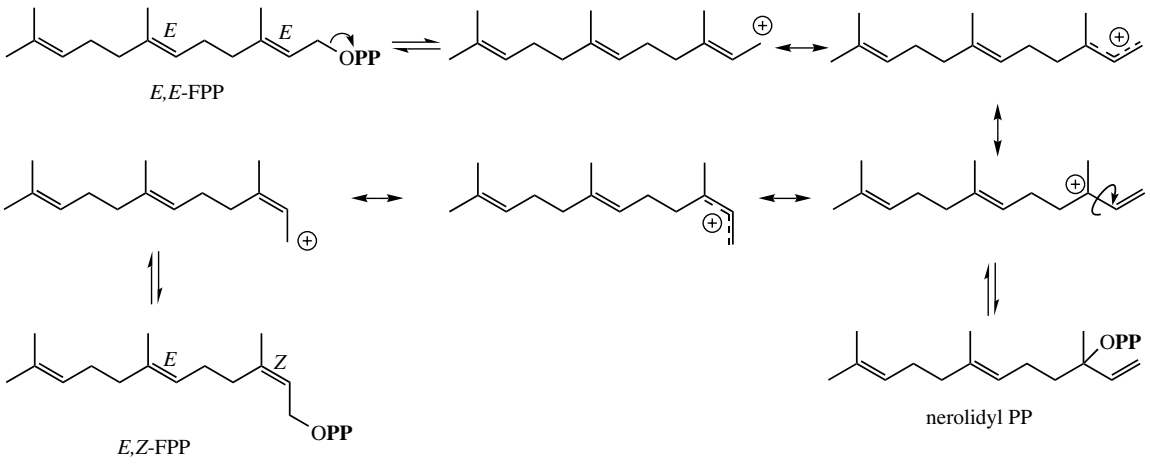

Figure 5.26

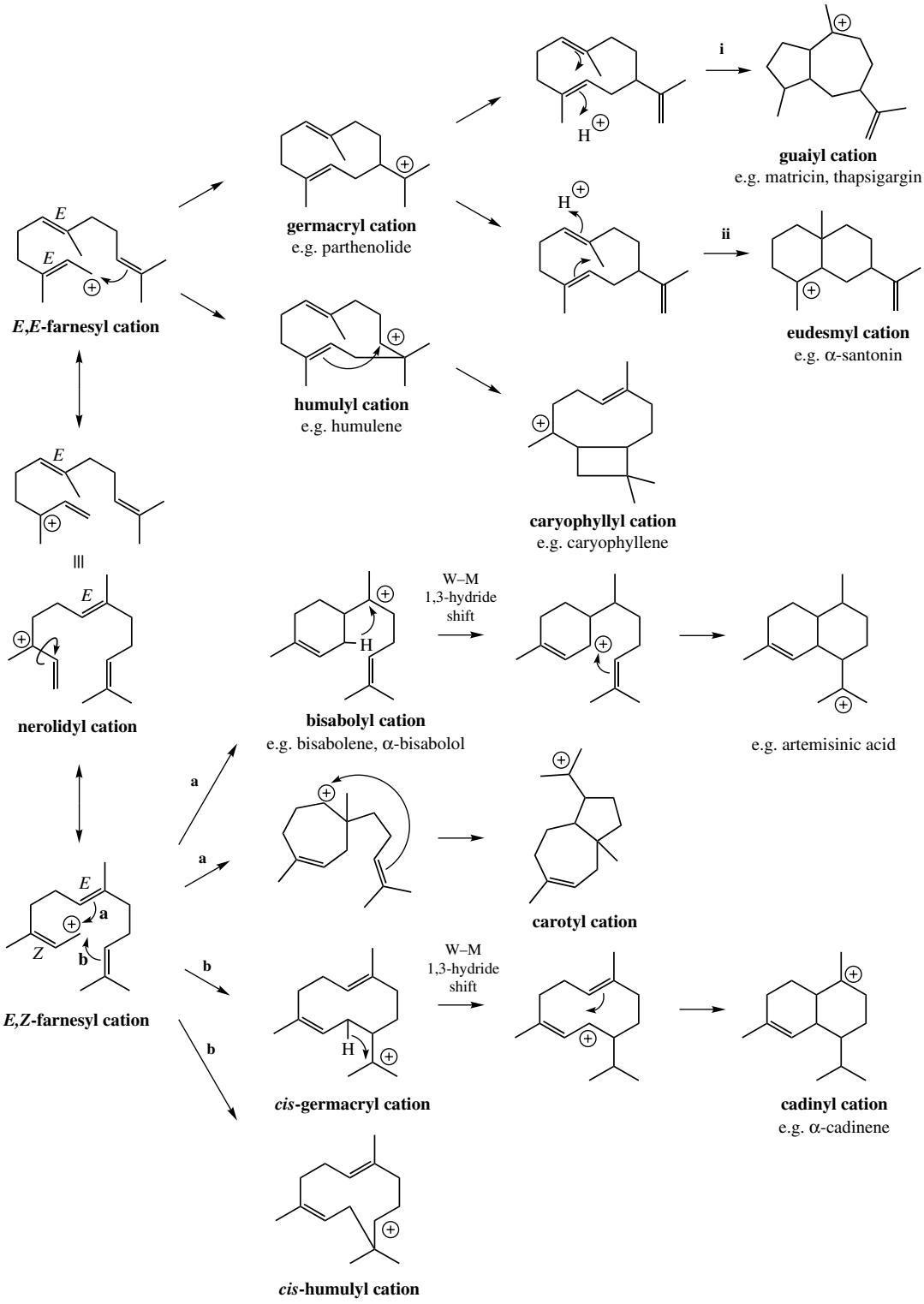
E
E
E,E-farnesyl cation
germacryl cation
e.g. parthenolide
humulyl cation
e.g. humulene
caryophyllyl cation
e.g. caryophyllene
i
guaiyl cation
e.g. matricin, thapsigargin
ii
eudesmyl cation
e.g. α-santonin
nerolidyl cation
bisabolyl cation
e.g. bisabolene, α-bisabolol
W–M
1,3-hydride
shift
e.g. artemisinic acid
E,Z-farnesyl cation
carotyl cation
cis-germacryl cation
cadinyl cation
e.g. α-cadinene
cis-humulyl cation
a
b

Figure 5.27

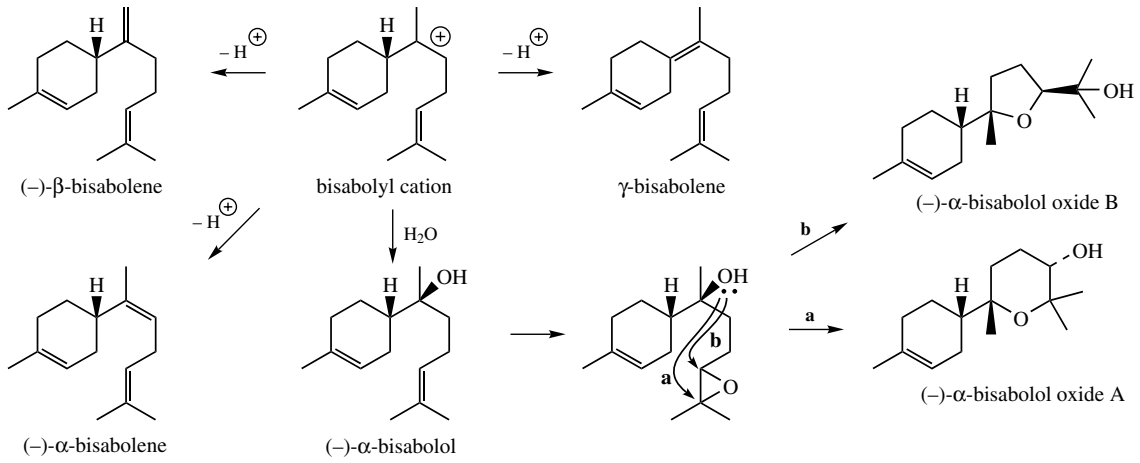

Figure 5.28

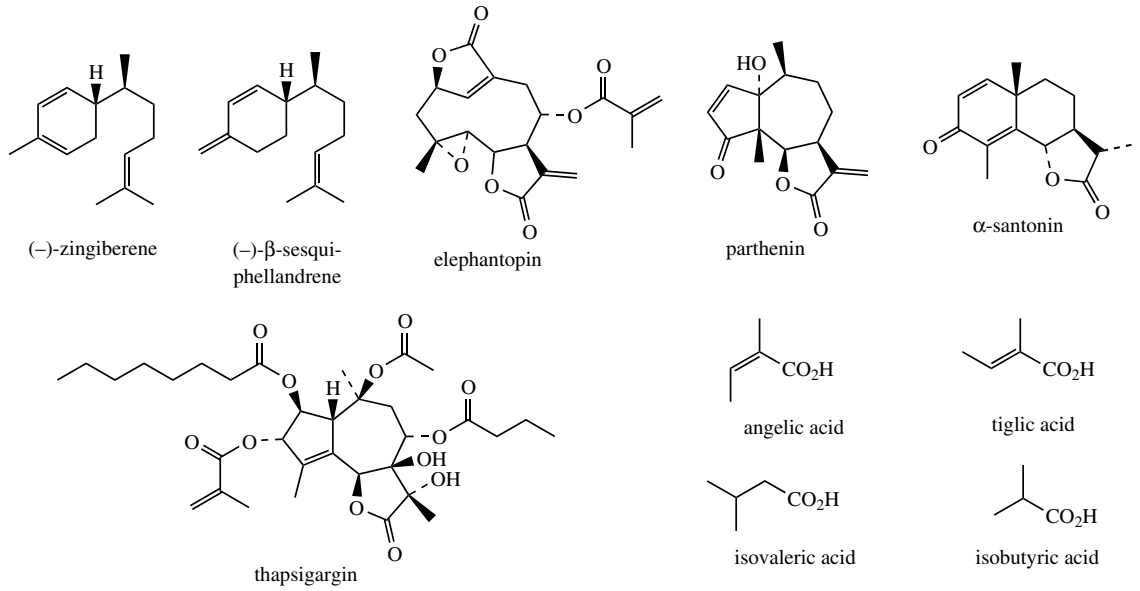

Figure 5.29

active terpenoids. The activity frequently manifests itself as a toxicity, especially cytotoxicity as seen with the germacranolide **elephantopin** (Figure 5.29) from *Elephantopus elatus* (Compositae/Asteraceae), or skin allergies, as caused by the pseudoguaianolide (a rearranged guaianolide) **parthenin** (Figure 5.29) from *Parthenium hysterophorus* (Compositae/Asteraceae), a highly troublesome weed in India. These compounds can be considered as powerful alkylating agents by a Michael-type addition of a suitable nucleophile, e.g. thiols, on to the α,β-unsaturated lactone. Such alkylation reactions are believed to explain biological activity, and, indeed, activity is typically lost if either the double bond or the carbonyl group is chemically reduced. In some structures, additional electrophilic centres offer further scope for alkylation reactions. In **parthenolide** (Figure 5.31), an electrophilic epoxide group is also present, allowing transannular cyclization and generation of a

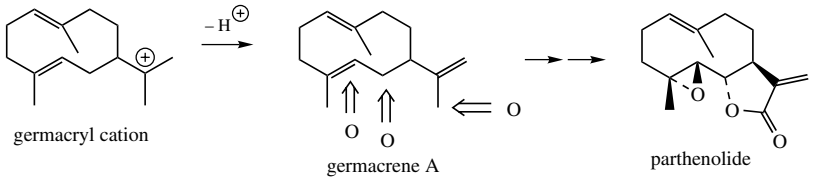

Figure 5.30

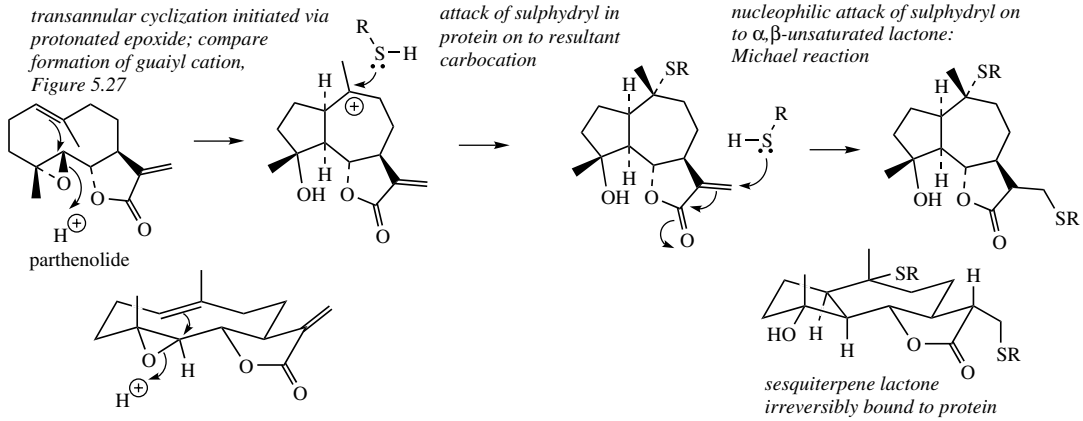

Figure 5.31

second alkylation site. Cytotoxic agents may irreversibly alkylate critical enzymes that control cell division, whilst allergenic compounds may conjugate with proteins to form antigens which trigger the allergic response. The beneficial effects of parthenolide and structurally related compounds in feverfew have been demonstrated to relate to alkylation of thiol groups.

α-Santonin (Figure 5.29) has been identified as the principal anthelmintic component of

Feverfew

Feverfew is a traditional herbal remedy for the relief of arthritis, migraine, toothache, and menstrual difficulties. The plant is a perennial, strongly aromatic herb of the Compositae/Asteraceae family, and has been classified variously as *Tanacetum parthenium*, *Chrysanthemum parthenium*, *Leucanthemum parthenium*, or *Pyrethrum parthenium*, the former name being currently favoured. Studies have confirmed that feverfew is an effective prophylactic treatment in about 70% of migraine sufferers. It reduces the frequency of attacks, the vomiting associated with attacks, and the severity of attacks. The herb has been shown to inhibit blood platelet aggregation, the release of 5-hydroxytryptamine (serotonin) from platelets, the release of histamine from mast cells, and the production of prostaglandins, thromboxanes and leukotrienes. Of a range of sesquiterpene lactones of the germacrane and guianane groups characterized in the leaf material, the principal constituent and major active component is parthenolide (Figure 5.30) (up to about 1% in dried leaves). The powerful pungent odour of the plant arises from the volatile oil constituents, of which the monoterpene camphor (Figure 5.14) is a major constituent. Feverfew may be taken as the fresh leaf, often eaten with bread in the form of a sandwich to minimize the bitter taste, or it can be obtained

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in dosage forms as tablets or capsules of the dried powdered leaf. The parthenolide content of dried leaf deteriorates on storage, and many commercial preparations of feverfew have been shown to contain little parthenolide, or to be well below the stated content. This may be a consequence of complexation with plant thiols via Michael addition. Consumers of fresh leaf can be troubled by sore mouth or mouth ulcers, caused by the sesquiterpenes. Parthenolide is also known to be capable of causing some allergic effects, e.g. contact dermatitis. The proposed mechanism of action of parthenolide via alkylation of thiol groups in proteins is shown in Figure 5.31.

various *Artemisia* species, e.g. wormseed (*A. cinia*; Compositae/Asteraceae), and has found considerable use for removal of roundworms, although potential toxicity limits its application. Structurally, α-santonin bears much similarity to parthenolide, and the most marked difference lies in the presence of the bicyclic decalin ring system. This basic skeleton, the eudesmane system, is formed from the germacryl cation by protonation and cyclization via the eudesmyl cation (Figure 5.27, route ii), whereas protonation at the more substituted end of a double bond (anti-Markovnikov addition, route i), could generate the guaiyl cation and guaiane skeleton. This latter skeleton is found in **matricin** (Figure 5.32), again from matricaria* flowers. This compound degrades on heating, presumably by elimination of acetic acid and water, and then decarboxylation to the azulene derivative **chamazulene**, responsible for the blue coloration of oil distilled from the flowers. **Thapsigargin** (Figure 5.29) from *Thapsia garganica* (Umbelliferae/Apiaceae) provides a further example of a guaianolide, esterified with a variety of acid groups. This compound is of considerable pharmacological interest as a tumour promoter, and as a potent activator of cells involved in the inflammatory response.

Another type of decalin-containing sesquiterpene is seen in the structures of α-cadinene and amorpha-4,11-diene. **α-Cadinene** (Figure 5.33) is

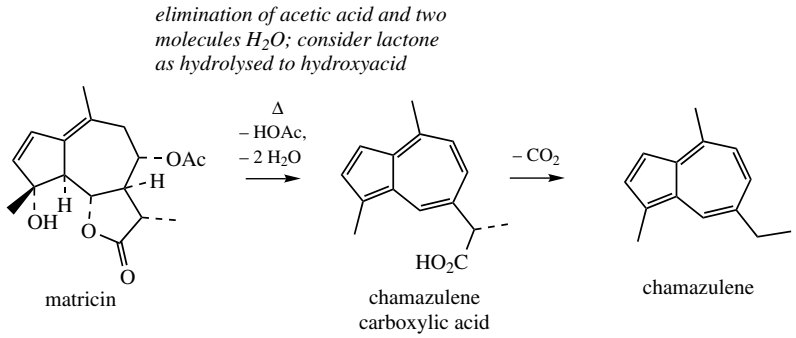

Figure 5.32

Chamomile and Matricaria

Two types of **chamomile** (**camomile**) are commonly employed in herbal medicine, Roman chamomile *Chamaemelum nobile* (formerly *Anthemis nobilis*) (Compositae/Asteraceae), and German chamomile *Matricaria chamomilla* (*Chamomilla recutica*) (Compositae/Asteraceae). German chamomile, an annual plant, is the more important commercially, and is often called **matricaria** to distinguish it from the perennial Roman chamomile. Both plants are cultivated

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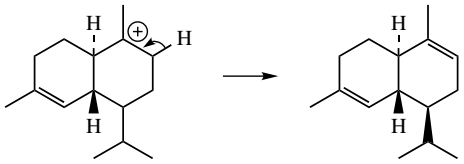
in various European countries to produce the flowerheads, which are then dried for drug use. Volatile oils obtained by steam distillation or solvent extraction are also available.

Roman chamomile is usually taken as an aqueous infusion (chamomile tea) to aid digestion, curb flatulence, etc, but extracts also feature in mouthwashes, shampoos, and many pharmaceutical preparations. It has mild antiseptic and anti-inflammatory properties. The flowerheads yield 0.4–1.5% of volatile oil, which contains over 75% of aliphatic esters of angelic, tiglic, isovaleric, and isobutyric acids (Figure 5.29), products of isoleucine, leucine, and valine metabolism (see pages 100, 295, 306), with small amounts of monoterpenes and sesquiterpenes. Matricaria is also used as a digestive aid, but is mainly employed for its anti-inflammatory and spasmolytic properties. Extracts or the volatile oil find use in creams and ointments to treat inflammatory skin conditions, and as an antibacterial and antifungal agent. Taken internally, matricaria may help in the control of gastric ulcers. The flowers yield 0.5–1.5% volatile oil containing the sesquiterpenes α-bisabolol (10–25%), bisabolol oxides A and B (10–25%) (Figure 5.28), and chamazulene (0–15%) (Figure 5.32). Chamazulene is a thermal decomposition product from matricin, and is responsible for the dark blue coloration of the oil (Roman chamomile oil contains only trace amounts of chamazulene). α-Bisabolol has some anti-inflammatory, antibacterial, and ulcer-protective properties, but chamazulene is probably a major contributor to the anti-inflammatory activity of matricaria preparations. It has been found to block the cyclooxygenase enzyme in prostaglandin biosynthesis (see page 55) and the anti-inflammatory activity may result from the subsequent inhibition of leukotriene formation.



cadinyl cation α-cadinene

Figure 5.33

one of the many terpenoids found in juniper berries (*Juniperus communis*; Cupressaceae) used in making gin, and this compound is derived from the ten-carbon ring-containing *cis*-germacryl cation. The double bonds in the *cis*-germacryl cation are unfavourably placed for a cyclization reaction as observed with the germacryl cation, and available evidence points to an initial 1,3-shift of hydride to the isopropyl side-chain generating a new cation, and thus allowing cyclization (Figure 5.27). **Amorpha-4,11-diene** (Figure 5.34) is structurally related to α-cadinene, but the different stereochemistry of ring fusion and site of the second double bond is a consequence of a different cyclization mechanism operating to produce the decalin ring system. In this case, a six-membered ring is most likely formed first giving the bisabolyl cation, and, again, a 1,3-hydride shift is implicated prior to forming the decalin system (Figure 5.27). Amorpha-4,11-diene is an intermediate in the pathway leading to **artemisinin** in *Artemisia annua* (Compositae/Asteraceae) (Figure 5.34). This proceeds through **artemisinic acid** and **dihydroartemisinic acid** via modest oxidation and reduction processes. Dihydroartemisinic acid may be converted chemically into artemisinin by an oxygen-mediated photochemical oxidation under conditions that might normally be present in the plant, suggesting that all further transformations may in fact be non-enzymic. An intermediate in this process also found naturally in *A. annua* is the hydroperoxide of dihydroartemisinic acid. The further modifications postulated in Figure 5.34 include ring expansion by cleavage of this hydroperoxide and a second oxygen-mediated hydroperoxidation. The 1,2,4-trioxane system in artemisinin can be viewed more simply as a combination of hemiketal, hemiacetal, and lactone functions, and the later stages of the pathway merely reflect their construction. Artemisinin* is an important

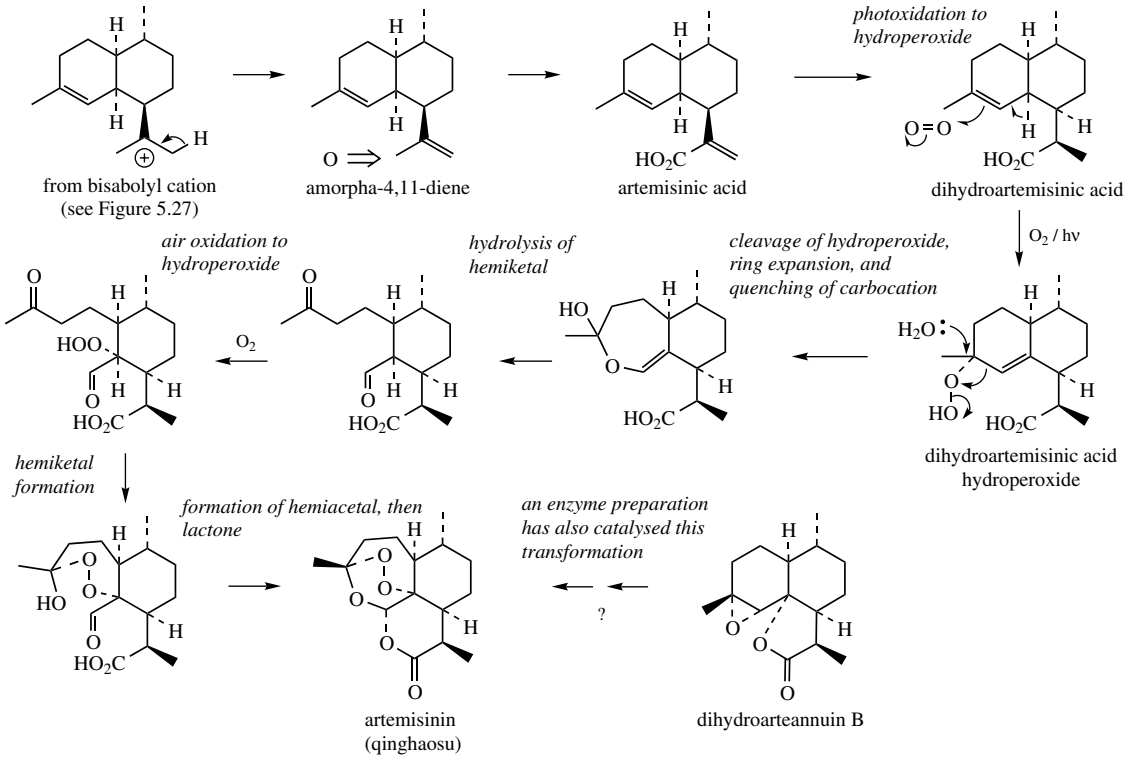


Figure 5.34

antimalarial component in *Artemisia annua**, a Chinese herbal drug. There is currently strong research effort to produce artemisinin or analogues as new antimalarial drugs, since many of the current drugs have become less satisfactory due to resistance (see quinine, page 362).

The 11-carbon ring of the humulyl carbocation may be retained, as in the formation of **humulene** (Figure 5.36), or modified to give the caryophyllyl cation containing a nine-membered ring fused to a four-membered ring, as in **β-caryophyllene** (Figure 5.36). Humulene is found

Artemisia annua and Artemisinin

Artemisia annua (Compositae/Asteraceae) is known as qinghao in Chinese traditional medicine, where it has been used for centuries in the treatment of fevers and malaria. The plant is sometimes called annual or sweet wormwood, and is quite widespread, being found in Europe, North and South America, as well as China. Artemisinin (qinghaosu) (Figure 5.34) was subsequently extracted and shown to be responsible for the antimalarial properties, being an effective blood schizontocide in humans infected with malaria, and showing virtually no toxicity. Malaria is caused by protozoa of the genus *Plasmodium*, especially *P. falciparum*, entering the blood system from the salivary glands of mosquitoes, and world-wide is responsible for 2–3 million deaths each year. Established antimalarial drugs such as chloroquine (see page 363) are proving less effective in the treatment of malaria due to the appearance of drug-resistant strains of *P. falciparum*. Artemisinin is currently effective against these drug-resistant strains.

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Artemisinin is a sesquiterpene lactone containing a rare peroxide linkage which appears essential for activity. Some plants of *Artemisia annua* have been found to produce as much as 1% artemisinin, but the yield is normally very much less, typically 0.05–0.2%. Apart from one or two low-yielding species, the compound has not been found in any other species of the genus *Artemisia* (about 400 species). Small amounts (about 0.01%) of the related peroxide structure artemisitene (Figure 5.35) are also present in *A. annua*, though this has a lower antimalarial activity. The most abundant sesquiterpenes in the plant are artemisinic acid (arteannuic acid, qinghao acid) (typically 0.2–0.8%) (Figure 5.34), and lesser amounts (0.1%) of arteannuin B (qinghaosu-II) (Figure 5.35). Fortunately, arteannuic acid may be converted chemically into artemisinin by a relatively simple and efficient process. Artemisinin may be reduced to the lactol (hemiacetal) dihydroartemisinin (Figure 5.35), and this has been used for the semi-synthesis of a range of analogues, of which the acetals artemether and arteether (Figure 5.35), and the water-soluble sodium salts of artelinic acid and artesunic acid (Figure 5.35), appear very promising antimalarial agents. These materials have increased activity compared with artemisinin and the chances of infection recurring are also reduced. **Artemether** has rapid action against chloroquinine-resistant *P. falciparum* malaria, and is currently being used as injection formulations. Arteether has similar activity. Being acetals, artemether and arteether are both extensively decomposed in acidic conditions, but are stable in alkali. The ester **artesunic acid** is also used in injection form, but is rather unstable in alkaline solution, hydrolysing to dihydroartemisinin. The ether artelinic acid is considerably more stable. These two compounds have a rapid action and particular application in the treatment of potentially fatal cerebral malaria. Dihydroartemisinin is a more active antimalarial than artemisinin and appears to be the main metabolite of these drugs in the body. They rapidly clear the blood of parasites, but do not have a prophylactic effect. Chemically, these agents are quite unlike any other class of current antimalarial agent, and when thoroughly evaluated, they may well become an important group of drugs in the fight against this life-threatening disease.

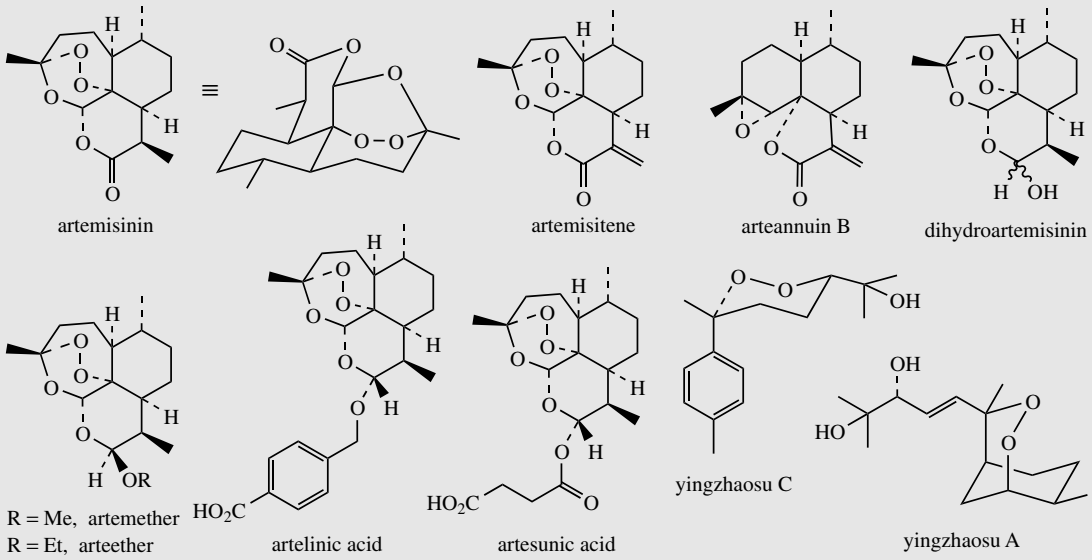

Figure 5.35

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The relationship between a peroxide linkage and antimalarial activity is strengthened by the isolation of other sesquiterpene peroxides which have similar levels of activity as artemisinin. Thus, roots of the vine yingzhao (*Artabotrys uncinatus*; Annonaceae), which is also used as a traditional remedy for malaria, contain the bisabolyl derivatives yingzhaosu A and yingzhaosu C (Figure 5.35), the latter containing an aromatic ring of isoprenoid origin (compare the monoterpenes thymol and carvacrol, page 186). Artemisinin, and other peroxide-containing antimalarial agents, appear to complex with haemin, which is a soluble iron–porphyrin material released from haemoglobin as a result of proteolytic digestion by the malarial parasite. This material is toxic to *Plasmodium*, so is normally converted into an insoluble non-toxic form haemozoin (malarial pigment) by enzymic polymerization. Agents like chloroquine (see page 363) interfere with the polymerization process. Complexation of haemin with artemisinin by coordination of the peroxide bridge with the iron atom interrupts the detoxification process and leads to the generation of free radical species through homolytic cleavage of the peroxide. The resulting radicals ultimately damage proteins in *Plasmodium*.

in hops (*Humulus lupulus*; Cannabaceae), and β-caryophyllene is found in a number of plants, e.g. in the oils from cloves (*Syzygium aromaticum*; Myrtaceae) and cinnamon (*Cinnamomum zeylanicum*; Lauraceae).

Gossypol* (Figure 5.37) is an interesting and unusual example of a dimeric sesquiterpene in which loss of hydrogen has led to an aromatic system (compare the phenolic monoterpenes thymol and carvacrol, page 186). This material is found in immature flower buds and seeds of the cotton plant (*Gossypium* species; Malvaceae), though originally isolated in small amounts from cottonseed oil. It can function as a male infertility agent, and is used in China as a male contraceptive. The cadinyl carbocation via **δ-cadinene** is involved in generating the basic aromatic sesquiterpene unit hemigossypol, and then dimerization is simply an example of phenolic oxidative coupling *ortho* to the phenol groups (Figure 5.37).

The formation of sesquiterpenes by a carbocation mechanism means that there is considerable scope for rearrangements of the Wagner–Meerwein type. So far, only occasional hydride migrations have been invoked in rationalizing the examples considered. Obviously, fundamental skeletal rearrangements will broaden the range of natural sesquiterpenes even further. That such processes do occur has been proven beyond doubt by appropriate labelling experiments, and

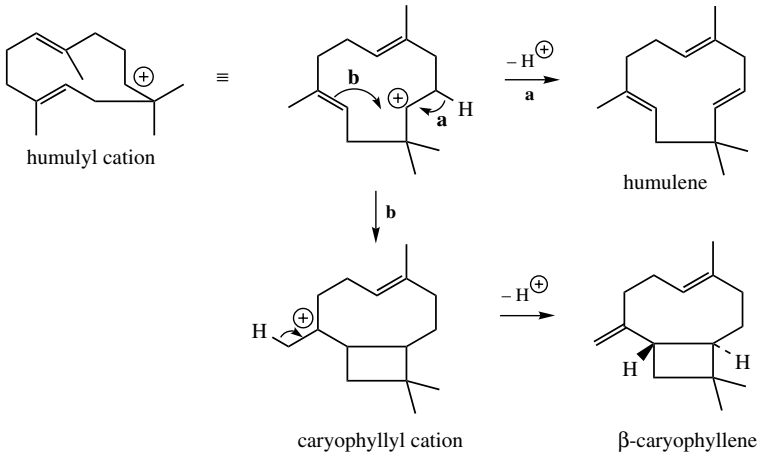


Figure 5.36

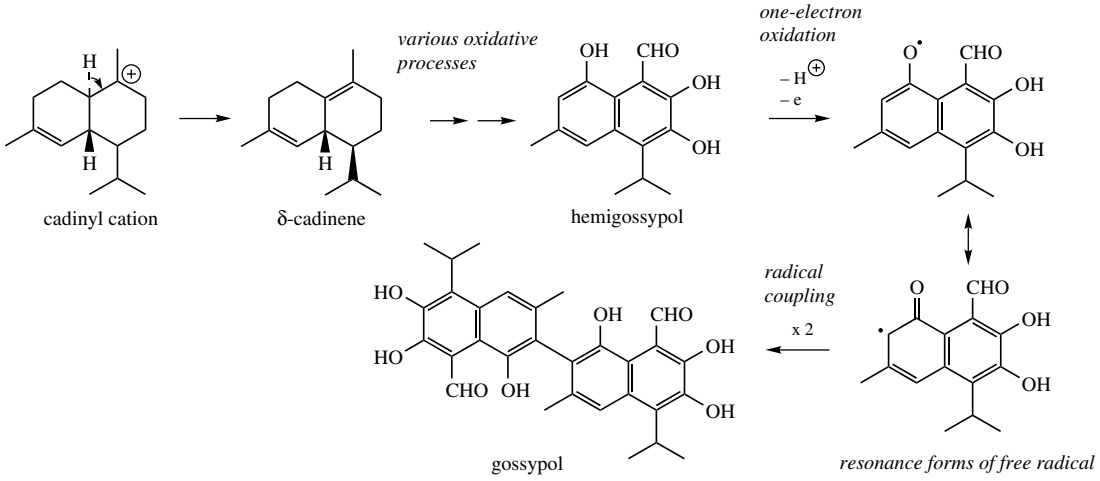
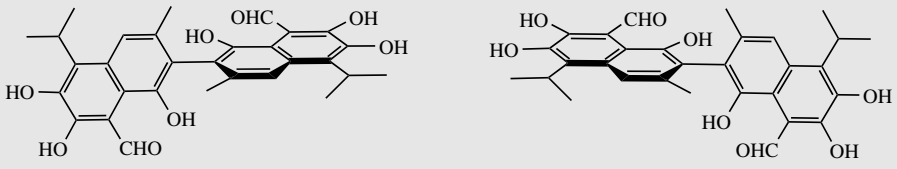


Figure 5.37

Gossypol

Gossypol occurs in the seeds of cotton (*Gossypium* species, e.g. *G. hirsutum*, *G. herbaceum*, *G. arboreum*, *G. barbadense*; Malvaceae) in amounts of 0.1–0.6%. Its contraceptive effects were discovered when subnormal fertility in some Chinese rural communities was traced back to the presence of gossypol in dietary cottonseed oil. Gossypol acts as a male contraceptive, altering sperm maturation, spermatozoid motility, and inactivation of sperm enzymes necessary for fertilization. Extensive clinical trials in China have shown the antifertility effect is reversible after stopping the treatment provided consumption has not been too prolonged. Cases of irreversible infertility have resulted from longer periods of drug use. The molecule is chiral due to restricted rotation, and can thus exist as two atropisomers which do not easily racemize (Figure 5.38). Only the (−)-isomer is pharmacologically active as a contraceptive, whereas most of the toxic symptoms appear to be associated with the (+)-isomer, which also displays antitumour and antiviral activities. Most species of *Gossypium* (except *G. barbadense*) produce gossypol where the (+)-isomer predominates over the (−)-isomer, with amounts varying according to species and cultivar. Racemic (±)-gossypol (but neither of the enantiomers) complexes with acetic acid, so that suitable treatment of cotton seed extracts actually separates the racemate from the excess of (+)-isomer. The racemic form can then be resolved. Other plants in the Gossypieae tribe of the Malvaceae also produce gossypol, with the barks of *Thespia populnea* (3.3%) and *Montezuma speciosissima* (6.1%) being particularly rich sources. Unfortunately, gossypol from these is almost entirely the inactive (+)-form.



(+)-gossypol (−)-gossypol

Figure 5.38

a single example will be used as illustration. The **trichothecenes*** are a group of fungal toxins found typically in infected grain foodstuffs. Their name comes from the fungal genus *Trichothecium*, but most of the known structures are derived from cultures of *Fusarium* species. A particularly prominent trichothecene contaminant is **deoxynivalenol** (vomitoxin), which is produced from the less substituted trichothecene **isotrichodermol** by a sequence of oxygenation reactions (Figure 5.39). The trichothecenes have their origins in **nerolidyl diphosphate**, and ring closure of the bisabolyl cation derived from it generates a new carbocation with a five-membered ring (Figure 5.39). At this stage, a series of one hydride and two methyl migrations occur to give a cation, which loses a proton to produce the specific trichothecene precursor **trichodiene**. These migrations are fully backed up by experimental data, and although not immediately predictable, can be rationalized satisfactorily by consideration of the cation suitably bound to the enzyme surface as shown in Figure 5.39. The sequence is initiated by a 1,4-hydride shift which is spatially allowed by the relative proximity of the centres. Two 1,2-methyl shifts then follow, and it is important to note that each migrating group attacks the opposite side of the centre from which the previous group is departing, i.e. inverting the configuration at these centres. Accordingly, a concerted sequence of migrations is feasible, such a process being seen more vividly in the formation of triterpenoids and steroids (see page 216). Loss of a proton and generation of a double bond terminates the process giving trichodiene. Oxygenation of trichodiene gives, in several steps, **isotrichotriol**. Two of the hydroxylations are at activated allylic positions; hydroxylation on the five-membered ring will therefore occur before the epoxidation. Ether formation, involving perhaps protonation, loss of water and generation of an allylic cation, completes the pathway to the basic trichothecene structure as in **isotrichodermol**.

Finally, it is worth noting how many of the sesquiterpene derivatives described above are found in plants belonging to the daisy family, the Compositae/Asteraceae. Whilst sesquiterpenes are by no means restricted to this family, the Compositae/Asteraceae undoubtedly provides a very rich source.

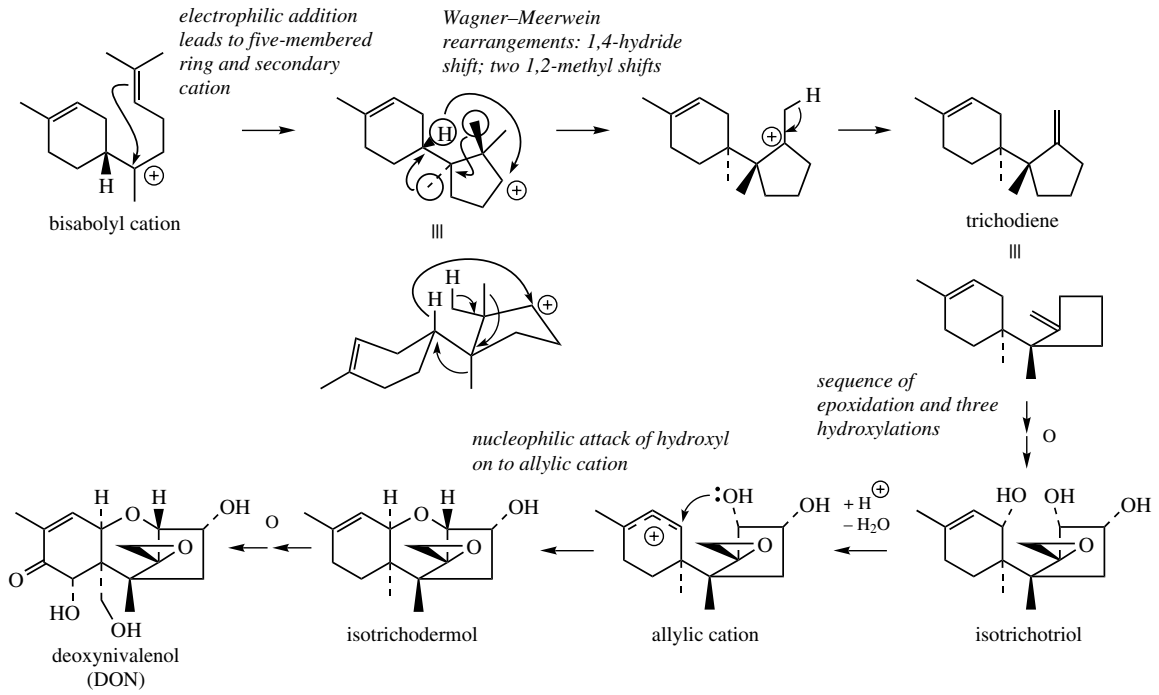


Figure 5.39

Trichothecenes

The trichothecenes are a group of sesquiterpene toxins produced by several fungi of the genera *Fusarium*, *Myrothecium*, *Trichothecium*, and *Trichoderma*, which are parasitic on cereals such as maize, wheat, rye, barley, and rice. About 150 different structures have been identified, with some of these being isolated from plants of the genus *Baccharis* (Compositae/Asteraceae), where a symbiotic plant–fungus relationship may account for their production. Examples of trichothecene structures commonly encountered as food contaminants include deoxynivalenol (DON) (Figure 5.39), and diacetoxyscirpenol (DAS), T-2 toxin, and verrucarin A (Figure 5.40). The double bond and the epoxide group in the basic trichothecene skeleton are essential for toxicity, and the number of oxygen substituents and ester functions also contribute. Macrocyclic ester functions as seen in verrucarin A tend to produce the most toxic examples. Although these compounds are more toxic when injected, oral toxicity is relatively high, and lethal amounts can easily be consumed because of the nature of the host plants. They are sufficiently toxic to warrant routine analysis of foodstuffs such as wheat and flour, and also flour-derived products, e.g. bread, since they survive processing and the high temperatures used in baking. DON levels above 1 ppm are considered hazardous for human consumption. It is relevant to note that when mammals ingest these compounds, a degree of de-epoxidation can occur, ascribed to gut microflora, thus providing some detoxification by removing a structural feature necessary for toxicity.

As their main mechanism of action, these compounds inhibit protein biosynthesis by binding to the ribosome and inhibiting peptidyl transferase activity (see page 407). They also inhibit DNA biosynthesis. A major human condition known to be caused by trichothecenes is alimentary toxic aleukia (ATA), characterized by destruction of the skin, haemorrhaging, inflammation, sepsis, a decrease in red and white blood corpuscles, bone marrow atrophy, and a high mortality rate. A severe outbreak of ATA was recorded in the former Soviet Union shortly after the Second World War when food shortages necessitated the consumption of grain that had overwintered in the field. This had become badly contaminated with *Fusarium sporotrichioides* and hence T-2 toxin. It is estimated that tens of thousands died as a result.

Many trichothecene derivatives have been tested as potential anticancer agents but have proved too toxic for clinical use.

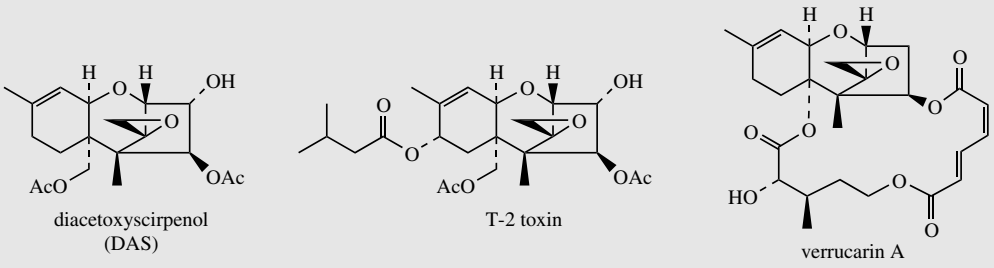


Figure 5.40

DITERPENES (C_{20})

The diterpenes arise from **geranylgeranyl diphosphate** (**GGPP**), which is formed by addition of a further IPP molecule to farnesyl diphosphate in the same manner as described for the lower terpenoids (Figure 5.41). One of the simplest and most important of the diterpenes is **phytol** (Figure 5.42), a reduced form of geranylgeraniol, which forms the lipophilic side-chain of the chlorophylls, e.g. **chlorophyll *a*** (Figure 5.42). Related haem molecules, porphyrin components

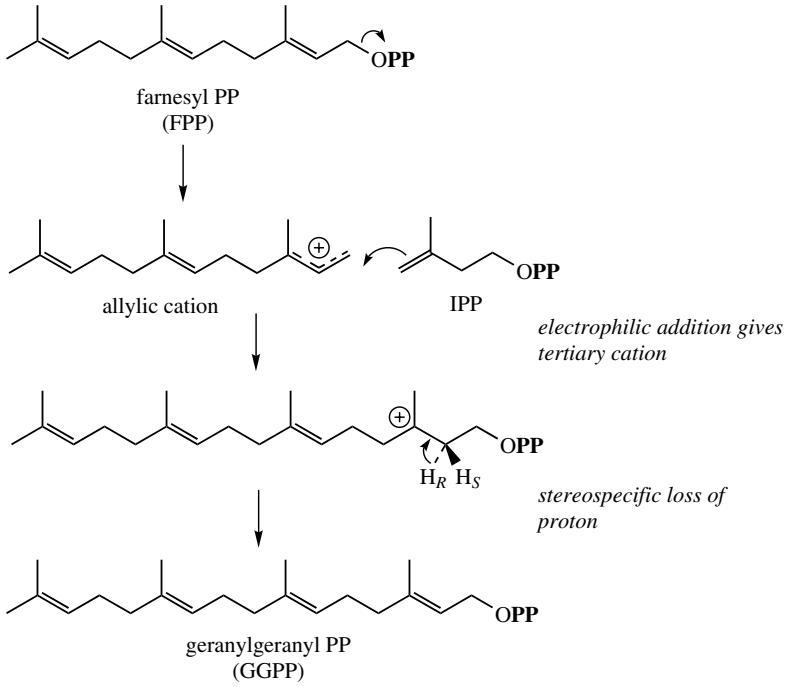


Figure 5.41

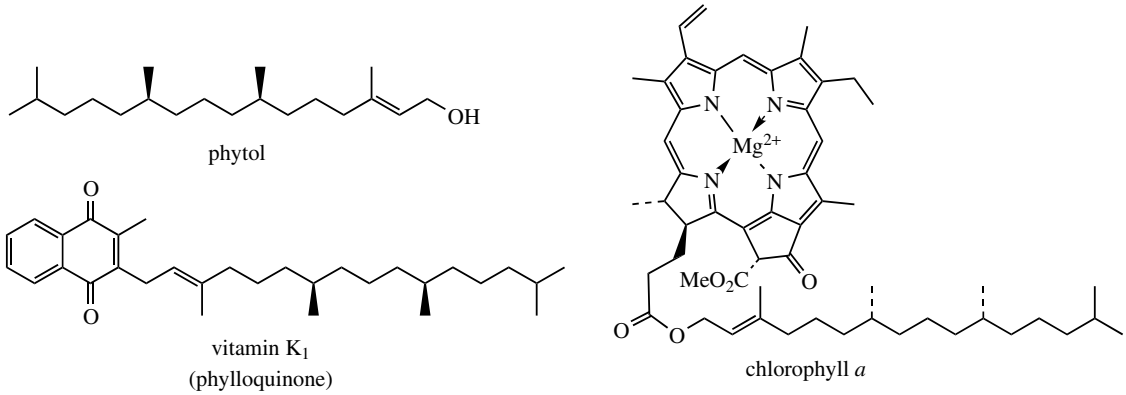


Figure 5.42

of haemoglobin, lack such lipophilic side-chains. Available evidence suggests that geranylgeranyl diphosphate is involved in forming the ester linkage, and the three reduction steps necessary to form the phytol ester occur after attachment to the chlorophyll molecule. A phytyl substituent is also found in **vitamin K_1** (**phylloquinone**) (Figure 5.42), a naphthoquinone derivative found in plants, though other members of the vitamin K group (**menaquinones**) from bacteria have unsaturated terpenoid side-chains of variable length. The phytyl group of phylloquinone is introduced by alkylation of dihydroxynaphthoic acid with phytyl diphosphate and a similar phytylation of homogentisic acid features in the formation of the E group vitamins (tocopherols). These compounds are discussed further under shikimate derivatives (see page 158).

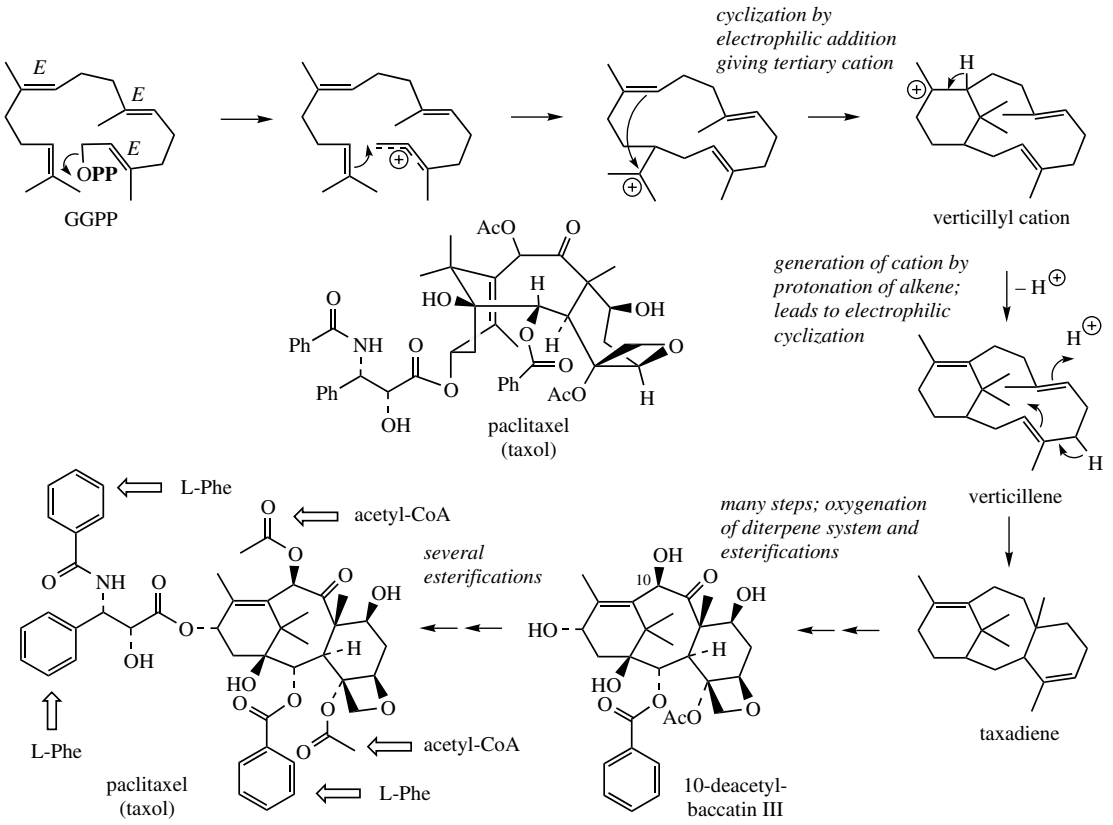


Figure 5.43

Cyclization reactions of GGPP mediated by carbocation formation, plus the potential for Wagner–Meerwein rearrangements, will allow many structural variants of diterpenoids to be produced. The toxic principle 'taxine' from common yew (*Taxus baccata*; Taxaceae) has been shown to be a mixture of at least eleven compounds based on the **taxadiene** skeleton which can be readily rationalized as in Figure 5.43, employing the same mechanistic principles as seen with mono- and sesqui-terpenes. Although these compounds are sometimes classified as diterpenoid alkaloids, the nitrogen atom is not incorporated into the diterpene skeleton, as exemplified by **taxol** (**paclitaxel**)* (Figure 5.43) from Pacific yew (*Taxus brevifolia*)*. The side-chains in taxol containing aromatic rings are derived from shikimate via phenylalanine. Taxol is an important new anticancer agent, with a broad spectrum of activity against some cancers which do not respond to other agents.

Taxus brevifolia and Taxol (Paclitaxel)

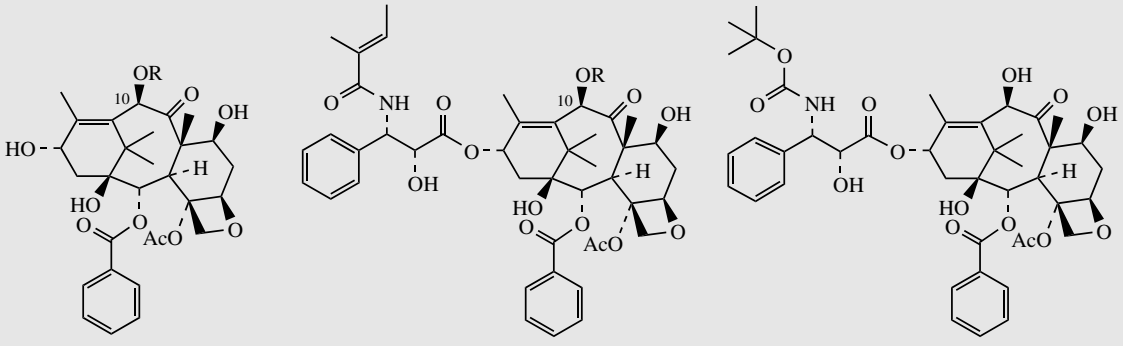
A note on nomenclature: the name taxol was given to a diterpene ester with anticancer properties when it was first isolated in 1971 from *Taxus brevifolia*. When the compound was subsequently exploited commercially as a drug, Taxol was registered as a trademark. Accordingly, the generic name paclitaxel has been assigned to the compound. The literature now contains an unhappy mixture of the two names, though the original name taxol is most often employed.

(*Continues*)

(*Continued*)

The anticancer drug taxol (Figure 5.43) is extracted from the bark of the Pacific yew, *Taxus brevifolia* (Taxaceae), a slow growing shrub/tree found in the forests of North-West Canada (British Columbia) and the USA (Washington, Oregon, Montana, Idaho, and North California). Although the plant is not rare, it does not form thick populations, and needs to be mature (about 100 years old) to be large enough for exploitation of its bark. The wood of *T. brevifolia* is not suitable for timber, and in some areas, plants have been systematically destroyed to allow cultivation of faster-growing commercially exploitable conifers. Harvesting is now strictly regulated, but it is realized that this will not provide a satisfactory long term supply of the drug. The bark from about three mature 100-year-old trees is required to provide one gram of taxol, and a course of treatment may need 2 grams of the drug. Current demand for taxol is in the region of 100–200 kg per annum.

All parts of *Taxus brevifolia* contain a wide range of diterpenoid derivatives termed taxanes, which are structurally related to the toxic constituents found in other *Taxus* species, e.g. the common yew, *Taxus baccata*. Over a hundred taxanes have been characterized from various *Taxus* species, and taxol is a member of a small group of compounds possessing a four-membered oxetane ring and a complex ester side-chain in their structures, both of which are essential for antitumour activity. Taxol is found predominantly in the bark of *T. brevifolia*, but in relatively low amounts (about 0.01–0.02%). Up to 0.033% of taxol has been recorded in some samples of leaves and twigs, but generally the taxol content is much lower than in the bark. The content of some other taxane derivatives in the bark is considerably higher, e.g. up to 0.2% baccatin III (Figure 5.44). Other taxane derivatives characterized include 10-deacetyltaxol, 10-deacetylbaccatin III, cephalomannine and 10-deacetylcephalomannine. A more satisfactory solution currently exploited for the supply of taxol and derivatives for drug use is to produce these compounds by semi-synthesis from more accessible structurally related materials. Both baccatin III and 10-deacetylbaccatin III (Figure 5.44) have been efficiently transformed into taxol. 10-Deacetylbaccatin III is readily extracted from the leaves and twigs of *Taxus baccata*, and, although the content is variable, it is generally present at much higher levels (up to 0.2%) than taxol can be found in *T. brevifolia. Taxus baccata,* the common yew, is widely planted as an ornamental tree in Europe and the USA and is much faster growing than the Pacific yew. Cell cultures of *T. baccata* also offer excellent potential for production of taxol or 10-deacetylbaccatin III but are not yet economic; taxol yields of up to 0.2% dry weight



R = Ac, baccatin III
R = H, 10-deacetylbaccatin III

R = Ac, cephalomannine
R = H, 10-deacetylcephalomannine

docetaxel
(taxotere)

Figure 5.44

(*Continues*)

(*Continued*)

cultured cells have been obtained. The use of microorganisms and enzymes to specifically hydrolyse ester groups from the mixture of structurally related taxanes in crude extracts and thus improve the yields of 10-deacetylbaccatin III has also been reported.

There is further optimism for new methods of obtaining taxol by microbial culture. Thus, a fungus, *Taxomyces adreanae*, isolated from the inner bark of *Taxus brevifolia* appears to have inherited the necessary genes from the tree (or vice versa) and is able to synthesize taxol and other taxanes in culture, though at only very low levels (20–50 ng l^{-1}). A fungus, *Pestalotiopsis microspora*, recently isolated from the inner bark of the Himalayan yew (*Taxus wallachiana*) produces higher levels (60–70 μg l^{-1}), and if this could be optimized further it might form the basis for commercial production.

Paclitaxel (**Taxol**®) is being used clinically in the treatment of ovarian and breast cancers, non-small-cell lung cancer, small-cell lung cancer, and cancers of the head and neck. **Docetaxel** (**Taxotere**®) (Figure 5.44) is a side-chain analogue of taxol, which has also been produced by semi-synthesis from 10-deacetylbaccatin III. It has improved water-solubility compared with taxol, and is being used clinically against ovarian and breast cancers. Taxol acts as an antimitotic by binding to microtubules, promoting their assembly from tubulin, and stabilizing them against depolymerization during cell division. The resultant abnormal tubulin–microtubule equilibrium disrupts the normal mitotic spindle apparatus and blocks cell proliferation. Taxol thus has a different mechanism of action to other antimitotics such as vincristine (see page 356) or podophyllotoxin (see page 136), which bind to the protein tubulin in the mitotic spindle, preventing polymerization and assembly into microtubules. Taxol has also been shown to bind to a second target, a protein which normally blocks the process of apoptosis (cell death). Inhibition of this protein allows apoptosis to proceed.

The latex of some plants in the genus *Euphorbia* (Euphorbiaceae) is toxic, and can cause poisoning in humans and animals, skin dermatitis, cell proliferation, and tumour promotion (co-carcinogen activity). Many species of *Euphorbia* are regarded as potentially toxic, and the latex can produce severe irritant effects, especially on mucous membranes and the eye. Most of the biological effects are due to diterpene esters, e.g. esters of **phorbol** (Figure 5.45), which activate protein kinase C, an important and widely distributed enzyme responsible for phosphorylating many biochemical entities. The permanent activation of protein kinase C is thought to lead to the uncontrolled cancerous growth. The most commonly encountered ester of phorbol is 12-*O*-myristoylphorbol 13-acetate (Figure 5.45). The origins of phorbol are not fully delineated, but may be rationalized as in Figure 5.45. Cyclization of GGPP generates a cation containing a 14-membered ring system. Loss of a proton via cyclopropane ring formation leads to **casbene**, an antifungal metabolite produced by the castor oil plant, *Ricinus communis* (Euphorbiaceae). Casbene, via the ring closures shown in Figure 5.45, is then likely to be the precursor of the phorbol ring system.

In contrast to the cyclization mechanisms shown in Figures 5.43 and 5.45, where loss of diphosphate generates the initial carbocation, many of the natural diterpenes have arisen by a different mechanism. Carbocation formation is initiated by protonation of the double bond at the head of the chain leading to a first cyclization sequence. Loss of the diphosphate later on also produces a carbocation and facilitates further cyclization. The early part of the sequence resembles that involved in hopanoid biosynthesis (see page 218), and to some extent triterpenoid and steroid biosynthesis (see page 214), though in the latter cases opening of the epoxide ring of the precursor squalene oxide is responsible for generation of the cationic intermediates. Protonation of GGPP can initiate a concerted cyclization sequence, terminated by loss of a proton from a methyl, yielding **copalyl PP** (Figure 5.46, a). The stereochemistry in this product is controlled by the folding of the substrate on the enzyme surface, though an alternative folding can lead to **labdadienyl PP**,

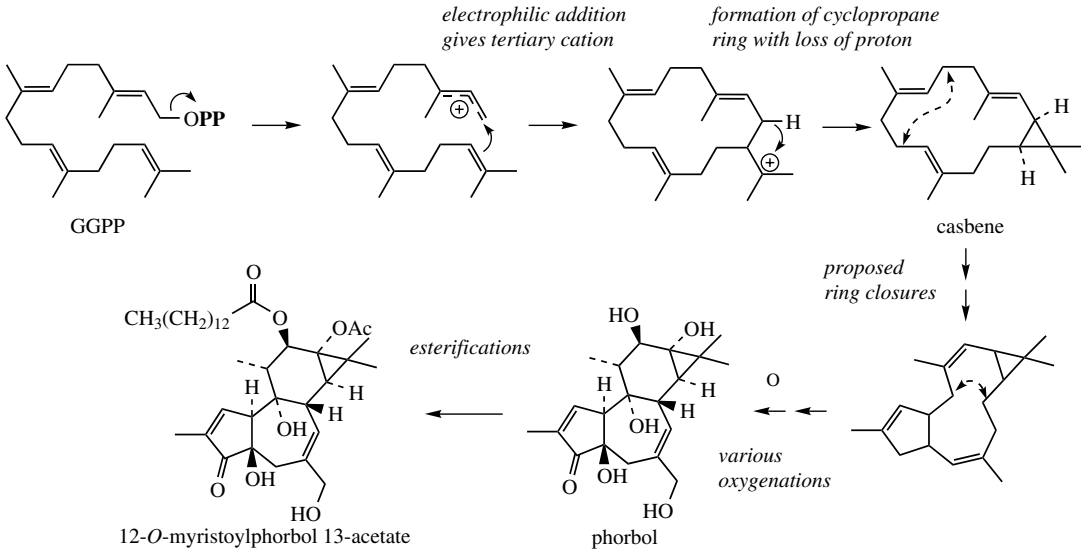


Figure 5.45

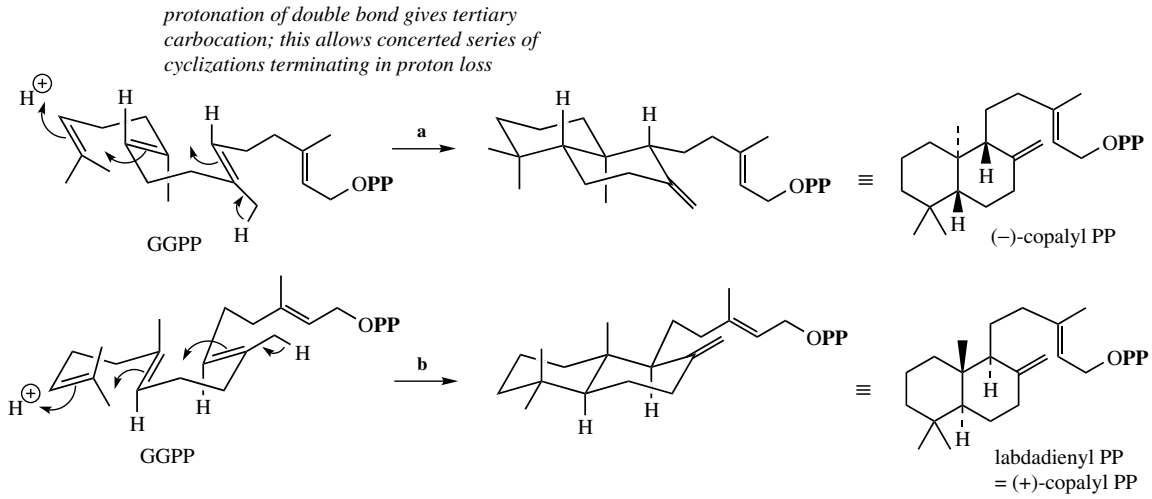


Figure 5.46

the enantiomeric product having opposite configurations at the newly generated chiral centres (Figure 5.46, b). From copalyl PP, a sequence of cyclizations and a rearrangement, all catalysed by a single enzyme, leads to ***ent*-kaurene** (Figure 5.47). As shown, this involves loss of the diphosphate leaving group enabling carbocation-mediated formation of the third ring system, and subsequent production of the fourth ring. Then follows a Wagner–Meerwein migration, effectively contracting the original six-membered ring to a five-membered one, whilst expanding the five-membered ring to give a six-membered ring. The driving force is transformation of a secondary carbocation to give a tertiary one, but this also results in the methyl group no longer being at a bridgehead, and what appears at first glance to be merely a confusing change in stereochemistry. Loss of a proton from this methyl generates the exocyclic double bond of ***ent*-kaurene** and provides an exit from the carbocationic system. The prefix *ent* is used to indicate enantiomeric; the most common stereochemistry

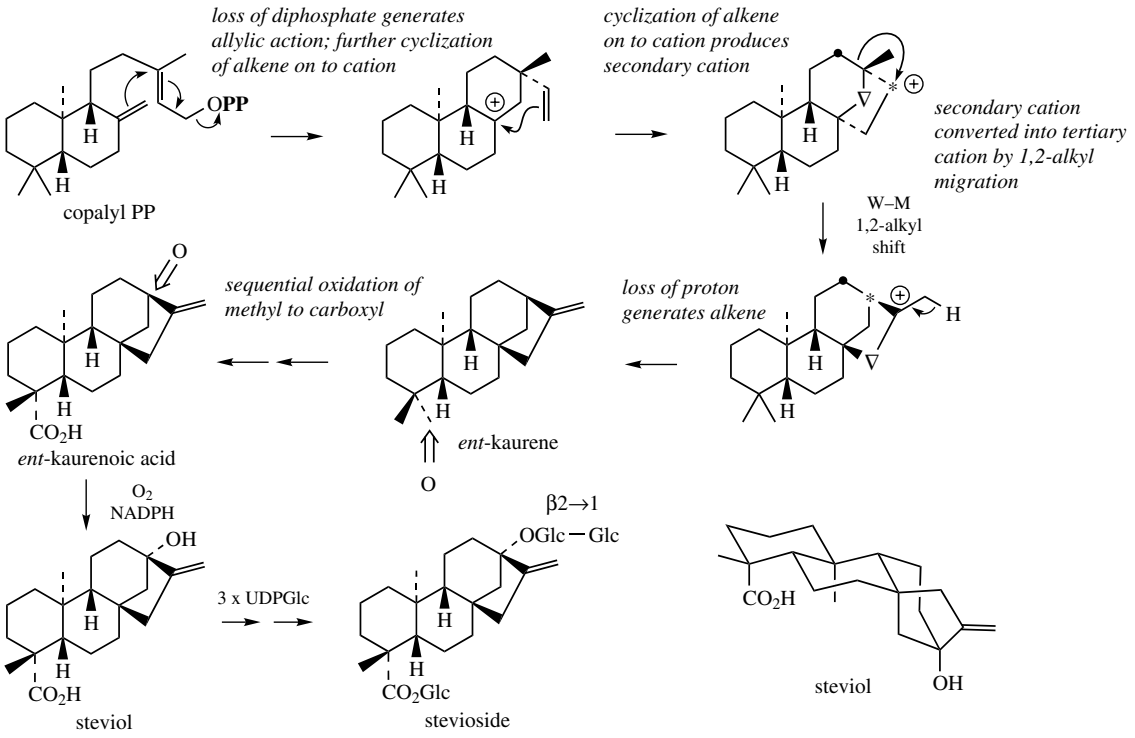


Figure 5.47

is that found in labdadienyl PP (Figure 5.46) and derivatives, so the kaurene series is termed enantiomeric.

ent-Kaurene is the precursor of **stevioside** (Figure 5.47) in the plant *Stevia rebaudiana* (Compositae/Asteraceae) by relatively simple hydroxylation, oxidation, and glucosylation reactions. Both glucosyl ester and glucoside linkages are present in stevioside, which help to confer an intensely sweet taste to this and related compounds. Stevioside is present in the plant leaf in quite large amounts (3–10%), is some 100–300 times as sweet as sucrose, and is being used commercially as a sweetening agent.

The alternative stereochemistry typified by labdadienyl PP can be seen in the structure of **abietic acid** (Figure 5.48), the major component of the rosin fraction of turpentine from pines and other conifers (Table 5.1). Initially, the tricyclic system is built up as in the pathway to *ent*-kaurene (Figure 5.47), via the same mechanism, but generating the enantiomeric series of compounds. The cation loses a proton to give **sandaracopimaradiene** (Figure 5.48), which undergoes a methyl migration to modify the side-chain, and further proton loss to form the diene **abietadiene**. **Abietic acid** results from sequential oxidation of the 4α-methyl. Wounding of pine trees leads to an accumulation at the wound site of both monoterpenes and diterpenes, which may be fractionated by distillation to give turpentine oil and rosin (Table 5.1). The volatile monoterpenes seem to act as a solvent to allow deposition of the rosin layer to seal the wound. The diterpenes in rosin have both antifungal and insecticidal properties.

Extensive modification of the labdadienyl diterpene skeleton is responsible for generation of the **ginkgolides**, highly oxidized diterpene trilactones which are the active principles of *Ginkgo biloba** (Ginkgoaceae). Several rearrangements, ring cleavage, and formation of lactone rings can broadly explain its origin (Figure 5.49), though this scheme is highly speculative and likely to be incorrect. Although detailed evidence is lacking, it is known that labdadienyl PP is a precursor, and most probably dehydroabietane also. The unusual *tert*-butyl substituent arises as a consequence of the A ring cleavage. **Bilobalide**

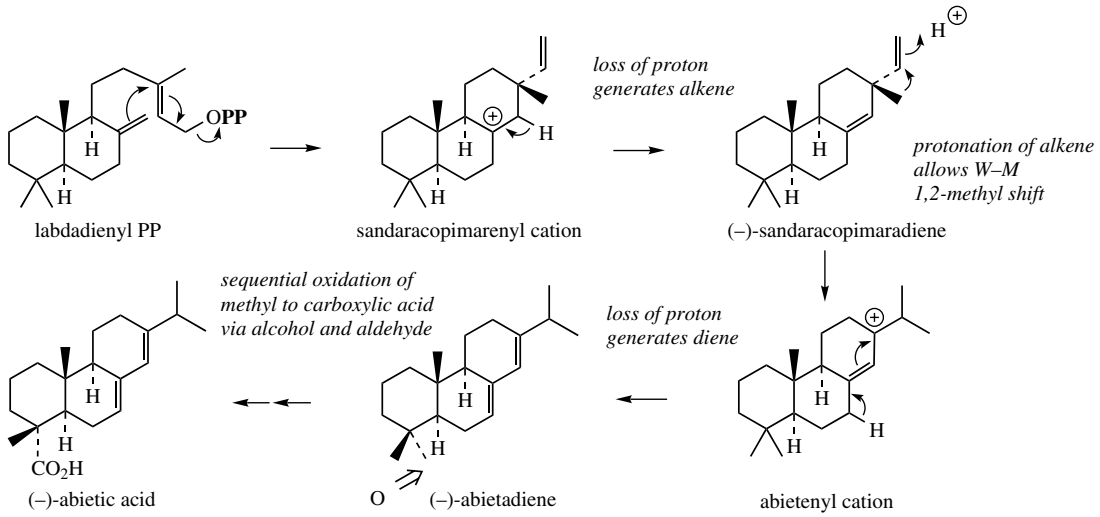


Figure 5.48

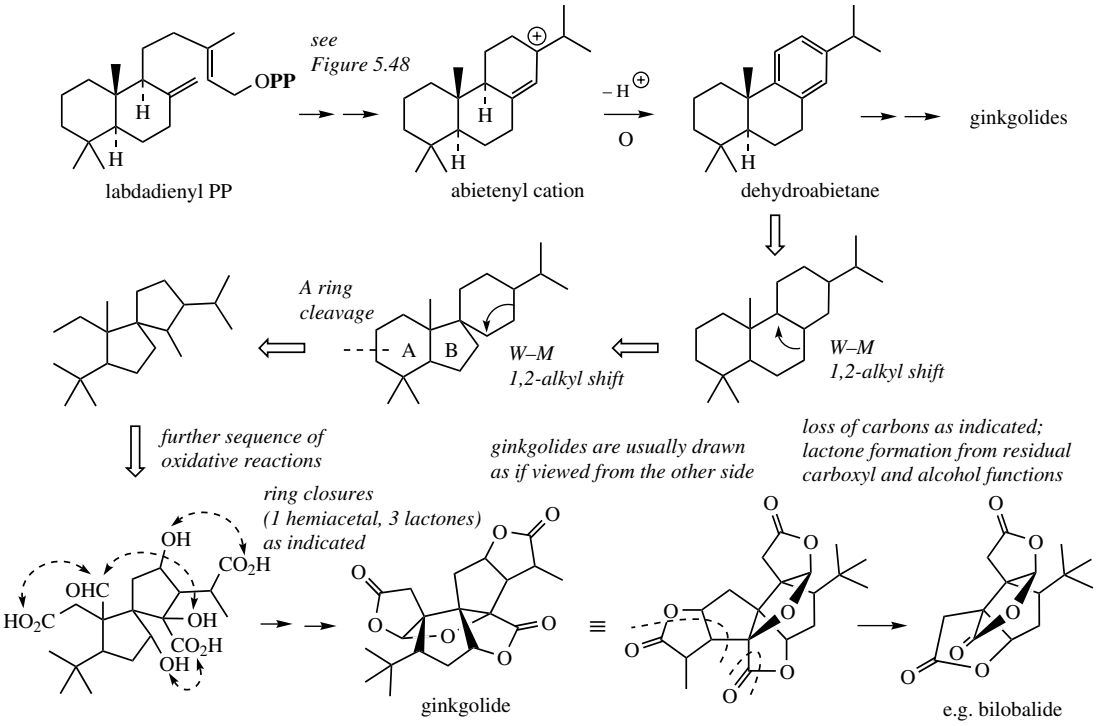


Figure 5.49

(Figure 5.49) contains a related C_{15}-skeleton, and is most likely a partially degraded ginkgolide. *Ginkgo* is the world's oldest tree species, and its leaves are now a currently fashionable health supplement, taken in the hope that it can delay some of the degeneration of the faculties normally experienced in old age.

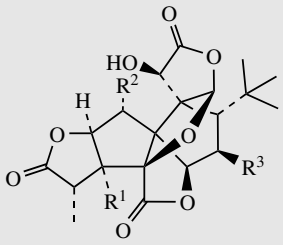
In **forskolin** (Figure 5.51), the third ring is heterocyclic rather than carbocyclic. The basic skeleton of forskolin can be viewed as the result

Ginkgo biloba

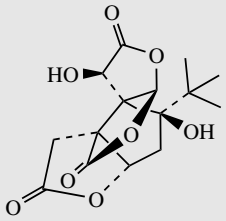
Ginkgo biloba is a primitive member of the gymnosperms and the only survivor of the Ginkgoaceae, all other species being found only as fossils. It is a small tree native to China, but widely planted as an ornamental, and cultivated for drug use in Korea, France, and the United States. Standardized extracts of the leaves are marketed against cerebral vascular disease and senile dementia. Extracts have been shown to improve peripheral and cerebrovascular circulation. The decline in cognitive function and memory processes in old age can be due to disturbances in brain blood circulation, and thus **ginkgo** may exert beneficial effects by improving this circulation, and assist with other symptoms such as vertigo, tinnitus, and hearing loss.

The active constituents have been characterized as mixtures of terpenoids and flavonoids. The dried leaves contain 0.1–0.25% terpene lactones, comprising five ginkgolides (A, B, C, J, and M) and bilobalide (Figure 5.50). Bilobalide comprises about 30–40% of the mixture, whilst ginkgolide A is the predominant ginkgolide (about 30%). The ginkgolides are diterpenoid in nature, whilst bilobalide is described as sesquiterpenoid. However, bilobalide bears such a structural similarity to the ginkgolides, it is most probably a degraded ginkgolide. The ginkgolides have been shown to have potent and selective antagonistic activity towards platelet-activating factor (PAF, see page 39), which is implicated in many physiological processes. The flavonoid content of the dried leaves is 0.5–1.0%, and consists of a mixture of mono-, di-, and tri-glycosides of the flavonols kaempferol and quercetin (Figure 5.50; see also page 151) and some biflavonoids. These probably also contribute to the activity of ginkgo, and may act as radical scavengers.

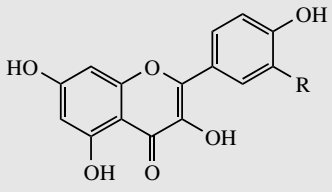
Extracts of ginkgo for drug use are usually standardized to contain flavonoid glycosides and terpene lactones in a ratio of 24% to 6%, or 27% to 7%. Ginkgo may be combined with ginseng (see page 222) in the treatment of geriatric disorders. Ginkgo and the ginkgolides are undergoing extensive investigation in conditions where there are high PAF levels, e.g. shock, burns, ulceration, and inflammatory skin disease.



	R^1	R^2	R^3
ginkgolide A	OH	H	H
ginkgolide B	OH	OH	H
ginkgolide C	OH	OH	OH
ginkgolide J	OH	H	OH
ginkgolide M	H	OH	OH



bilobalide



R = H, kaempferol
R = OH, quercetin

Figure 5.50

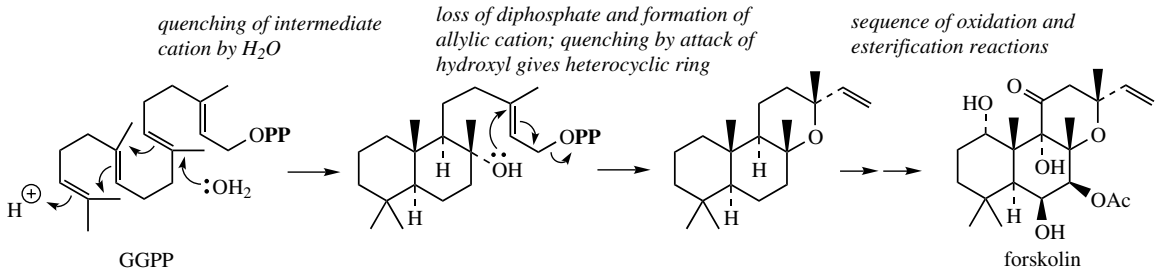


Figure 5.51

of quenching of the cation by water as opposed to proton loss, followed by S_N2' nucleophilic substitution on to the allylic diphosphate (or nucleophilic substitution on to the allylic cation generated by loss of diphosphate) (Figure 5.51). A series of oxidative modifications will then lead to forskolin. This compound has been isolated from *Coleus forskohlii* (Labiatae/Lamiaceae), a plant used in Indian traditional medicine and shown to have quite pronounced hypotensive and antispasmodic activities. Forskolin* is a valuable pharmacological tool as a potent stimulator of adenylate cyclase activity, and it is being investigated for its cardiovascular and bronchospasmolytic effects.

SESTERTERPENES (C_{25})

Although many examples of this group of natural terpenoids are now known, they are found principally in fungi and marine organisms, and span relatively few structural types. The origin of **ophiobolene** and **ophiobolin A** in the plant pathogen *Helminthosporium maydis* from cyclization of **geranylfarnesyl PP** (**GFPP**) is shown in Figure 5.52, and provides no novel features except for an experimentally demonstrated 1,5-hydride shift. GFPP arises by a continuation of the chain extension process, adding a further IPP unit to GGPP. Ophiobolin A shows a broad spectrum of biological activity against bacteria, fungi, and nematodes. The most common type of marine sesterterpenoid is exemplified by **sclarin** and this structure can be envisaged as the result of a concerted cyclization sequence (Figure 5.53) analogous to that seen with GGPP in the diterpenoids, and with squalene oxide in the triterpenoids (see below).

TRITERPENES (C_{30})

Triterpenes are not formed by an extension of the now familiar process of adding IPP to the growing chain. Instead, two molecules of farnesyl PP are joined tail to tail to yield the hydrocarbon **squalene** (Figure 5.54), originally isolated from the liver oil of shark (*Squalus* sp.). Squalene was subsequently found in rat liver and yeast, and these systems were used to study its biosynthetic role

Forskolin

In a screening programme of Indian medicinal plants, extracts from the roots of *Coleus forskohlii* (Labiatae/Lamiaceae) were discovered to lower blood pressure and have cardioactive properties. This led to the isolation of the diterpene forskolin (= coleonol) (Figure 5.51) as the active principle in yields of about 0.1%. Forskolin has been shown to exert its effects by direct stimulation of adenylate cyclase, and has become a valuable pharmacological tool in the study of this enzyme and its functions. It has shown promising potential for the treatment of glaucoma, congestive heart failure, hypertension, and bronchial asthma, though drug use is limited by poor water-solubility, and derivatives or analogues will need to be developed.

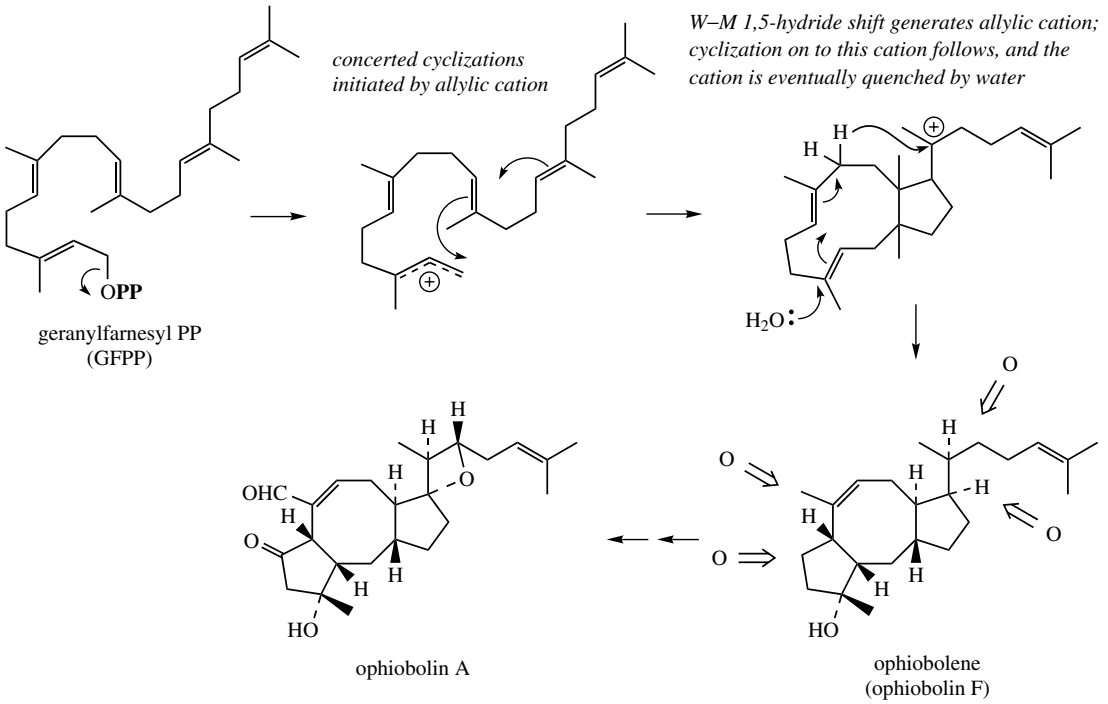


Figure 5.52

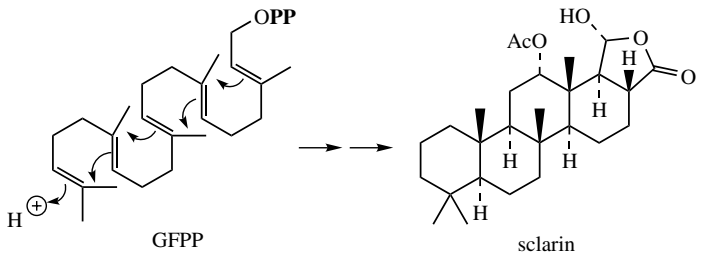


Figure 5.53

as a precursor of triterpenes and steroids; several seed oils are now recognized as quite rich sources of squalene, e.g. *Amaranthus cruentus* (Amaranthaceae). During the coupling process, which on paper merely requires removal of the two diphosphate groups, a proton from a C-1 position of one molecule of FPP is lost, and a proton from NADPH is inserted. Difficulties with formulating a plausible mechanism for this unlikely reaction were resolved when an intermediate in the process, **presqualene diphosphate**, was isolated from rat liver. Its characterization as a cyclopropane derivative immediately ruled out all the hypotheses current at the time.

The formation of presqualene PP is represented in Figure 5.54 as attack of the 2,3-double bond of FPP on to the farnesyl cation, analogous to the chain extension using IPP (see also the proposal for the origins of irregular monoterpenes, page 186). The resultant tertiary cation is discharged by loss of a proton and formation of the cyclopropane ring, giving presqualene PP. Obviously, to form squalene, carbons-1 of the two FPP units must eventually be coupled, whilst presqualene PP formation has actually joined C-1 of one molecule to C-2 of the other. To account for the subsequent change in bonding of the two FPP units, a further cyclopropane cationic intermediate is proposed. Loss of

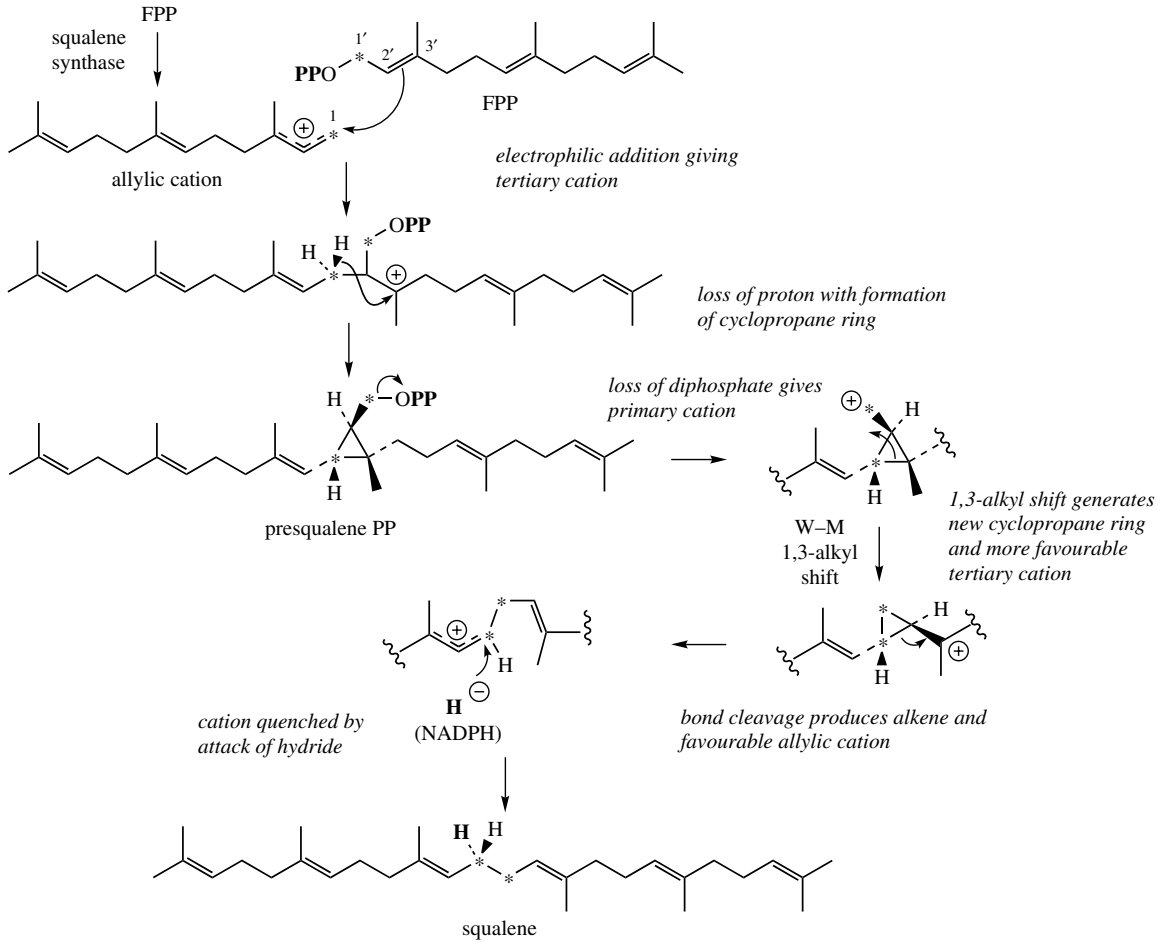


Figure 5.54

diphosphate from presqualene PP would give the unfavourable primary cation, which via Wagner–Meerwein rearrangement can generate a tertiary carbocation and achieve the required C-1–C-1′ bond. Breaking the original but now redundant C-1–C-2′ bond can give an allylic cation, and the generation of **squalene** is completed by supply of hydride from NADPH.

Cyclization of squalene is via the intermediate **squalene-2,3-oxide** (Figure 5.55), produced in a reaction catalysed by a flavoprotein requiring O_2 and NADPH cofactors. If squalene oxide is suitably positioned and folded on the enzyme surface, the polycyclic triterpene structures formed can be rationalized in terms of a series of cyclizations, followed by a sequence of concerted Wagner–Meerwein migrations of methyls and hydrides (Figure 5.55). The cyclizations are carbocation mediated and proceed in a step-wise sequence (Figure 5.56). Thus, protonation of the epoxide group will allow opening of this ring and generation of the preferred tertiary carbocation, suitably placed to allow electrophilic addition to a double bond, formation of a six-membered ring and production of a new tertiary carbocation. This process continues twice more, generating the preferred tertiary carbocation (Markovnikov addition) after each ring formation, though the third ring formed is consequently a five-membered one. This is expanded to a six-membered ring via a Wagner–Meerwein 1,2-alkyl shift, resulting in some relief of ring strain, though sacrificing a tertiary carbocation for a secondary one. A further electrophilic addition generates the tertiary protosteryl

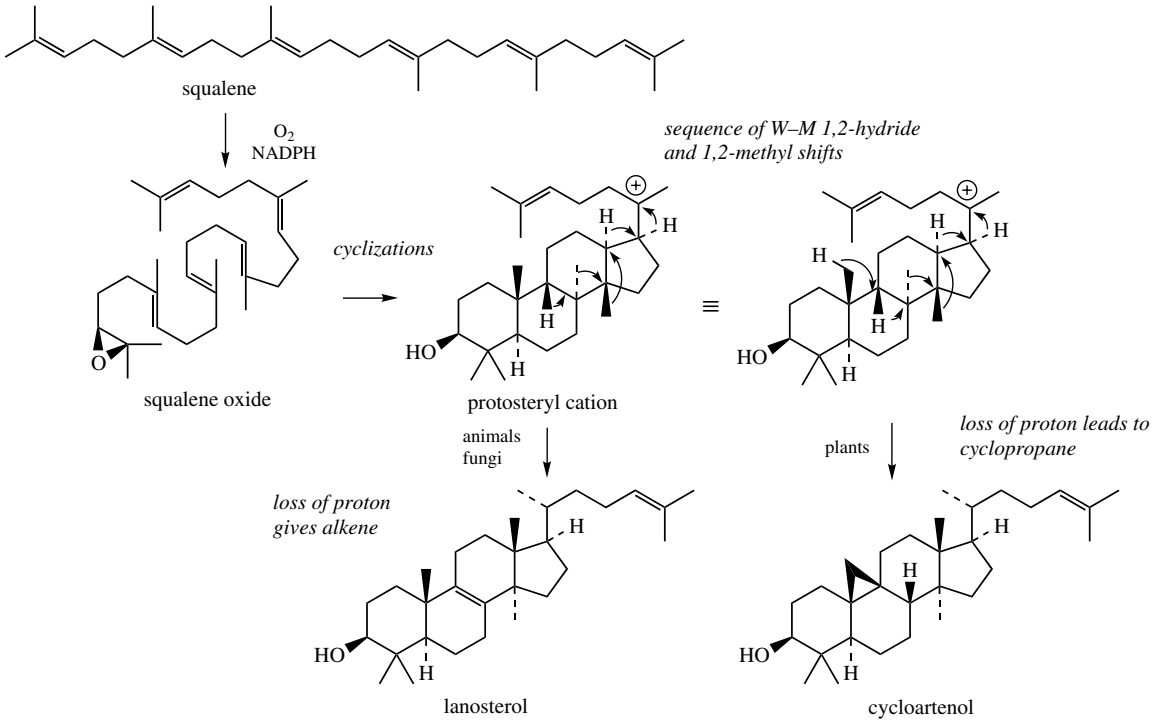


Figure 5.55

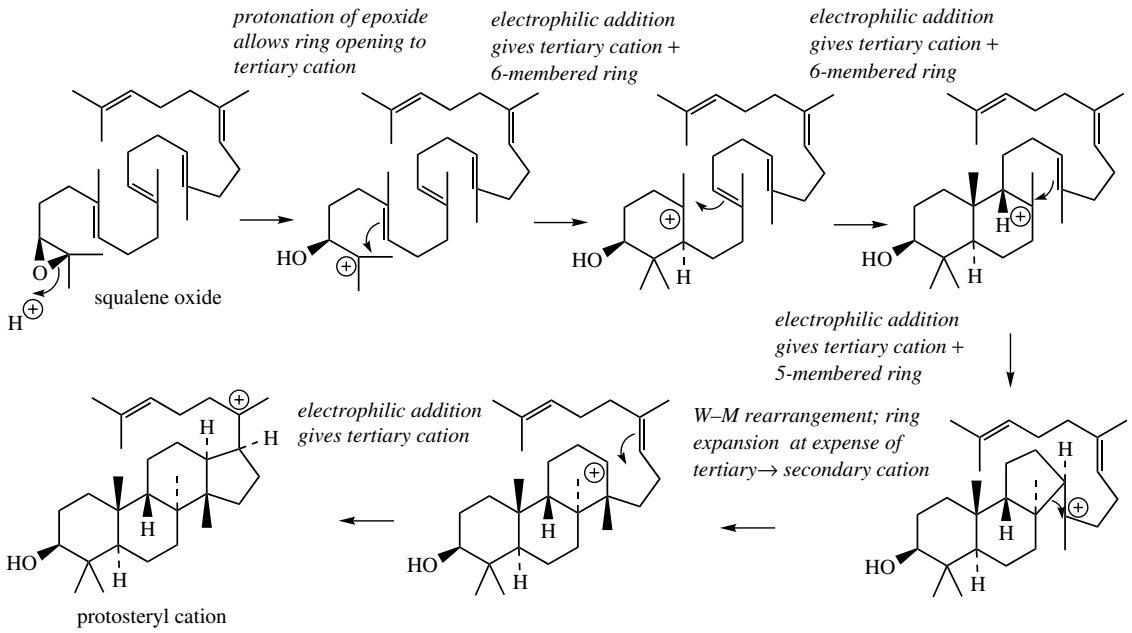


Figure 5.56

cation (Figure 5.56). The stereochemistries in this cation are controlled by the type of folding achieved on the enzyme surface, and this probably also limits the extent of the cyclization process. Thus, if the folded squalene oxide approximates to a chair–boat–chair–boat conformation (Figure 5.57), the transient **protosteryl cation** will be produced with these conformational characteristics. This cation then undergoes a series of Wagner–Meerwein 1,2-shifts, firstly migrating a hydride and generating a new cation, migrating the next hydride, then a methyl and so on until a proton is lost forming a double bond and thus creating **lanosterol** (Figure 5.57). The stereochemistry

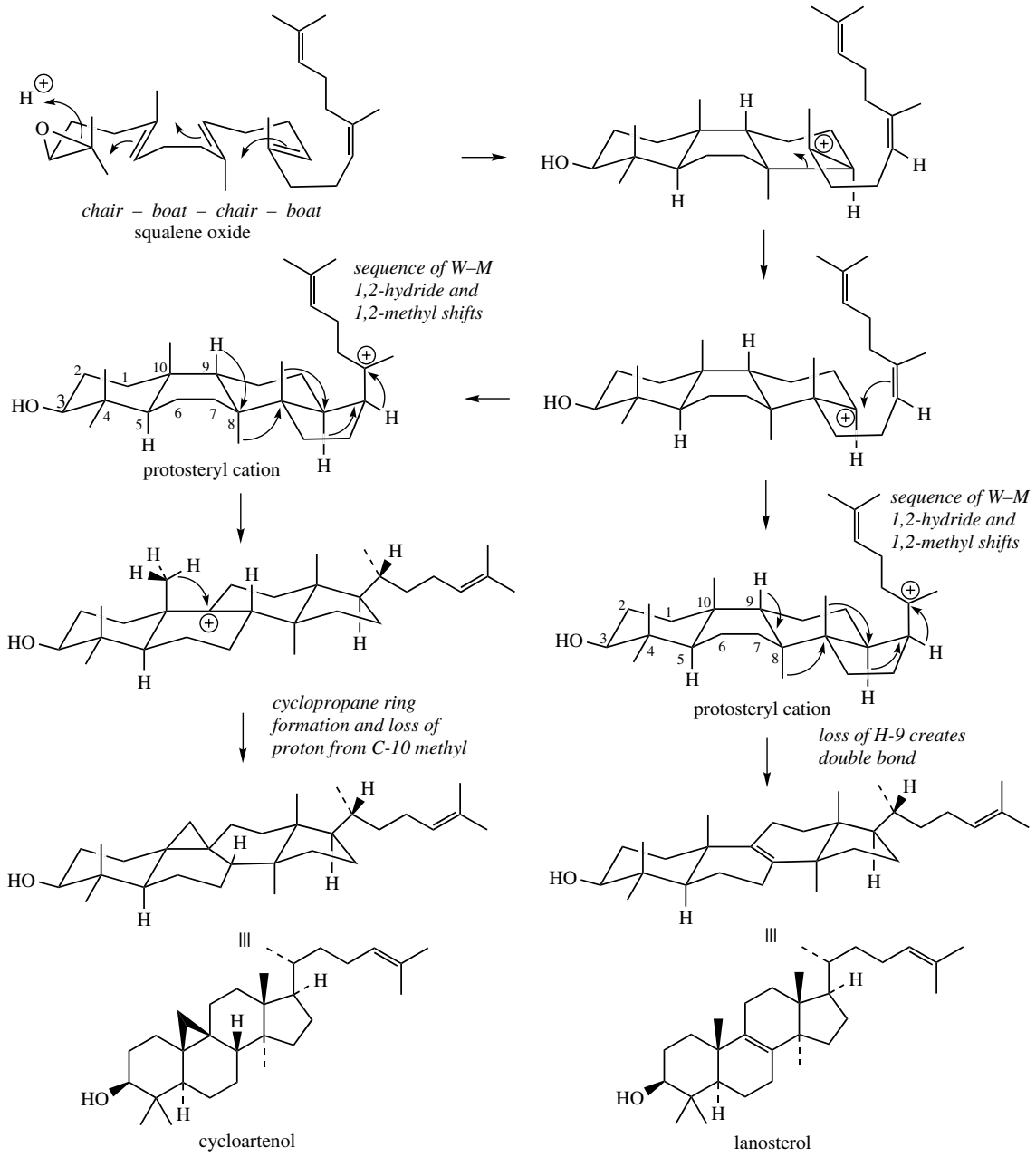


Figure 5.57

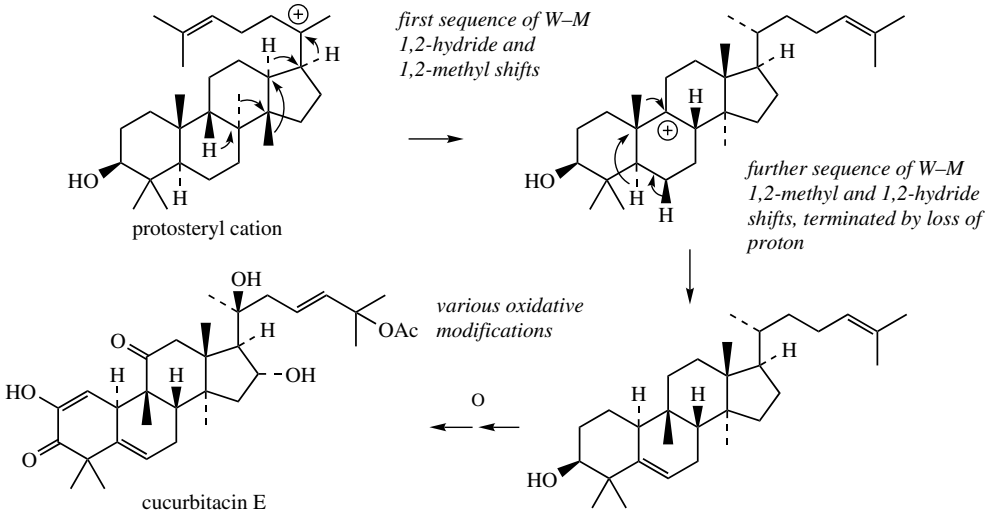


Figure 5.58

of the protosteryl cation in Figure 5.57 shows how favourable this sequence will be, and emphasizes that in the ring system, the migrating groups are positioned *anti* to each other, one group entering whilst the other leaves from the opposite side of the stereocentre. This, of course, inverts configurations at each appropriate centre. No *anti* group is available to migrate to C-9 (steroid numbering), and the reaction terminates by loss of proton H-9. Lanosterol is a typical animal triterpenoid, and the precursor for cholesterol and other sterols in animals (see page 233) and fungi (see page 254). In plants, its intermediate role is taken by **cycloartenol** (Figure 5.57), which contains a cyclopropane ring, generated by inclusion of carbon from the methyl at C-10. For cycloartenol, H-9 is not lost, but migrates to C-8, and the carbocation so formed is quenched by cyclopropane formation and loss of one of the methyl protons. For many plant steroids, this cyclopropane ring has then to be reopened (see page 235). Most natural triterpenoids and steroids contain a 3-hydroxyl group, the original epoxide oxygen from squalene oxide.

An additional feature of the protosteryl cation is that the C-10 methyl and H-5 also share an *anti*-axial relationship, and are also susceptible to Wagner–Meerwein rearrangements, so that the C-9 cation formed in the cycloartenol sequence may then initiate further migrations. This can be terminated by formation of a 5,6-double bond (Figure 5.58), as in the pathway to the **cucurbitacins**, a group of highly oxygenated triterpenes encountered in the cucumber/melon/marrow family, the Cucurbitaceae. These compounds are characteristically bitter tasting, purgative, and extremely cytotoxic.

Should **squalene oxide** be folded on to another type of cyclase enzyme, this time in a roughly chair–chair–chair–boat conformation (Figure 5.59), then an identical carbocation mechanism ensues, and the transient **dammarenyl cation** formed now has different stereochemical features to the protosteryl cation. Whilst a series of Wagner–Meerwein migrations can occur, there is relatively little to be gained on purely chemical grounds, since these would invert stereochemistry and destroy the already favourable conformation. Instead, the dammarenyl cation typically undergoes further carbocation promoted cyclizations, without any major changes to the ring system already formed. There are occasions in which the migrations do occur, however, and **euphol** from *Euphorbia* species (Euphorbiaceae) is a stereoisomer of lanosterol (Figure 5.55).

Should the Wagner–Meerwein rearrangements not occur, the dammarenyl cation could be quenched with water, giving the epimeric **dammarenediols**, as found in Dammar resin from *Balanocarpus heimii* (Dipterocarpaceae) and ginseng* (*Panax ginseng*; Araliaceae) (Figure 5.60). Alternatively, the migration shown to give the

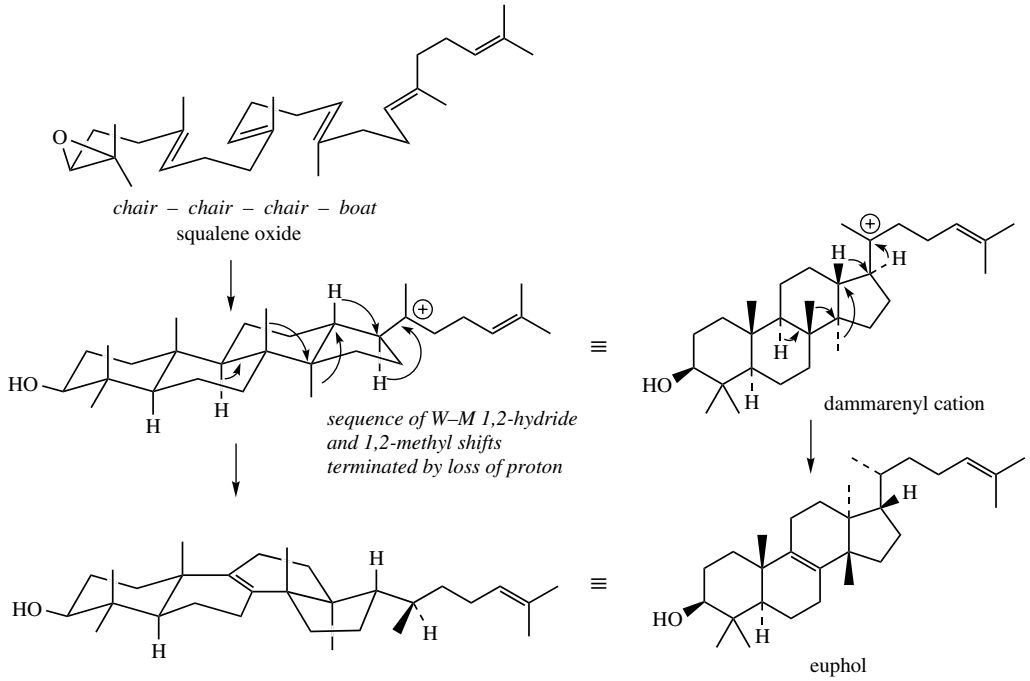


Figure 5.59

baccharenyl cation relieves some ring strain by creating a six-membered ring, despite sacrificing a tertiary carbocation for a secondary one. A pentacyclic ring system can now be formed by cyclization on to the double bond, giving a new five-membered ring and the tertiary lupenyl cation. Although this appears to contradict the reasoning used above for the dammarenyl → baccharenyl transformation, the contribution of the enzyme involved must also be considered in each case. A five-membered ring is not highly strained as evidenced by all the natural examples encountered. Loss of a proton from the lupenyl cation gives **lupeol**, found in lupin (*Lupinus luteus*; Leguminosae/Fabaceae). Ring expansion in the lupenyl cation by bond migration gives the oleanyl system, and labelling studies have demonstrated this ion is discharged by hydride migrations and loss of a proton, giving the widely distributed **β-amyrin**. Formation of the isomeric **α-amyrin** involves first the migration of a methyl in the oleanyl cation, then discharge of the new taraxasteryl cation by three hydride migrations and loss of a proton. Loss of a proton from the non-migrated methyl in the taraxasteryl cation is an alternative way of achieving a neutral molecule, and yields **taraxasterol** found in dandelion (*Taraxacum officinale*; Compositae/Asteraceae). Comparison with α-amyrin shows the subtly different stereochemistry present because the inversions of configuration caused by hydride migrations have not occurred. Where evidence is available, these extensive series of cyclizations and Wagner–Meerwein rearrangements appear to be catalysed by a single enzyme, which converts squalene oxide into the final product, e.g. lanosterol, cycloartenol, α-amyrin, or β-amyrin.

Bacterial membranes frequently contain **hopanoids** (Figure 5.61), triterpenoid compounds that appear to take the place of the sterols that are typically found in the membranes of higher organisms, helping to maintain the structural integrity and to control permeability. Hopanoids arise from squalene by a similar carbocation cyclization mechanism, but do not involve the initial epoxidation to squalene oxide. Instead, the carbocation is produced by protonation (compare the cyclization of GGPP to labdadienyl PP, page 207), and the resultant compounds tend to lack the characteristic 3-hydroxyl group, e.g. **hopan-22-ol** from *Alicyclobacillus acidocaldarius* (Figure 5.61). On the other hand, **tetrahymanol** from the protozoan

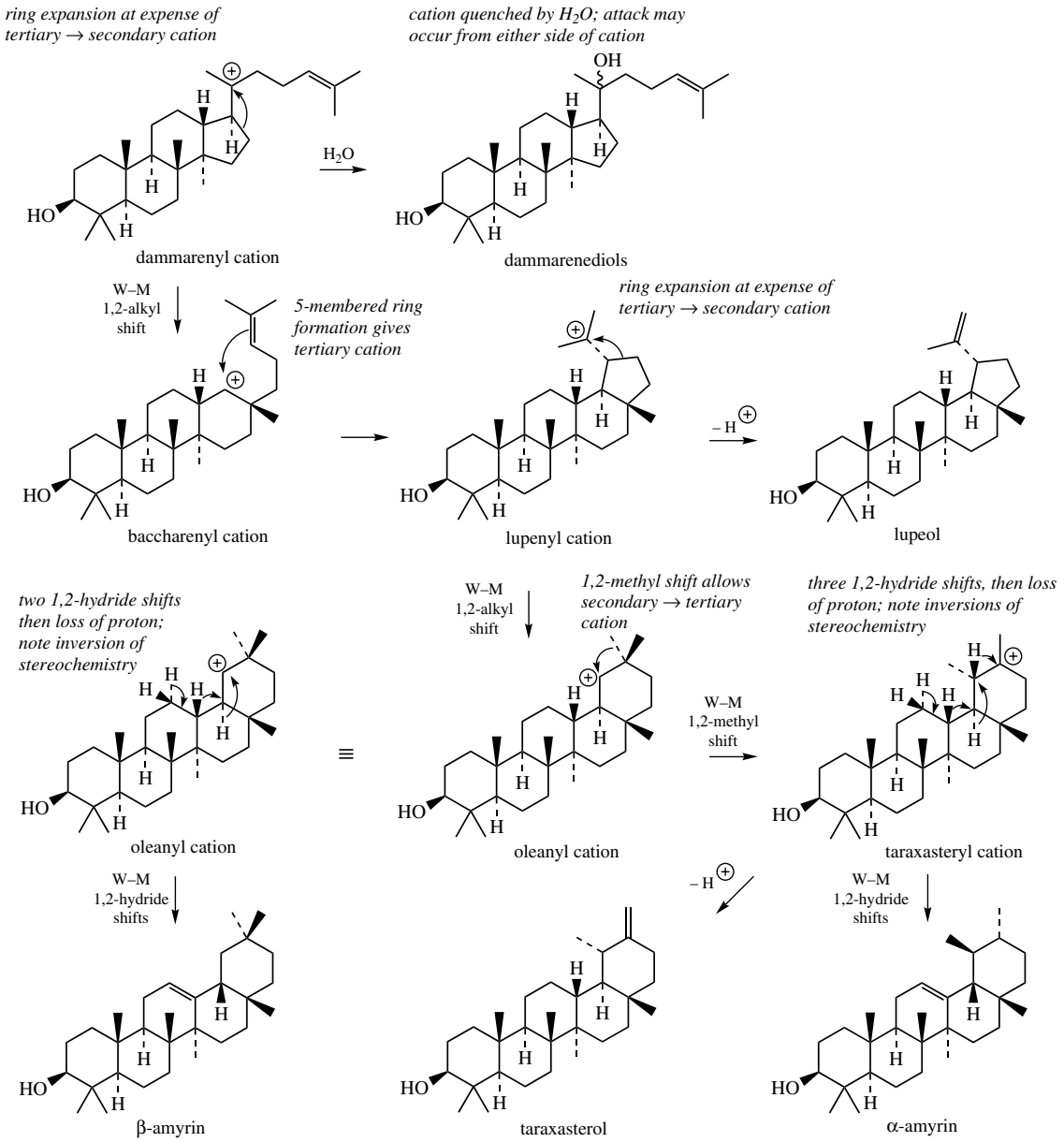


Figure 5.60

Tetrahymena pyriformis, because of its symmetry, might appear to have a 3-hydroxyl group, but this is derived from water, and not molecular oxygen as would be the case if squalene oxide were involved. As in formation of the protosteryl cation (page 214), Wagner–Meerwein ring expansions occur during the cyclization mechanisms shown in Figure 5.61 so that the first-formed tertiary carbocation/five-membered ring (Markovnikov addition) becomes a secondary carbocation/six-membered ring.

Triterpenoid Saponins

The pentacyclic triterpenoid skeletons exemplified by lupeol, α-amyrin, and β-amyrin (Figure 5.60) are frequently encountered in the form of triterpenoid saponin structures. Saponins are

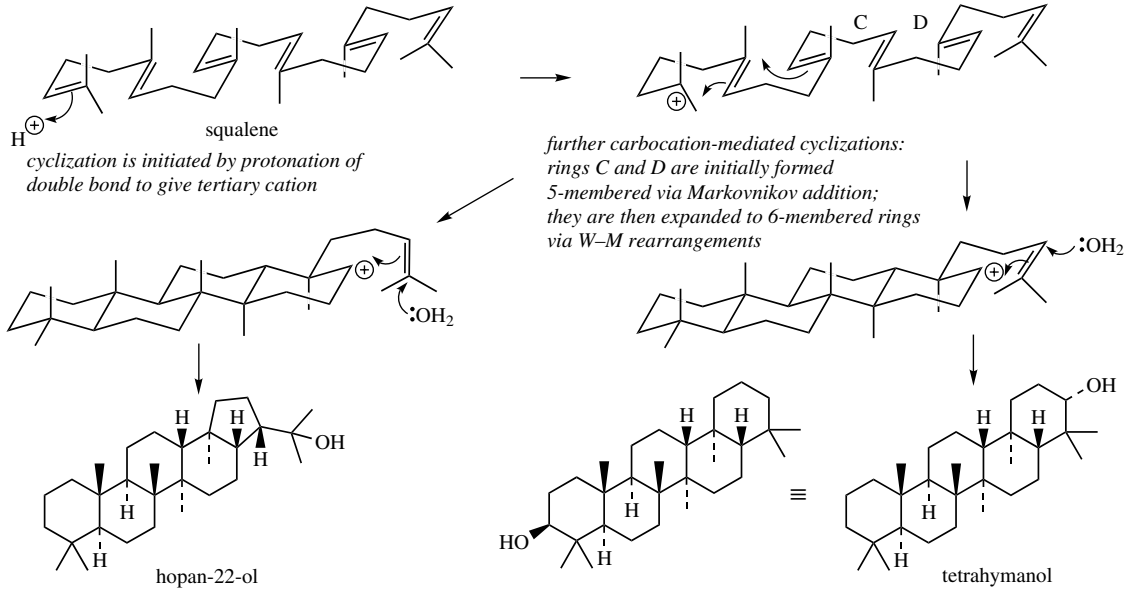


Figure 5.61

glycosides which, even at low concentrations, produce a frothing in aqueous solution, because they have surfactant and soaplike properties. The name comes from the Latin *sapo*, soap, and plant materials containing saponins were originally used for cleansing clothes, e.g. soapwort (*Saponaria officinalis*; Caryophyllaceae) and quillaia or soapbark (*Quillaja saponaria*; Rosaceae). These materials also cause haemolysis, lysing red blood cells by increasing the permeability of the plasma membrane, and thus they are highly toxic when injected into the blood stream. Some saponin-containing plant extracts have been used as arrow poisons. However, saponins are relatively harmless when taken orally, and some of our valuable food materials, e.g. beans, lentils, soybeans, spinach, and oats, contain significant amounts. Sarsaparilla (see page 242) is rich in steroidal saponins but is widely used in the manufacture of non-alcoholic drinks. Toxicity is minimized during ingestion by low absorption, and by hydrolysis. Acid-catalysed hydrolysis of saponins liberates sugar(s) and an aglycone (sapogenin), which can be either triterpenoid or steroidal (see page 237) in nature. Some plants may contain exceptionally high amounts of saponins, e.g. about 10% in quillaia bark.

Triterpenoid saponins are rare in monocotyledons, but abundant in many dicotyledonous families. Medicinally useful examples are mainly based on the β-amyrin subgroup (Figure 5.62), and many of these possess carboxylic acid groups derived by oxidation of methyl groups, those at positions 4 (C-23), 17 (C-28), and 20 (C-30) on the aglycone ring system being subject to such oxidation. In some structures, less oxidized formyl (−CHO) or hydroxymethyl ($-CH_2OH$) groups may also be encountered. Positions 11 and 16 may also be oxygenated. Sugar residues are usually attached to the 3-hydroxyl, with one to six monosaccharide units, the most common being glucose, galactose, rhamnose, and arabinose, with uronic acid units (glucuronic acid and galacturonic acid) also featuring (see page 467). Thus, quillaia* bark contains a saponin mixture with **quillaic acid** (Figure 5.62) as the principal aglycone, and the medicinally valuable root of liquorice* (*Glycyrrhiza glabra*; Leguminosae/Fabaceae) contains **glycyrrhizin**, a mixture of potassium and calcium salts of **glycyrrhizic acid** (Figure 5.63), which is composed of the aglycone **glycyrrhetic acid** and two glucuronic acid units. Seeds of horsechestnut (*Aesculus hippocastrum*; Hippocastanaceae), sometimes used in herbal preparations as an anti-inflammatory and anti-bruising remedy, contain a complex mixture of saponins termed **aescin**, based on the polyhydroxylated aglycones **protoaescigenin** and **barringtogenol**

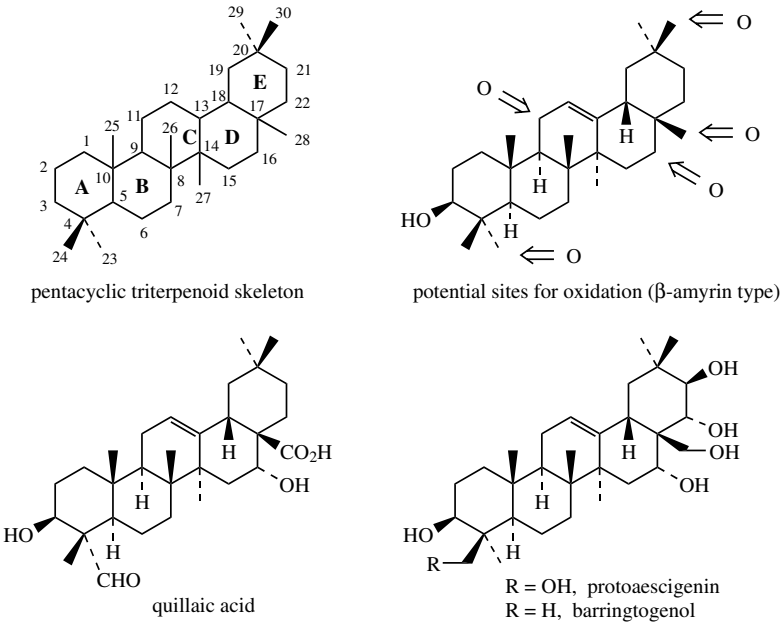


Figure 5.62

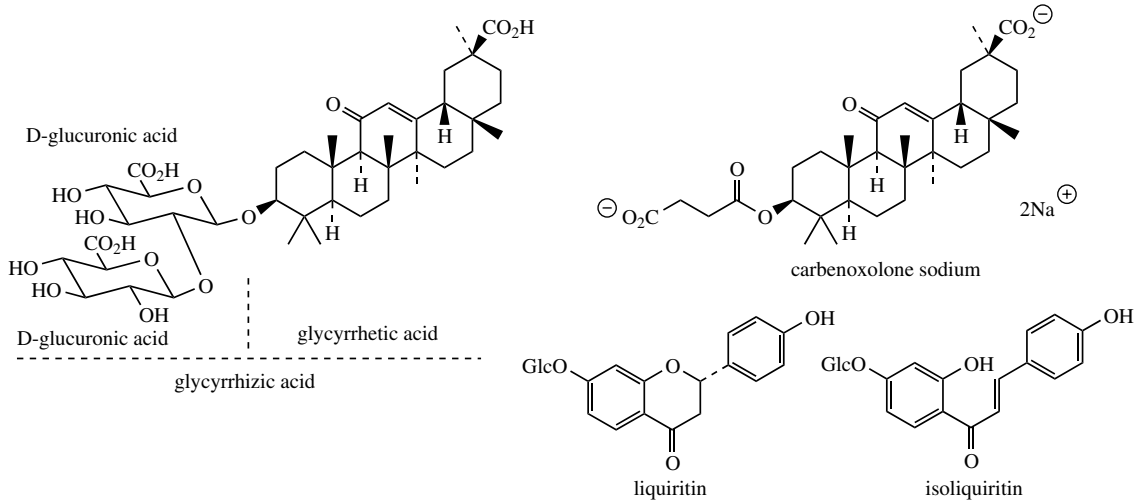


Figure 5.63

Liquorice

Liquorice (licorice; glycyrrhiza) is the dried unpeeled rhizome and root of the perennial herb *Glycyrrhiza glabra* (Leguminosae/Fabaceae). A number of different varieties are cultivated commercially, including *G. glabra* var. *typica* (Spanish liquorice) in Spain, Italy, and France, and *G. glabra* var. *glandulifera* (Russian liquorice) in Russia. Russian liquorice is usually peeled

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before drying. *Glycyrrhiza uralensis* (Manchurian liquorice) from China is also commercially important. Much of the liquorice is imported in the form of an extract, prepared by extraction with water, then evaporation to give a dark black solid. Most of the liquorice produced is used in confectionery and for flavouring, including tobacco, beers, and stouts. Its pleasant sweet taste and foaming properties are due to saponins. Liquorice root contains about 20% of water soluble extractives, and much of this (typically 3–5% of the root, but up to 12% in some varieties) is comprised of glycyrrhizin, a mixture of the potassium and calcium salts of glycyrrhizic (glycyrrhizinic) acid (Figure 5.63). Glycyrrhizic acid is a diglucuronide of the aglycone glycyrrhetic (glycyrrhetinic) acid. The bright yellow colour of liquorice root is provided by flavonoids (1–1.5%) including liquiritin and isoliquiritin (Figure 5.63), and their corresponding aglycones (see page 150). Considerable amounts (5–15%) of sugars (glucose and sucrose) are also present.

Glycyrrhizin is reported to be 50–150 times as sweet as sucrose, and liquorice has thus long been used in pharmacy to mask the taste of bitter drugs. Its surfactant properties have also been exploited in various formulations, as have its demulcent and mild expectorant properties. More recently, some corticosteroid-like activity has been recognized, liquorice extracts displaying mild anti-inflammatory and mineralocorticoid activities. These have been exploited in the treatment of rheumatoid arthritis, Addison's disease (chronic adrenocortical insufficiency), and various inflammatory conditions. Glycyrrhetic acid has been implicated in these activities, and has been found to inhibit enzymes that catalyse the conversion of prostaglandins and glucocorticoids into inactive metabolites. This results in increased levels of prostaglandins, e.g. PGE_2 and $PGF_{2\alpha}$ (see page 54), and of hydrocortisone (see page 268). Perhaps the most important current application is to give systematic relief from peptic ulcers by promoting healing through increased prostaglandin activity. A semi-synthetic derivative of glycyrrhetic acid, the hemisuccinate **carbenoxolone sodium** (Figure 5.63), is widely prescribed for the treatment of gastric ulcers, and also duodenal ulcers. The mineralocorticoid effects (sodium and water retention) may exacerbate hypertension and cardiac problems. Surprisingly, a **deglycyrrhizinized liquorice** preparation is also marketed for treatment of peptic ulcers, but its efficiency has been questioned.

Quillaia

Quillaia bark or soapbark is derived from the tree *Quillaja saponaria* (Rosaceae) and other *Quillaja* species found in Chile, Peru, and Bolivia. The bark contains up to 10% saponins, a mixture known as 'commercial saponin', which is used for its detergent properties. Quillaia's surfactant properties are occasionally exploited in pharmaceutical preparations where it is used in the form of quillaia tincture as an emulsifying agent, particularly for fats, tars, and volatile oils. The bark contains a mixture of saponins which on hydrolysis liberates quillaic acid (Figure 5.62) as the aglycone, together with sugars, uronic acids, and acids from ester functions.

Ginseng

The roots of the herbaceous plants *Panax ginseng* (Araliaceae) from China, Korea, and Russia, and related *Panax* species, e.g. *P. quinquefolium* (American ginseng) from the USA

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and Canada, and *P. notoginseng* (Sanchi-ginseng) from China, have been widely used in China and Russia for the treatment of a number of diseases including anaemia, diabetes, gastritis, insomnia, sexual impotence, and as a general restorative. Interest in the drug has increased considerably in recent years and ginseng is widely available as a health food in the form of powders, extracts, and teas. The dried and usually peeled root provides white ginseng, whereas red ginseng is obtained by steaming the root, this process generating a reddish-brown caramel-like colour, and reputedly enhancing biological activity. **Ginseng** is classified as an 'adaptogen', helping the body to adapt to stress, improving stamina and concentration, and providing a normalizing and restorative effect. It is also widely promoted as an aphrodisiac. The Korean root is highly prized and the most expensive. Long term use of ginseng can lead to symptoms similar to those of corticosteroid poisoning, including hypertension, nervousness, and sleeplessness in some subjects, yet hypotension and tranquillizing effects in others.

The benefits of ginseng treatment are by no means confirmed at the pharmacological level, though CNS-stimulating, CNS-sedative, tranquillizing, antifatigue, hypotensive, and hypertensive activities have all been demonstrated. Many of the secondary metabolites present in the root have now been identified. It contains a large number of triterpenoid saponins based on the dammarane subgroup, saponins that have been termed ginsenosides by Japanese investigators, or panaxosides by Russian researchers. These are derivatives of two main aglycones, protopanaxadiol and protopanaxatriol (Figure 5.64), though the aglycones liberated on acid hydrolysis are panaxadiol and panaxatriol respectively. Acid-catalysed cyclization in the side-chain produces an ether ring (Figure 5.64). Sugars are present in the saponins on the 3- and 20-hydroxyls in the diol series, and the 6- and 20-hydroxyls in the triol series. About 30 ginsenosides have been characterized from the different varieties of ginseng, with ginsenoside $R_{b\text{-}1}$ (Figure 5.64) of the diol series typically being the most abundant constituent. Ginsenoside $R_{g\text{-}1}$ (Figure 5.64) is usually the major component representative of the triol series. Other variants are shown in Figure 5.64. Particularly in white ginseng, many of the ginsenosides are also present as esters with malonic acid. Steaming to prepare red ginseng causes partial hydrolysis of esters and glycosides. Ginsenosides $R_{b\text{-}1}$ and $R_{g\text{-}1}$ appear to be the main representatives in *Panax ginseng*, ginsenosides $R_{b\text{-}1}$, $R_{g\text{-}1}$, and R_d in *P. notoginseng*, and ginsenosides $R_{b\text{-}1}$, R_e, and malonylated $R_{b\text{-}1}$ in *P. quinquefolium*. The pentacyclic triterpenoid sapogenin oleanolic acid (Figure 5.65) is also produced by hydrolysis of the total saponins of *P. ginseng*, and is present in some saponin structures (chikusetsusaponins). The saponin contents of *Panax notoginseng* (about 12%) and *P. quinquefolium* (about 6%) are generally higher than that of *P. ginseng* (1.5–2%).

The root of *Eleutherococcus senticosus* (*Acanthopanax senticosus*) (Araliaceae) is used as an inexpensive substitute for ginseng, and is known as **Russian** or **Siberian ginseng**. This material is held to have similar adaptogenic properties as *Panax ginseng* and a number of eleutherosides have been isolated. However, the term eleutheroside has been applied to compounds of different chemical classes, and the main active anti-stress constituents appear to be lignan glycosides, e.g. eleutheroside E (≡ syringaresinol diglucoside) (Figure 5.65) (see page 132) and phenylpropane glycosides, e.g. eleutheroside B (≡ syringin). The leaves of Russian ginseng contain a number of saponins based on oleanolic acid, but these are quite different to the ginsenosides/panaxosides found in *Panax*. Whilst there is sufficient evidence to support the beneficial adaptogen properties for *Eleutherococcus senticosus*, detailed pharmacological confirmation is not available.

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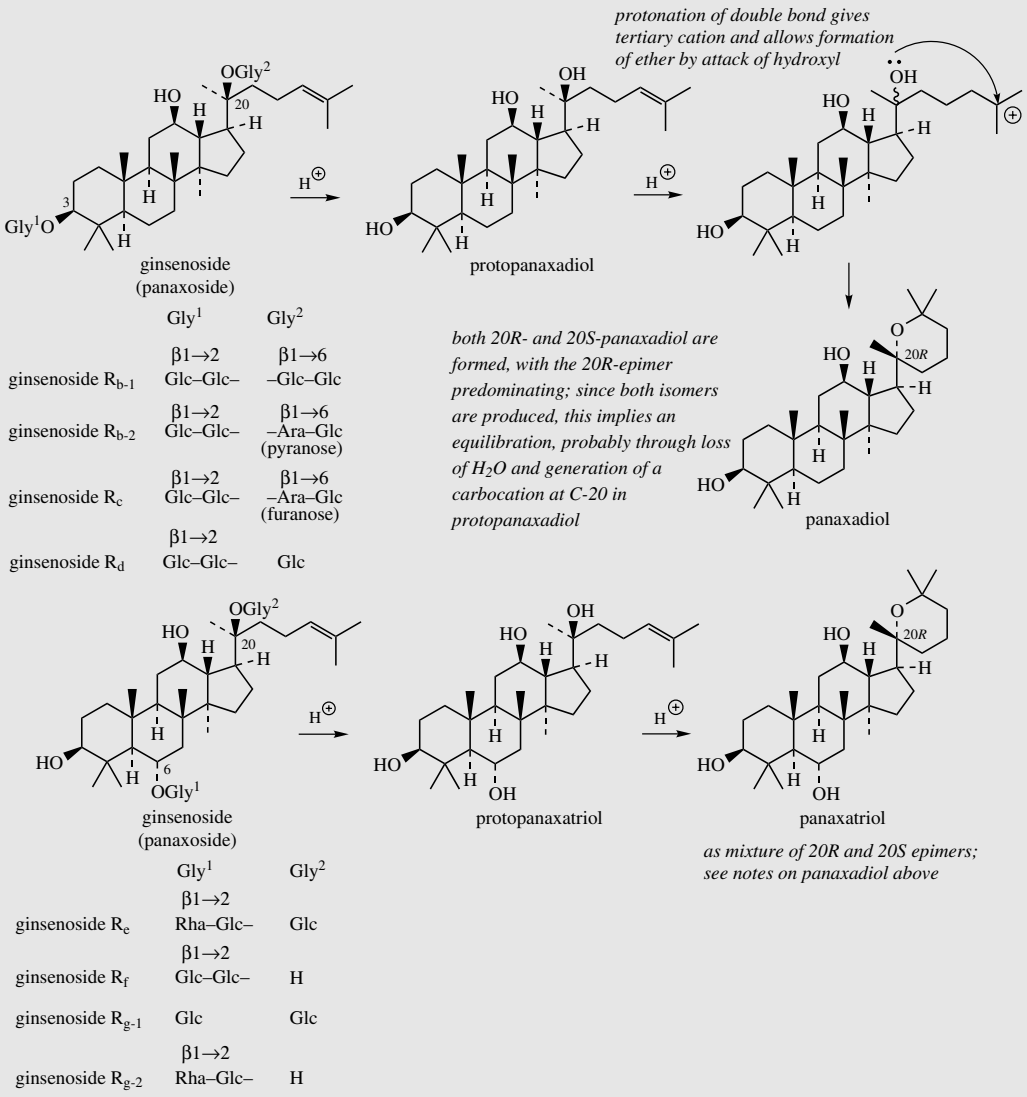

	Gly^1	Gly^2
	β1→2	β1→6
ginsenoside R_{b-1}	Glc–Glc–	–Glc–Glc
ginsenoside R_{b-2}	Glc–Glc–	–Ara–Glc (pyranose)
ginsenoside R_c	Glc–Glc–	–Ara–Glc (furanose)
ginsenoside R_d	Glc–Glc–	Glc
ginsenoside R_e	Rha–Glc–	Glc
ginsenoside R_f	Glc–Glc–	H
ginsenoside R_{g-1}	Glc	Glc
ginsenoside R_{g-2}	Rha–Glc–	H

Figure 5.64

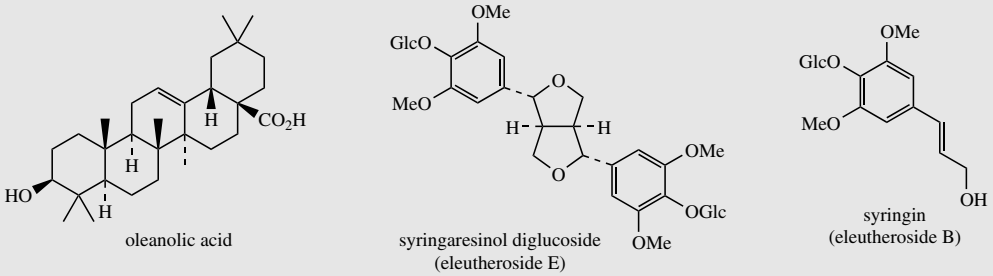

Figure 5.65

(Figure 5.62). Several of these hydroxyls are esterified with aliphatic acids, e.g. acetic, tiglic, and angelic acids.

Modified Triterpenoids

The triterpenoid skeletons may be subjected to a variety of structural modifications as already illustrated. However, the particular modifications considered in this section are those that lead to loss of several skeletal carbon atoms. Pre-eminent amongst such degraded triterpenoids are the steroids, and these are so important that they are considered separately. Other degraded triterpenoids include the **limonoids** (tetranortriterpenoids), in which four terminal carbons from the side-chain are removed, and the **quassinoids**, which have lost ten carbons, including one of the C-4 methyls. The quassinoids thus have a C_{20} skeleton which could be misinterpreted as a diterpene structure. Biosynthetic information is relatively sparse, but the relationship to precursors of the euphol type is outlined in Figure 5.66. Limonoids are found mainly in plants of the

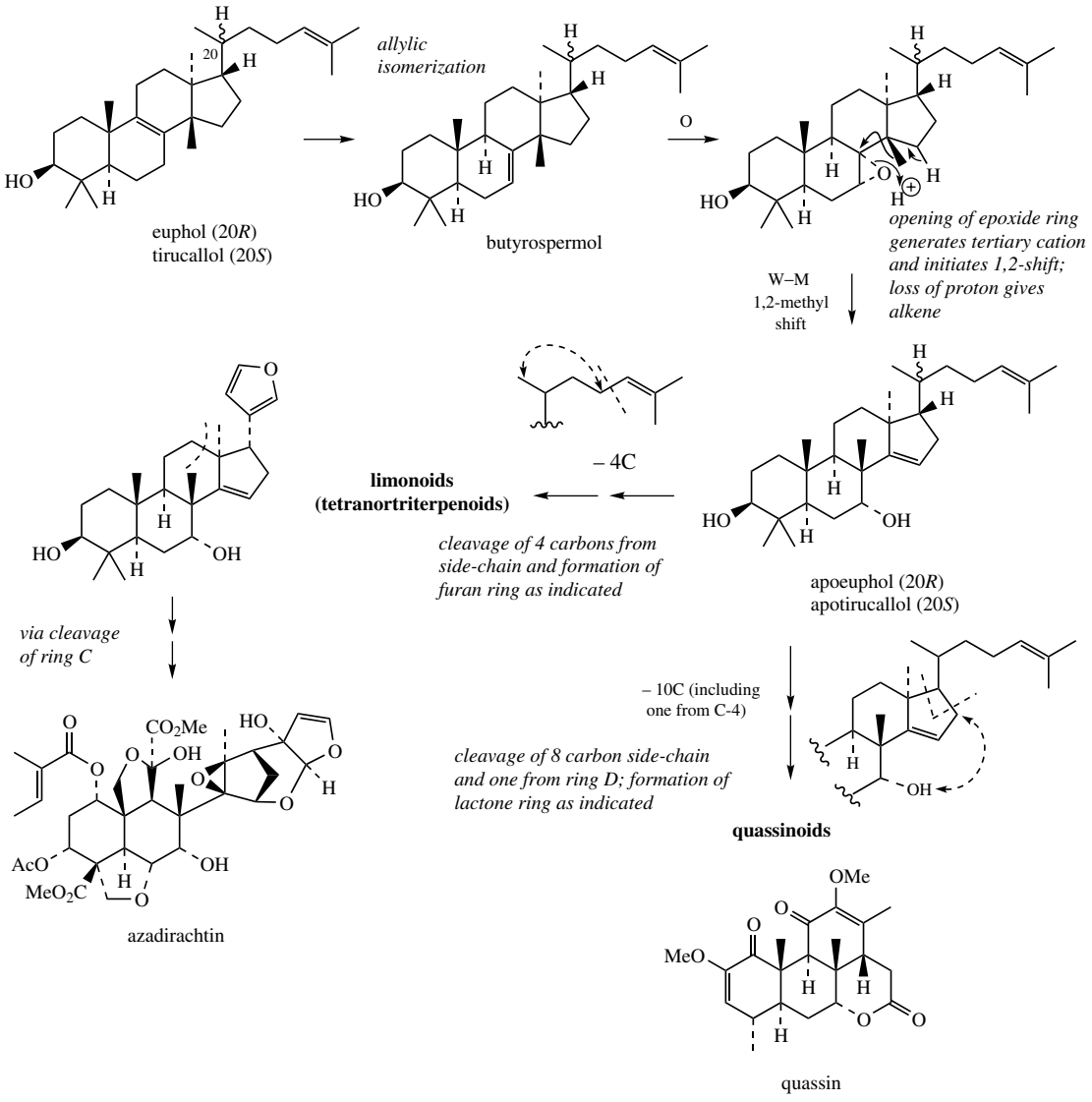

Figure 5.66

families Rutaceae, Meliaceae, and Simaroubaceae. **Azadirachtin** (Figure 5.66) is probably one of the most complex limonoid structures to be encountered, but is currently of considerable interest. This material has potent insect antifeedant properties and is extracted commercially from seeds of the Neem tree (*Azadirachta indica*; Meliaceae) for use as an agricultural pesticide to prevent insect damage to crops. It is a relatively inexpensive and ecologically sound pesticide. Quassinoids are produced by many plants in the Simaroubaceae family, in particular *Quassia*. **Quassin** (Figure 5.66) from *Q. amara* (quassia wood) is a typical example. They have attracted considerable study because of their cytotoxic, antimalarial, and amoebicidal properties.

TETRATERPENES (C_{40})

The tetraterpenes are represented by only one group of compounds, the **carotenoids**, though several hundred natural structural variants are known. These compounds play a role in photosynthesis, but they are also found in non-photosynthetic plant tissues, in fungi and bacteria. Formation of the tetraterpene skeleton, e.g. **phytoene**, involves tail-to-tail coupling of two molecules of **geranylgeranyl diphosphate** (GGPP) in a sequence essentially analogous to that seen for squalene and triterpenes (Figure 5.67). A cyclopropyl compound, **prephytoene diphosphate** (compare presqualene diphosphate, page 213) is an intermediate in the sequence, and the main difference between the tetraterpene and triterpene pathways is how the resultant allylic cation is discharged. For squalene formation, the allylic cation accepts a hydride ion from NADPH, but for phytoene biosynthesis, a proton is lost, generating a double bond in the centre of the molecule, and thus a short conjugated chain is developed. In plants and fungi, this new double bond has the *Z* (*cis*) configuration, whilst in bacteria, it is *E* (*trans*). This triene system prevents the type of cyclization seen with squalene. Conjugation is extended then by a sequence of desaturation reactions, removing pairs of hydrogens alternately from each side of the triene system, giving eventually **lycopene** (Figure 5.67), which, in common with the majority of carotenoids, has the all-*trans* configuration. This means that in plants and fungi, an additional isomerization step is involved to change the configuration of the central double bond.

The extended π-electron system confers colour to the carotenoids, and accordingly they contribute yellow, orange, and red pigmentations to plant tissues. Lycopene is the characteristic carotenoid pigment in ripe tomato fruit (*Lycopersicon esculente*; Solanaceae). The orange colour of carrots (*Daucus carota*; Umbelliferae/Apiaceae) is caused by **β-carotene** (Figure 5.68), though this compound is widespread in higher plants. β-Carotene and other natural carotenoids (Figure 5.68) are widely employed as colouring agents for foods, drinks, confectionery, and drugs. β-Carotene displays additional cyclization of the chain ends, which can be rationalized by the carbocation mechanism shown in Figure 5.69. Depending on which proton is lost from the cyclized cation, three different cyclic alkene systems can arise at the end of the chain, described as β-, γ-, or ε-ring systems. **α-Carotene** (Figure 5.68) has a β-ring at one end of the chain, and an ε-type at the other, and is representative of carotenoids lacking symmetry. **γ-Carotene** (a precursor of β-carotene) and **δ-carotene** (a precursor of α-carotene) illustrate carotenoids where only one end of the chain has become cyclized. Oxygenated carotenoids (termed xanthophylls) are also widely distributed, and the biosynthetic origins of the oxygenated rings found in some of these, such as **zeathanthin**, **lutein**, and **violaxanthin** (Figure 5.68), all common green leaf carotenoids, are shown in Figure 5.69. The epoxide grouping in violaxanthin allows further chemical modifications, such as ring contraction to a cyclopentane, exemplified by **capsanthin** (Figure 5.68), the brilliant red pigment of peppers (*Capsicum annuum*; Solanaceae), or formation of an allene as in **fucoxanthin**, an abundant carotenoid in brown algae (*Fucus* species; Fucaceae). **Astaxanthin** (Figure 5.68) is commonly found in marine animals and is responsible for the pink/red coloration of crustaceans, shellfish, and fish such as salmon. These animals are unable to synthesize carotenoids and astaxanthin is produced by modification of plant carotenoids, e.g. β-carotene, obtained in the diet.

Carotenoids function along with chlorophylls in photosynthesis as accessory light-harvesting

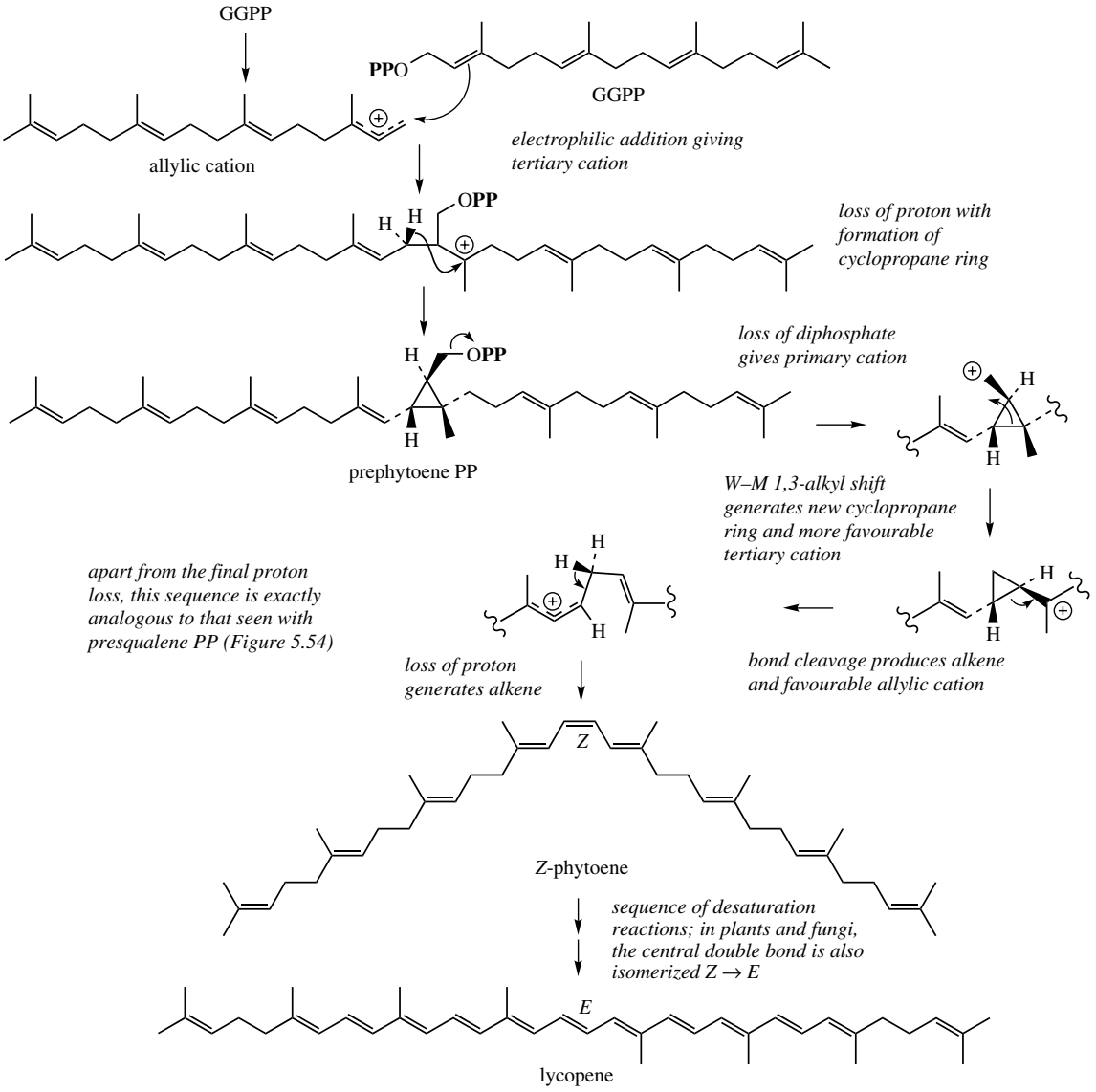

Figure 5.67

pigments, effectively extending the range of light absorbed by the photosynthetic apparatus. They also serve as important protectants for plants and algae against photo-oxidative damage, quenching toxic oxygen species. Some herbicides (bleaching herbicides) act by inhibiting carotenoid biosynthesis, and the unprotected plant is subsequently killed by photo-oxidation. Recent research also suggests carotenoids are important antioxidant molecules in humans, quenching singlet oxygen and scavenging peroxyl radicals, thus minimizing cell damage and affording protection against some forms of cancer. The most significant dietary carotenoid in this respect is **lycopene**, with tomatoes and processed tomato products featuring as the predominant source. The extended conjugated system allows free radical addition reactions and hydrogen abstraction from positions allylic to this chain.

The A group of vitamins* are important metabolites of carotenoids. **Vitamin A_1** (**retinol**) (Figure 5.70) effectively has a diterpene structure, but it is derived in mammals by oxidative

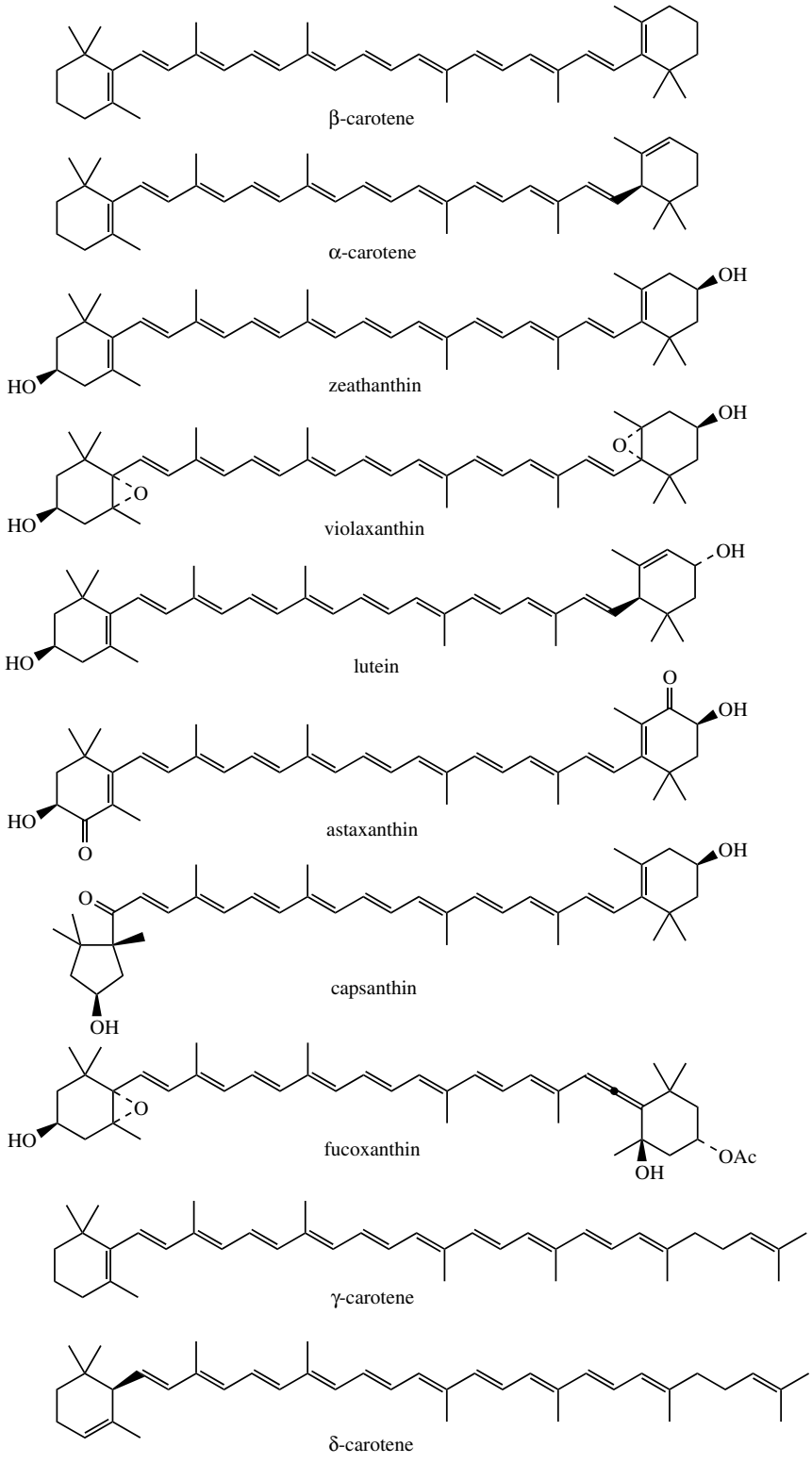
β-carotene
α-carotene
OH
HO
zeathanthin
O
violaxanthin
lutein
astaxanthin
capsanthin
fucoxanthin
OAc
γ-carotene
δ-carotene

Figure 5.68

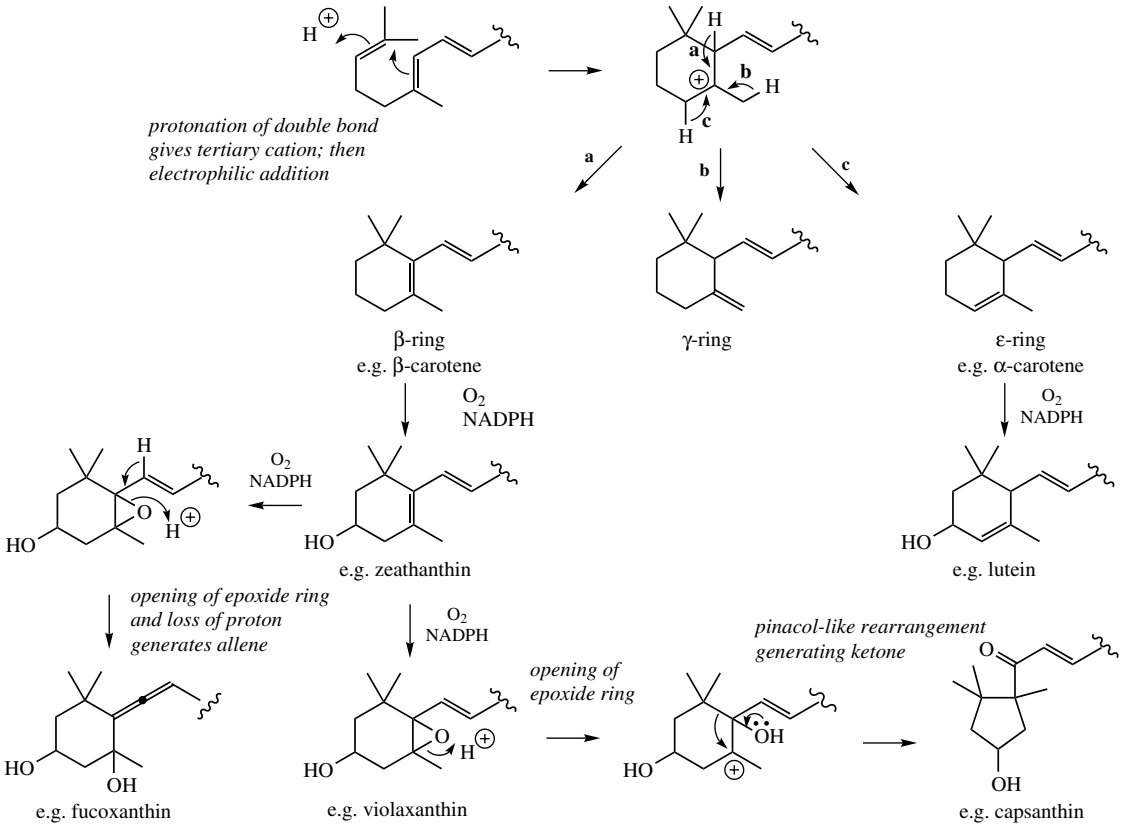

Figure 5.69

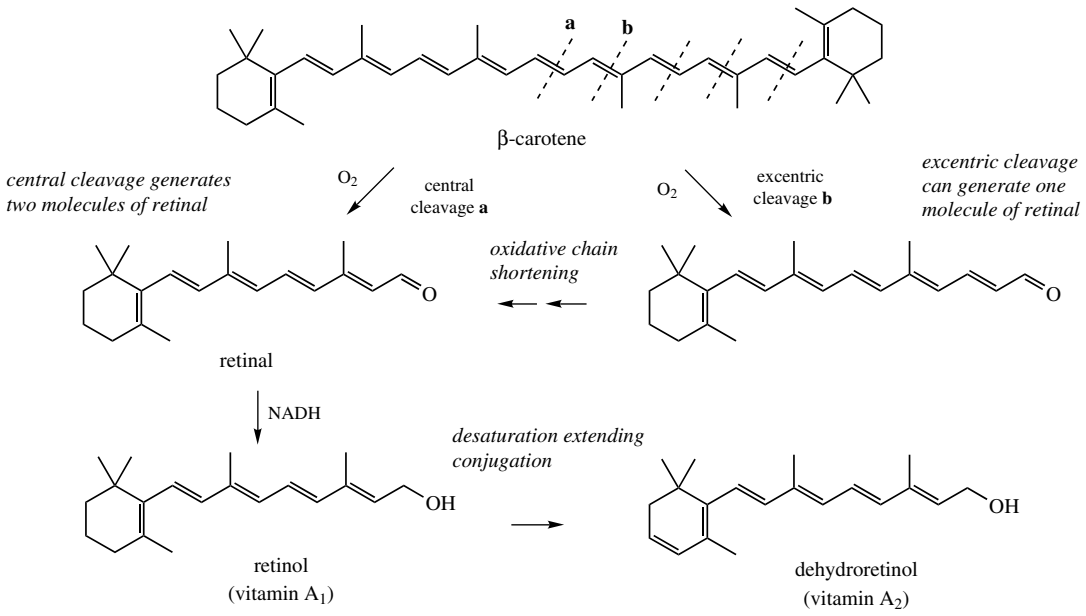

Figure 5.70

metabolism of a tetraterpenoid, mainly β-carotene, taken in the diet. Cleavage occurs in the mucosal cells of the intestine, and is catalysed by an O_2-dependent dioxygenase, probably via an intermediate peroxide. This can theoretically yield two molecules of the intermediate aldehyde, **retinal**, which is subsequently reduced to the alcohol, retinol (Figure 5.70). Although β-carotene cleaved at the central double bond is capable of giving rise to two molecules of retinol, there is evidence that cleavage can also occur at other double bonds, so-called excentric cleavage (Figure 5.70). Further chain shortening then produces retinal, but only one molecule is produced per molecule of β-carotene. **Vitamin A_2** (**dehydroretinol**) (Figure 5.70) is an analogue of retinol containing a cyclohexadiene ring system; the corresponding aldehyde, and retinal, are also included in the A group of vitamins. Retinol and its derivatives are found only in animal products, and these provide some of our dietary needs. Cod-liver oil and halibut-liver oil are rich sources used as dietary supplements. However, carotenoid sources are equally important. These need to have at least one non-hydroxylated ring system of the β-type, e.g. β-carotene, α-carotene, and γ-carotene.

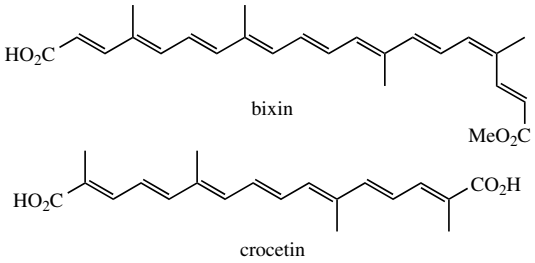

Figure 5.71

Cleavage of carotenoid precursors is likely to explain the formation of **bixin** and **crocetin** (Figure 5.71) and, indeed, these are classified as apocarotenoids. Large amounts (up to 10%) of the red pigment bixin are found in the seed

Vitamin A

Vitamin A_1 (**retinol**) and **vitamin A_2** (dehydroretinol) (Figure 5.72) are fat-soluble vitamins found only in animal products, especially eggs, dairy products, and animal livers and kidneys. Fish liver oils, e.g. cod liver oil and halibut liver oil (see Table 3.2) are particularly rich sources. They exist as the free alcohols, or as esters with acetic and palmitic acid. Vitamin A_2 has about 40% of the activity of vitamin A_1. Carotenoid precursors (provitamins) are widely distributed in plants, and after ingestion, these are subsequently transformed into vitamin A in the liver. Green vegetables and rich plant sources such as carrots help to provide adequate levels. A deficiency of vitamin A leads to vision defects, including impairment at low light levels (night blindness) and a drying and degenerative disease of the cornea. It also is necessary for normal growth of young animals. Retinoids (vitamin A and analogues) are now known to act as signalling molecules which regulate diverse aspects of cell differentiation, embryonic development, growth, and vision. For the processes of vision, retinol needs to be converted first by oxidation into the aldehyde all-*trans*-retinal, and then by enzymic isomerization to *cis*-retinal (Figure 5.73). *cis*-Retinal is then bound to the protein opsin in the retina via a Schiff base linkage to give the red visual pigment rhodopsin, and its sensitivity to light involves isomerization of the *cis*-retinal portion back to the all-*trans* form, thus translating the light energy into molecular change, which triggers a nerve impulse to the brain. The absorption of light energy promotes an electron from a π- to a π*-orbital, thus temporarily destroying the double bond character and allowing rotation. A similar *cis–trans* isomerization affecting cinnamic acids was discussed under coumarins (see page 142). All-*trans*-retinal is then subsequently released for the process to continue. Vitamin A is relatively unstable, and sensitive to oxidation and light. Antioxidant stabilizers such as vitamin E and vitamin C are sometimes added. It is more stable in oils such as the fish liver oils, which are thus good vehicles for administering the vitamin. Synthetic material is also used. Excessive intake of

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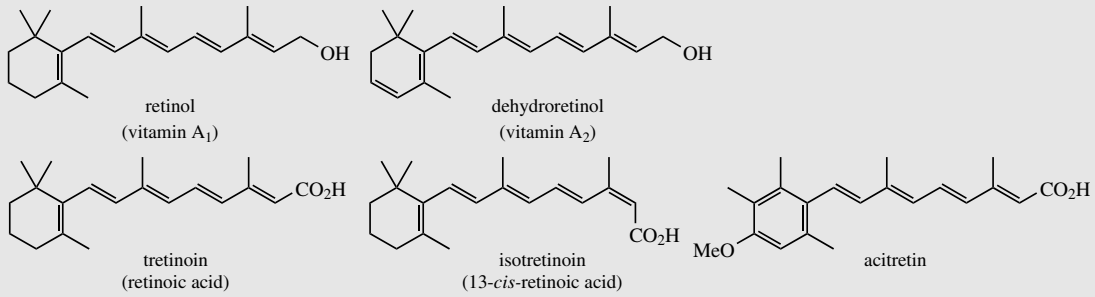

Figure 5.72

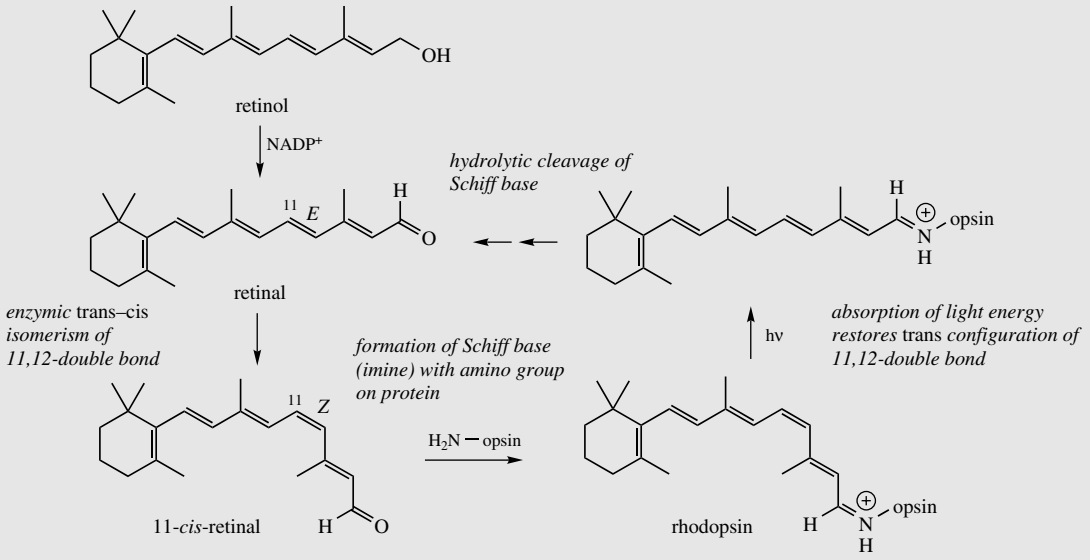

Figure 5.73

vitamin A can lead to toxic effects, including pathological changes in the skin, hair loss, blurred vision, and headaches.

The synthetic retinoic acids **tretinoin** (retinoic acid) and **isotretinoin** (13-*cis*-retinoic acid) (Figure 5.72) are retinoids that are used as topical or oral treatments for acne vulgaris, reducing levels of dehydroretinol and modifying skin keratinization. Dehydroretinol levels in the skin become markedly elevated in conditions such as eczema and psoriasis. **Acitretin** (Figure 5.72) is an aromatic analogue which can give relief in severe cases of psoriasis. All these materials can produce toxic side-effects.

coats of annatto (*Bixa orellana*; Bixaceae), and bixin is widely used as a natural food colorant. Crocetin, in the form of esters with gentiobiose [D-Glc($\beta 1 \rightarrow 6$)D-Glc], is the major pigment in stigmas of *Crocus sativus* (Iridaceae), which comprise the spice saffron.

HIGHER TERPENOIDS

Terpenoid fragments containing several isoprene units are found as alkyl substituents in shikimate-derived quinones (see page 158). Thus ubiquinones typically have $C_{40}-C_{50}$ side-chains,

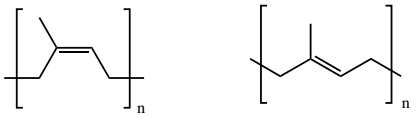

Figure 5.74

plastoquinones usually C_{45}, and menaquinones up to C_{65}. The alkylating agents are polyprenyl diphosphates, formed simply by an extension of the prenyltransferase reaction (see page 172), repeatedly adding IPP residues. Even longer polyisoprene chains are encountered in some natural polymers, especially rubber and gutta percha. **Rubber** (Figure 5.74), from the rubber tree *Hevea brasiliensis* (Euphorbiaceae), is unusual in possessing an extended array of *cis* (*Z*) double bonds rather than the normal *trans* configuration. **Gutta percha**, from *Palaquium gutta* (Sapotaceae) on the other hand, has *trans* (*E*) double bonds. The *cis* double bonds in rubber are known to arise by loss of the *pro-S* proton (H_S) from C-2 of IPP (contrast loss of H_R, which gives a *trans* double bond) (Figure 5.75). However, a small (up to C_{20}) *trans*-allylic diphosphate initiator is actually used for the beginning of the chain before the extended *cis* chain is elaborated.

STEROIDS

Stereochemistry

The steroids are modified triterpenoids containing the tetracyclic ring system of lanosterol (Figure 5.55), but lacking the three methyl groups at C-4 and C-14. **Cholesterol** (Figure 5.76) typifies the fundamental structure, but further modifications, especially to the side-chain, help to create a wide range of biologically important natural products, e.g. sterols, steroidal saponins, cardioactive glycosides, bile acids, corticosteroids, and mammalian sex hormones. Because of the profound biological activities encountered, many natural steroids together with a considerable number of synthetic and semi-synthetic steroidal compounds are routinely employed in medicine. The markedly different biological activities observed emanating from compounds containing a common structural skeleton are in part ascribed to the functional groups attached to the steroid nucleus, and in part to the overall shape conferred on this nucleus by the stereochemistry of ring fusions.

Ring systems containing six-membered or five-membered rings can be *trans*-fused as exemplified by *trans*-decalin or *cis*-fused as in *cis*-decalin (Figure 5.76). The *trans*-fusion produces a flattish molecule when two chair conformations are present. The only conformational mobility allowed is to less favourable boat forms. Both bridgehead hydrogens (or other substituents) are axial to both of the rings. In contrast, the *cis*-fused decalin is basically a bent molecule, and is found to be flexible in that alternative conformers are possible, both rings still being in chair form. However, this flexibility will be lost if either ring is then *trans*-fused to a third ring. Bridgehead substituents are axial to one ring, whilst being equatorial to the other, in each conformer.

In natural steroids, there are examples of the A/B ring fusion being *trans* or *cis*, or having unsaturation, either Δ^4 or Δ^5. In some compounds, notably the oestrogens, ring A can even be aromatic; clearly there can then be no bridgehead substituent at C-10 and the normal C-10 methyl (C-19) must therefore be lost. All natural steroids have a *trans* B/C fusion, though *cis* forms can be

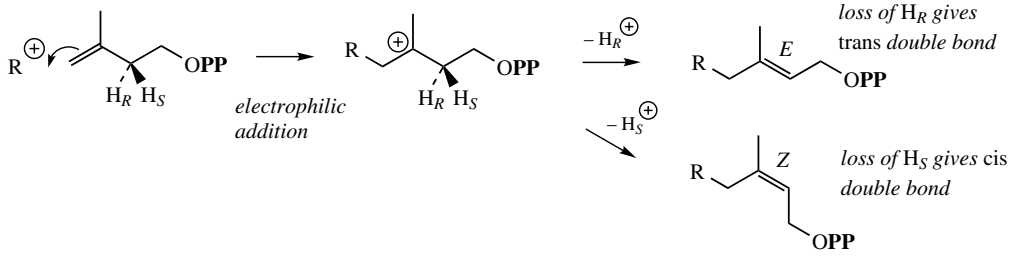

Figure 5.75

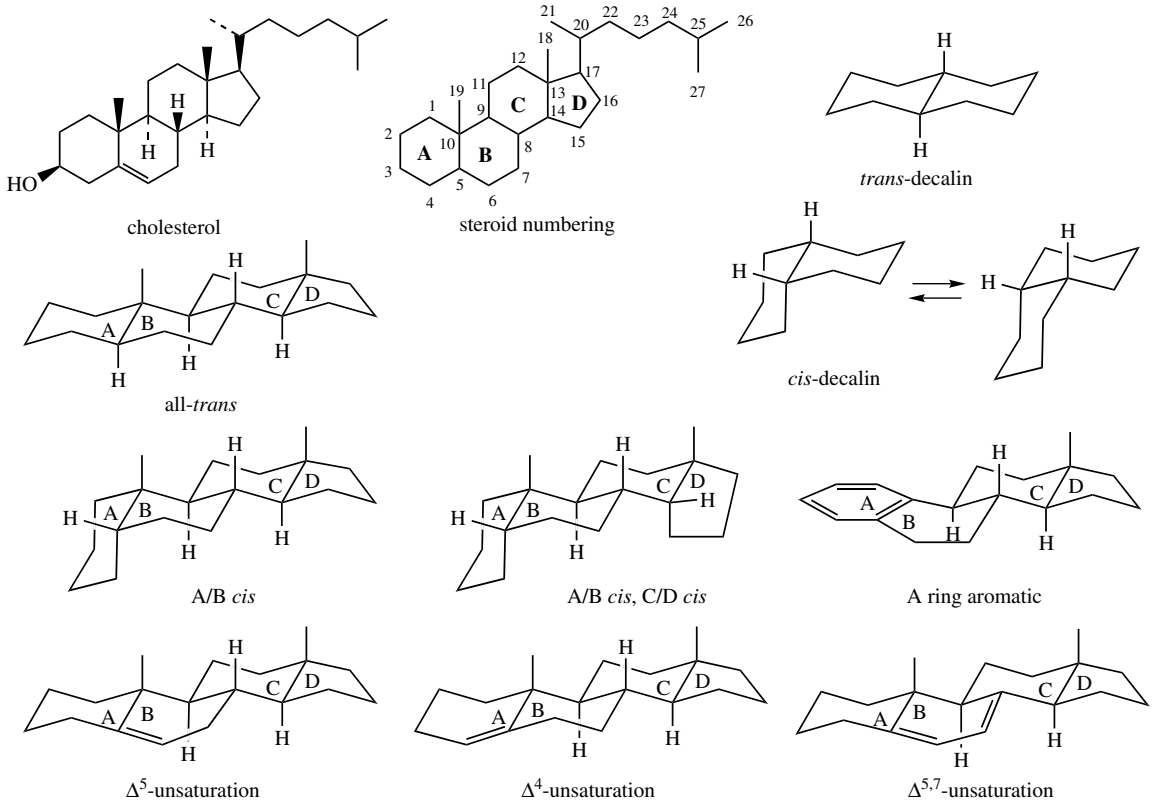

Figure 5.76

made synthetically. The C/D fusion is also usually *trans*, though there are notable exceptions such as the cardioactive glycosides. Of course, such comments apply equally to some of the triterpenoid structures already considered. However, it is in the steroid field that the relationship between stereochemistry and biological activity is most marked. The overall shapes of some typical steroid skeletons are shown in Figure 5.76.

Systematic **steroid nomenclature** is based on a series of parent hydrocarbons, including **estrane**, **androstane**, **pregnane**, **cholane**, **cholestane**, **ergostane**, **campestane**, **stigmastane** and **poriferastane** (Figure 5.77). The triterpenoid hydrocarbons **lanostane** and **cycloartane** are similarly used in systematic nomenclature and are also included in Figure 5.77. It is usual to add only unsaturation (ene/yne) and the highest priority functional group as suffixes to the root name; other groups are added as prefixes. Stereochemistry of substituents is represented by α (on the lower face of the molecule when it is drawn according to customary conventions as in Figure 5.77), or β (on the upper face). Ring fusions may be designated by using α or β for the appropriate bridgehead hydrogen, particularly those at positions 5 and 14, which will define the A/B and C/D fusions respectively, e.g. 5β-cholestane has the A/B rings *cis*-fused. Since the parent hydrocarbon assumes that ring fusions are *trans*, the stereochemistry for ring fusions is usually only specified where it is *cis*. Cholesterol is thus cholest-5-en-3β-ol or 5-cholesten-3β-ol. The term *nor* is affixed to indicate loss of a carbon atom, e.g. 19-norsteroids (see page 274) lack C-19 (the methyl at C-10).

Cholesterol

In animals, the triterpenoid alcohol **lanosterol** (Figure 5.55) is converted into **cholesterol*** (Figure 5.76), a process requiring, as well as the loss of three methyl groups, reduction of the side-chain double bond, and generation of a $\Delta^{5,6}$

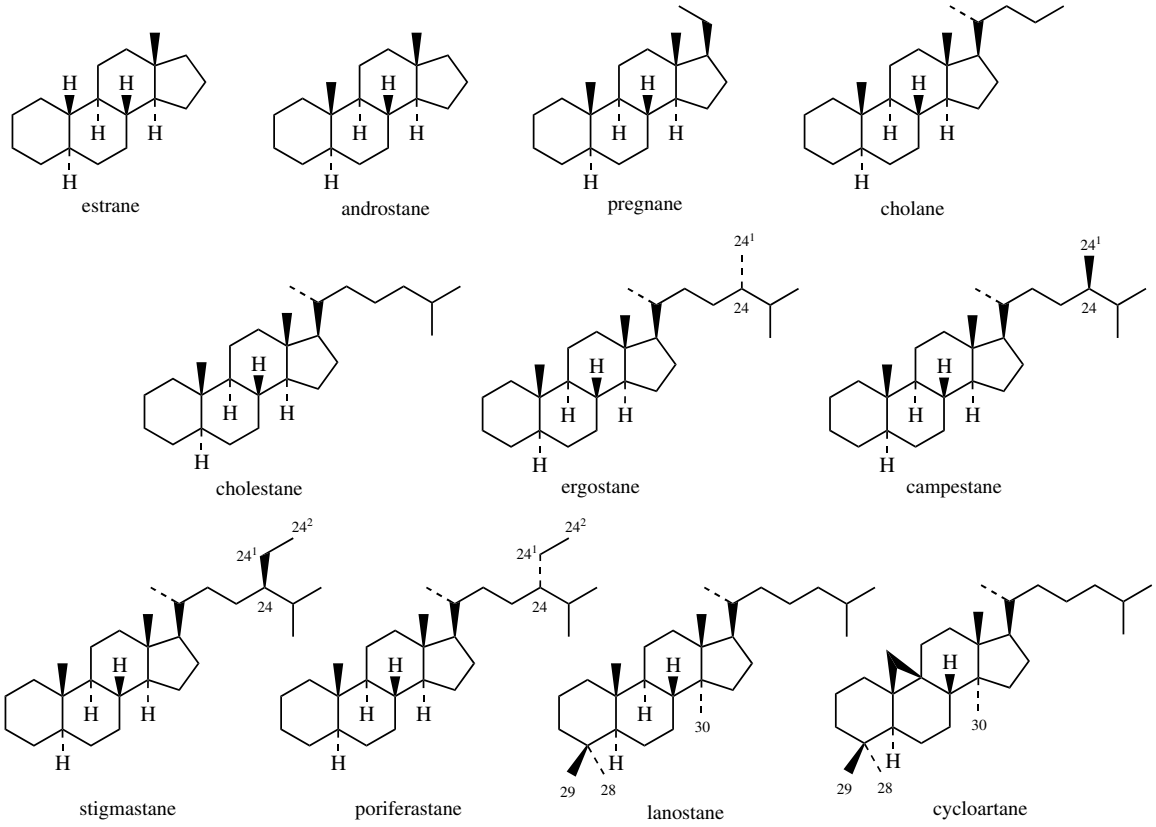

Figure 5.77

double bond in place of the $\Delta^{8,9}$ double bond. The sequence of these steps is to some extent variable, and dependent on the organism involved. Accordingly, these individual transformations are considered rather than the overall pathway.

The methyl at C-14 is usually the one lost first, and this is removed as formic acid. The reaction is catalysed by a cytochrome P-450 monooxygenase, which achieves two oxidation reactions to give the 14α-formyl derivative (Figure 5.78), and loss of this formyl group giving the $\Delta^{8,14}$ diene, most probably via homolytic cleavage of the peroxy adduct as indicated (compare similar peroxy adducts involved in side-chain cleavage from ring D, page 278, and in A ring aromatization, page 278). The 14-demethyl sterol is then obtained by an NADPH-dependent reduction step, the 15-proton being derived from water.

Loss of the C-4 methyls occurs sequentially, usually after removal of the 14α-methyl, with both carbons being cleaved off via a decarboxylation mechanism (Figure 5.79). This is facilitated by oxidizing the 3-hydroxyl to a ketone, thus generating intermediate β-keto acids. In this sequence, the enolate is restored to a ketone, in which the remaining C-4 methyl takes up the more favourable equatorial (4α) orientation.

The side-chain Δ^{24} double bond is reduced by an NADPH-dependent reductase, hydride from the coenzyme being added at C-25, with H-24 being derived from water (Figure 5.80). The Δ^{8} double bond is effectively migrated to Δ^{5} via Δ^{7} and the $\Delta^{5,7}$ diene (Figure 5.81). This sequence involves an allylic isomerization, a dehydrogenation, and a reduction. Newly introduced protons at C-9 and C-8 originate from water, and that at C-7 from NADPH.

The role of lanosterol in non-photosynthetic organisms (animals, fungi) is taken in photosynthetic organisms (plants, algae) by the cyclopropane triterpenoid **cycloartenol** (Figure 5.55). This cyclopropane feature is found in a number of

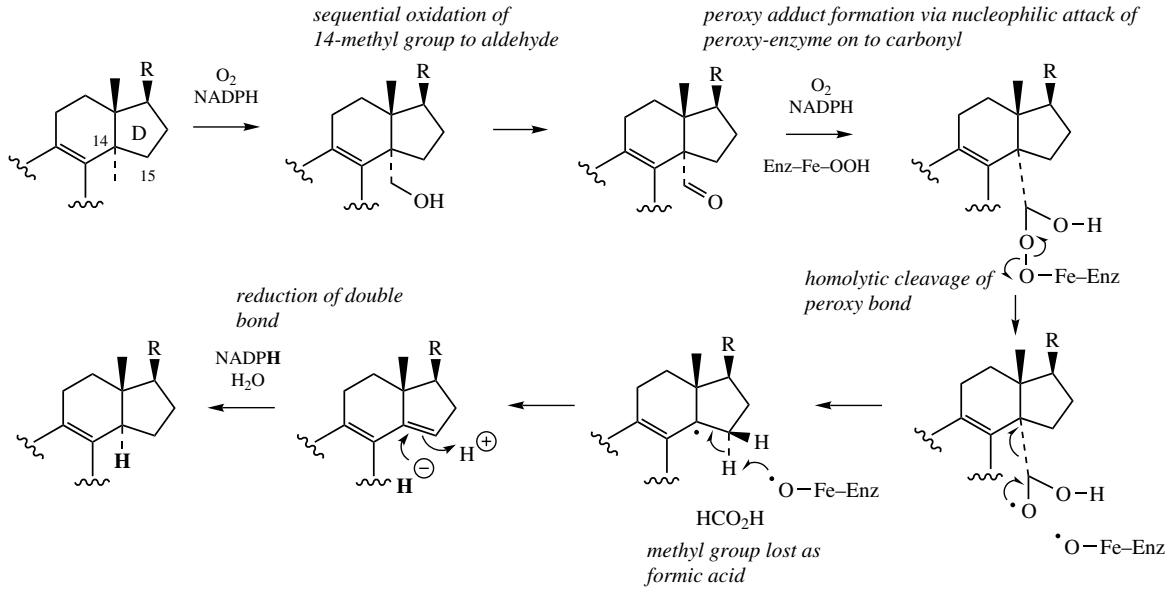

Figure 5.78

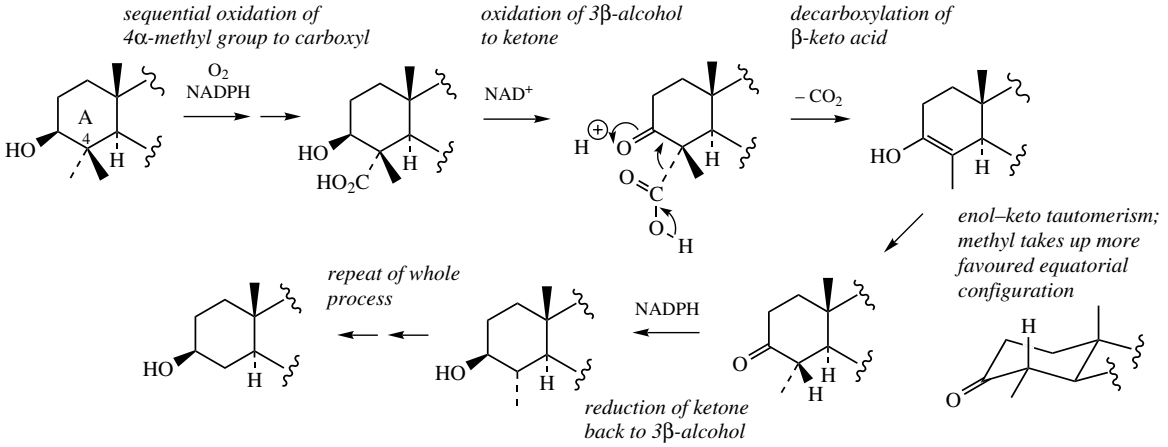

Figure 5.79

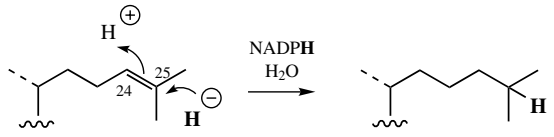

Figure 5.80

plant sterols, but the majority of plant steroids contain the normal methyl at C-10. This means that, in addition to the lanosterol → cholesterol modifications outlined above, a further mechanism to reopen the cyclopropane ring is necessary. This is shown in Figure 5.82. The stereochemistry at C-8 (Hβ) is unfavourable for a concerted mechanism involving loss of H-8 with cyclopropane ring opening. It is suggested therefore that a nucleophilic group from the enzyme attacks C-9, opening the cyclopropane ring and incorporating a proton from water. A *trans* elimination then generates the Δ^8 double bond. The cyclopropane ring-opening process seems specific to 4α-monomethyl sterols. In plants, removal of the first 4-methyl group (4α;

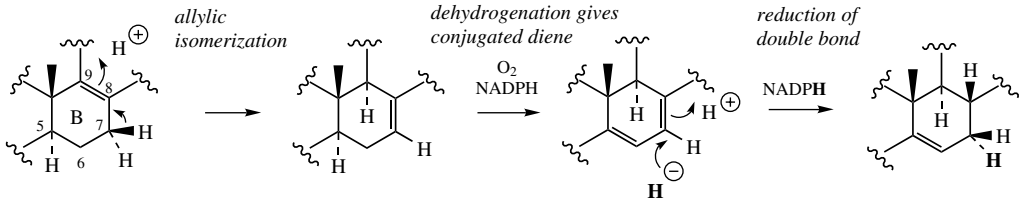

Figure 5.81

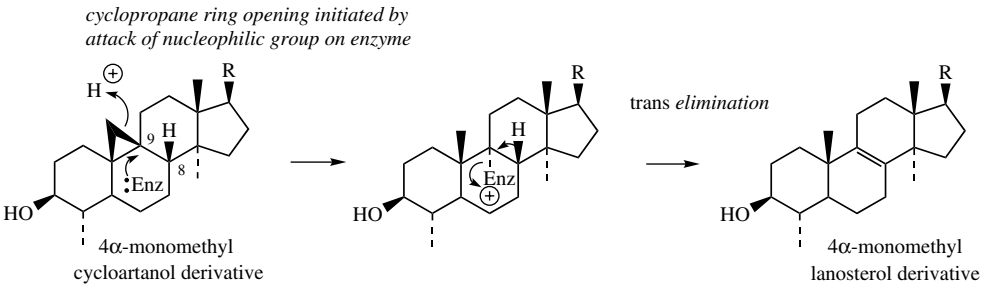

Figure 5.82

note the remaining 4β-methyl group then takes up the α-orientation) is also known to precede loss of the 14α-methyl. Accordingly, the substrate shown in Figure 5.82 has both 4α- and 14α-methyl groups. The specificity of the cyclopropane ring-opening enzyme means cycloartenol is not converted into lanosterol, and lanosterol is thus absent from virtually all plant tissues. Cholesterol is almost always present in plants, though often in only trace amounts, and is formed via cycloartenol.

Cholesterol

Cholesterol (Figure 5.76) is the principal animal sterol and since it is a constituent of cell membranes has been found in all animal tissues. Human gallstones are almost entirely composed of cholesterol precipitated from the bile. Cholesterol is currently available in quantity via the brains and spinal cords of cattle as a by-product of meat production, and these form one source for medicinal steroid semi-synthesis. Large quantities are also extractable from lanolin, the fatty material coating sheep's wool. This is a complex mixture of esters of long chain fatty acids (including straight-chain, branched-chain, and hydroxy acids) with long chain aliphatic alcohols and sterols. Cholesterol is a major sterol component. Saponification of crude lanolin gives an alcohol fraction (lanolin alcohols or wool alcohols) containing about 34% cholesterol and 38% lanosterol/dihydrolanosterol. Wool alcohols are also used as an ointment base.

Although the processes involved are quite complex, there appears to be a clear correlation between human blood cholesterol levels and heart disease. Atherosclerosis is a hardening of the arteries caused by deposition of cholesterol, cholesterol esters, and other lipids in the artery wall, causing a narrowing of the artery and thus an increased risk of forming blood clots (thrombosis). Normally, most of the cholesterol serves a structural element in cell walls, whilst the remainder is transported via the blood and is used for synthesis of steroid hormones, vitamin D (page 259), or bile acids (page 261). Transport of cholesterol is facilitated by formation of lipoprotein carriers, comprising protein and phospholipid shells

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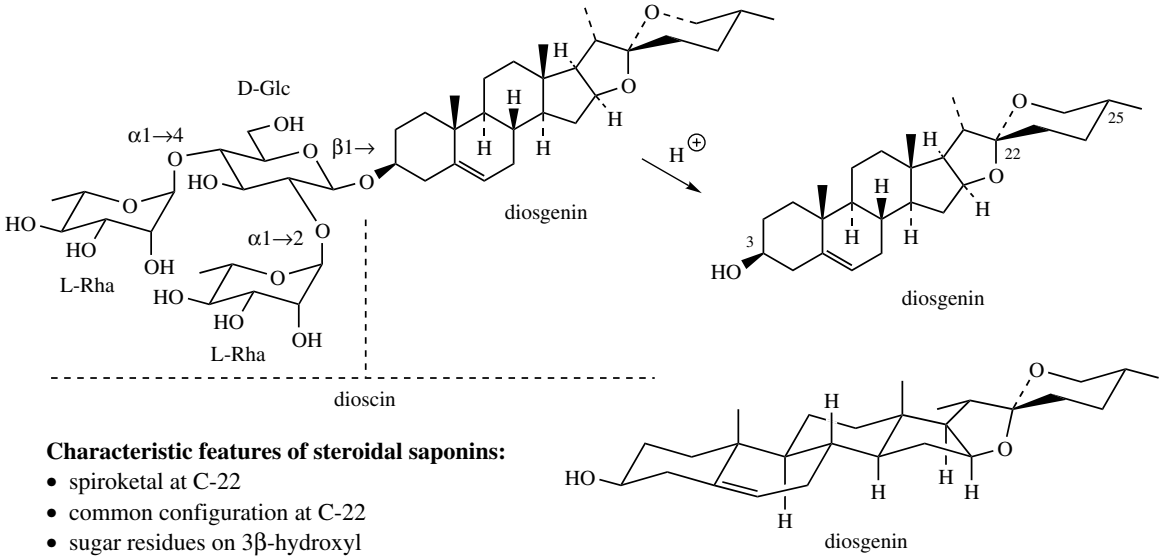
surrounding a core of cholesterol, in both free and esterified forms. Risk of atherosclerosis increases with increasing levels of low density lipoprotein (LDL) cholesterol, and is reduced with increasing levels of high density lipoprotein (HDL) cholesterol. Blood LDL cholesterol levels are thus a good statistical indicator of the potential risk of a heart attack. The risks can be lessened by avoiding foods rich in cholesterol, e.g. eggs, reducing the intake of foods containing high amounts of saturated fatty acids such as animal fats, and replacing these with vegetable oils and fish that are rich in polyunsaturated fatty acids (see page 40). Blood LDL cholesterol levels may also be reduced by incorporating into the diet plant sterol esters or plant stanol esters, which reduce the absorption of cholesterol (see page 256). In humans, dietary cholesterol is actually a smaller contributor to LDL cholesterol levels than is dietary saturated fat. Cholesterol biosynthesis may also be inhibited by drug therapy using specific inhibitors of the mevalonate pathway, e.g. lovastatin and related compounds (see page 112).

Steroidal Saponins

Steroidal saponins have similar biological properties to the triterpenoid saponins (see page 219), but are less widely distributed in nature. They are found in many monocotyledon families, especially the Dioscoreaceae (e.g. *Dioscorea*), the Agavaceae (e.g. *Agave, Yucca*) and the Liliaceae (e.g. *Smilax, Trillium*). Their sapogenins are C_{27} sterols in which the side-chain of cholesterol has undergone modification to produce a spiroketal, e.g. **dioscin** (Figure 5.83) from *Dioscorea*. Acid hydrolysis of dioscin liberates the aglycone **diosgenin**. All the steroidal saponins have the same configuration at the *spiro* centre C-22, but stereoisomers at C-25 exist, e.g. **yamogenin** (Figure 5.84), and often mixtures of the C-25 stereoisomers cooccur in the plant. The sugar moiety is usually at position 3, and typically contains fewer monosaccharide units than found with triterpenoid saponins. One to three monosaccharide units are most common. The three-dimensional shape of diosgenin is indicated in Figure 5.83.

The spiroketal function is derived from the cholesterol side-chain by a series of oxygenation reactions, hydroxylating C-16 and one of the



Characteristic features of steroidal saponins:

- spiroketal at C-22
- common configuration at C-22
- sugar residues on 3β-hydroxyl

Figure 5.83

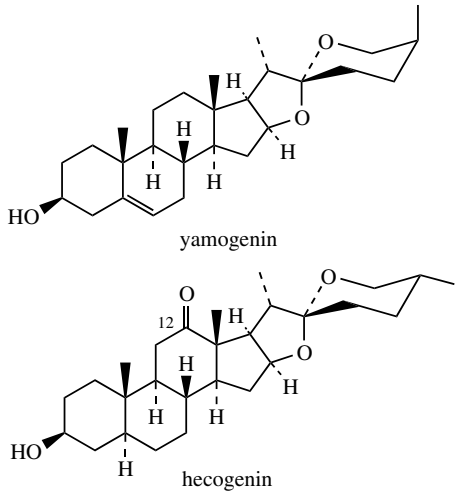


Figure 5.84

terminal methyls, and then producing a ketone function at C-22 (Figure 5.85). This proposed intermediate is transformed into the hemiketal and then the spiroketal. The chirality at C-22 is fixed by the stereospecificity in the formation of the ketal whilst the different possible stereochemistries at C-25 are dictated by whether C-26 or C-27 is hydroxylated in the earlier step. Glycoside derivatives, e.g. **protodioscin** (Figure 5.86) have been isolated from plants. These are readily hydrolysed, and then spontaneously cyclize to the spiroketal (Figure 5.86). Allowing homogenized fresh plant tissues to stand and autolyse through the action of endogenous glycosidase enzymes not only achieves cyclization of such open-chain saponins, but can hydrolyse off the sugar units at C-3, thus yielding the aglycone or sapogenin. This is a standard approach employed in commercial production of steroidal sapogenins, important starting materials for the semi-synthesis of steroidal drugs. **Diosgenin** is the principal example and is obtained from Mexican yams (*Dioscorea* spp.; Dioscoreaceae)*. Fenugreek* (*Trigonella foenum-graecum*; Leguminosae/Fabaceae) is another potentially useful commercial source. Sisal* (*Agave sisalana*; Agavaceae) is also used commercially, yielding **hecogenin** (Figure 5.84), a 12-keto derivative with *trans*-fused A/B rings, the result of reduction of the Δ^5 double bond.

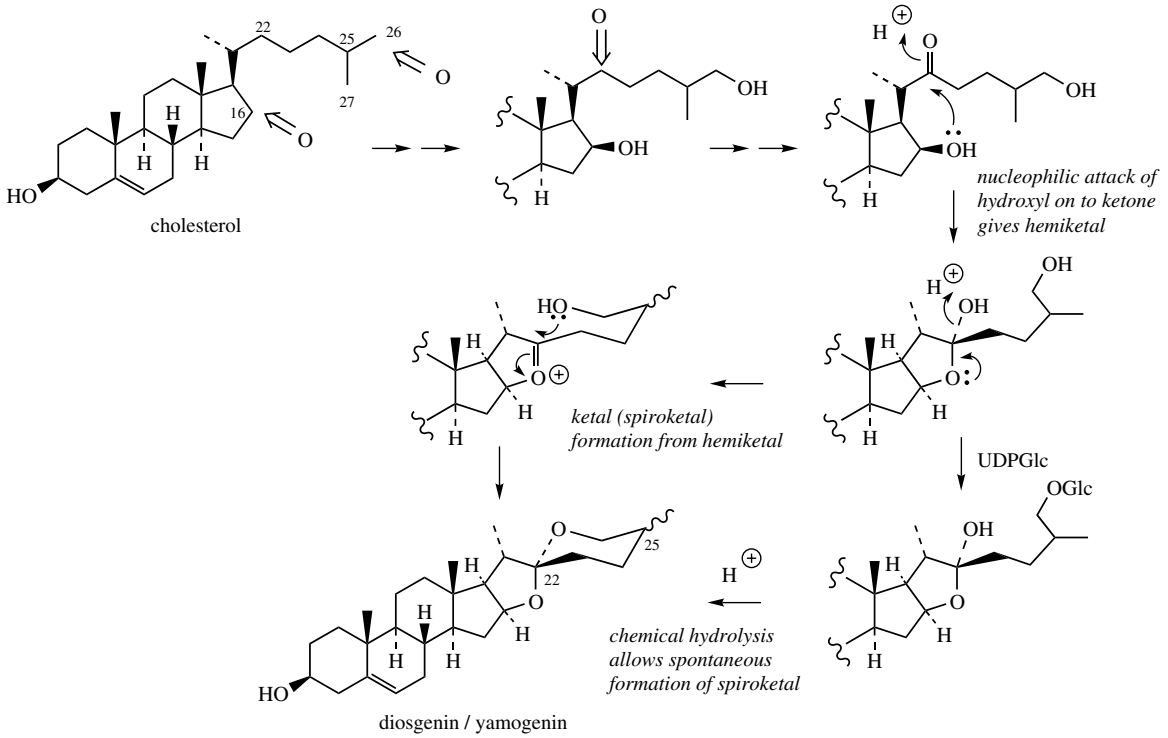


Figure 5.85

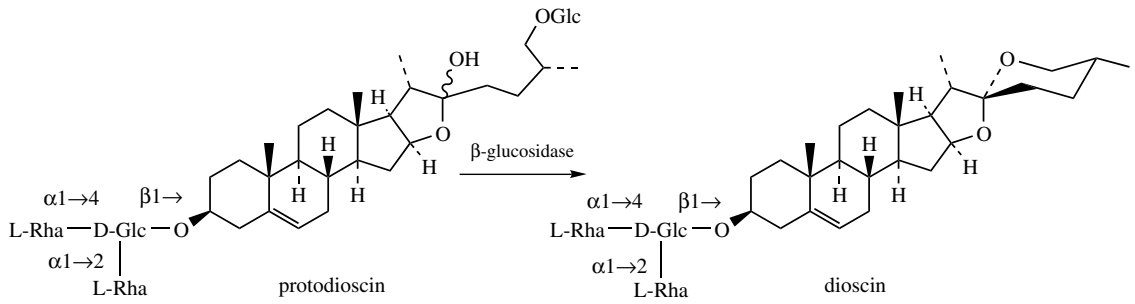


Figure 5.86

Dioscorea

About 600 species of *Dioscorea* (Dioscoreaceae) are known, and a number of these are cultivated for their large starchy tubers, commonly called yams, which are an important food crop in many parts of the world. Important edible species are *Dioscorea alata* and *D. esculenta* (S E Asia), *D. rotundata* and *D. cayenensis* (W Africa) and *D. trifida* (America). A number of species accumulate quite high levels of saponins in their tubers, which make them bitter and inedible, but these provide suitable sources of steroidal material for drug manufacture.

Dioscoreas are herbaceous, climbing, vinelike plants, the tuber being totally buried, or sometimes protruding from the ground. Tubers weigh anything up to 5 kg, but in some species, tubers have been recorded to reach weights as high as 40–50 kg. Drug material is obtained from both wild and cultivated plants, with plants collected from the wild having been exploited considerably more than cultivated ones. Commercial cultivation is less economic, requiring a 4–5 year growing period, and some form of support for the climbing stems. Much of the world's production has come from Mexico, where tubers from *D. composita* (barbasco), *D. mexicana*, and *D. floribunda*, mainly harvested from wild plants, are utilized. The saponin content of the tubers varies, usually increasing as tubers become older. Typically, tubers of *D. composita* may contain 4–6% total saponins, and *D. floribunda* 6–8%. Other important sources of *Dioscorea* used commercially now include India (*D. deltoidea*), South Africa (*D. sylvatica*) and China (*D. collettii*, *D. pathaica*, and *D. nipponica*).

Sapogenins are isolated by chopping the tubers, allowing them to ferment for several days, then completing the hydrolysis of saponins by heating with aqueous acid. The sapogenins can then be solvent extracted. The principal sapogenin in the species given above is diosgenin (Figure 5.83), with small quantities of the 25β-epimer yamogenin (Figure 5.84). Demand for diosgenin for pharmaceuticals is huge, equivalent to 10 000 tonnes of *Dioscorea* tuber per annum, and it is estimated that about 60% of all steroidal drugs are derived from diosgenin.

Powdered Dioscorea (wild yam) root or extract is also marketed to treat the symptoms of menopause as an alternative to hormone replacement therapy (see page 279). Although there is a belief that this increases levels of progesterone, which is then used as a biosynthetic precursor of other hormones, there is no evidence that diosgenin is metabolized in the human body to progesterone, and any beneficial effects may arise from diosgenin itself.

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Fenugreek

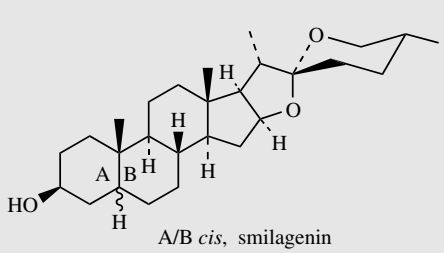
The seeds of fenugreek (*Trigonella foenum-graecum*; Leguminosae/Fabaceae) are an important spice material, and are ingredients in curries and other dishes. The plant is an annual, and is grown widely, especially in India, both as a spice and as a forage crop. Seeds can yield, after hydrolysis, 1–2% of sapogenins, principally diosgenin (Figure 5.83) and yamogenin (Figure 5.84). Although yields are considerably lower than from *Dioscorea*, the ease of cultivation of fenugreek and its rapid growth make the plant a potentially viable crop for steroid production in temperate countries. Field trials of selected high-yielding strains have been conducted.

Sisal

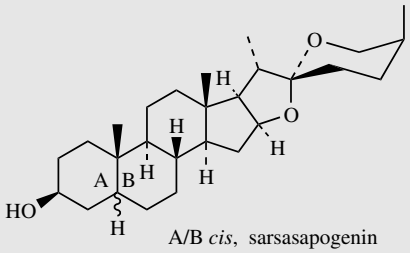
Sisal (*Agave sisalana*; Agavaceae) has long been cultivated for fibre production, being the source of sisal hemp, used for making ropes, sacking and matting. The plant is a large, rosette-forming succulent with long, tough, spine-tipped leaves containing the very strong fibres. The main area of sisal cultivation is East Africa (Tanzania, Kenya), with smaller plantations in other parts of the world. The sapogenin hecogenin (Figure 5.84) was initially produced from the leaf waste (0.1–0.2% hecogenin) after the fibres had been stripped out. The leaf waste was concentrated, allowed to ferment for several days, then treated with steam under pressure to complete hydrolysis of the saponins. Filtration then produced a material containing about 12% hecogenin, plus other sapogenins. This was refined further in the pharmaceutical industry. Other sapogenins present include tigogenin and neotigogenin (Figure 5.87).

As the demand for natural fibres declined due to the availability of synthetics, so did the supply of sisal waste and thus hecogenin. In due course, hecogenin became a more valuable commodity than sisal, and efforts were directed specifically towards hecogenin production. This has resulted in the cultivation of *Agave* hybrids with much improved hecogenin content.

The fermented sap of several species of Mexican *Agave* provides the alcoholic beverage pulque. Distillation of the fermented sap produces tequila.



A/B *cis*, smilagenin
A/B *trans*, tigogenin



A/B *cis*, sarsasapogenin
A/B *trans*, neotigogenin

Figure 5.87

Some steroidal alkaloids are nitrogen analogues of steroidal saponins, and display similar properties such as surface activity and haemolytic activity, but these compounds *are* toxic when ingested. These types of compound, e.g. **solasonine** (Figure 5.88) (aglycone **solasodine**), are found in many plants of the genus *Solanum* (Solanaceae), and such plants must thus be regarded as potentially toxic. In contrast to the oxygen analogues, all compounds have the same stereochemistry at C-25 (methyl always equatorial), whilst isomers at C-22 do exist, e.g. **tomatine**

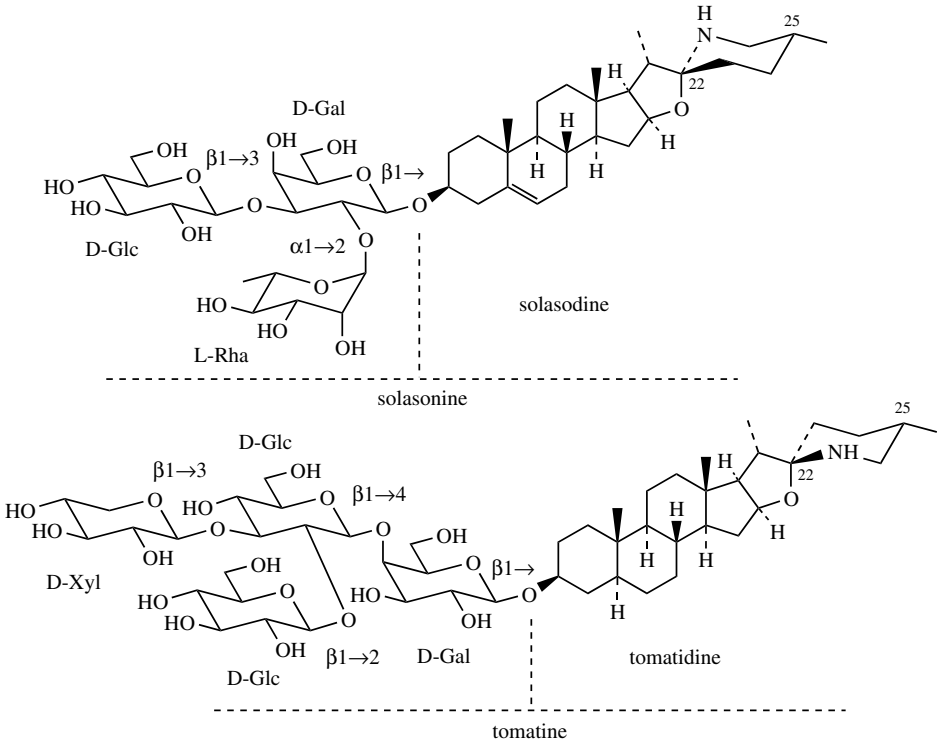


Figure 5.88

(Figure 5.88) (aglycone **tomatidine**) from tomato (*Lycopersicon esculente*; Solanaceae). The nitrogen atom is introduced by a transamination reaction, typically employing an amino acid as donor (see page 20). Since the production of medicinal steroids from steroidal saponins 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 (see page 391).

Smilagenin and **sarsasapogenin** (Figure 5.87) found in sarsaparilla* (*Smilax* spp.; Liliaceae/ Smilacaceae) are reduced forms of diosgenin and yamogenin respectively. These contain *cis*-fused A/B rings, whilst the corresponding *trans*-fused systems are present in **tigogenin** and **neotigogenin** (Figure 5.87) found in *Digitalis purpurea* along with cardioactive glycosides (see page 246). All four stereoisomers are derived from cholesterol, and the stereochemistry of the A/B ring fusion appears to be controlled by the nature of the substrate being reduced. Direct enzymic reduction of the Δ^5 double bond yields the *trans*-fused system, whereas reduction of a Δ^4 double bond gives the alternative *cis*-fused system (Figure 5.89). Accordingly, to obtain the A/B *cis* fusion, the Δ^5 unsaturation of cholesterol is changed to Δ^4 by oxidation of the 3-hydroxyl and allylic isomerization to the conjugated 4-ene-3-one system, and this is followed by reduction of both functional groups (Figure 5.89) (compare biosynthesis of progesterone, page 243). The sarsaparilla saponins are not present in sufficient quantities to be commercially important for steroid production, but quite large amounts of sarsasapogenin can be extracted from the seeds of *Yucca brevifolia** (Agavaceae).

Cardioactive Glycosides

Many of the plants known to contain cardiac or cardiotonic glycosides have long been used as arrow poisons (e.g. *Strophanthus*) or as heart drugs (e.g. *Digitalis*). They are used to strengthen a weakened heart and allow it to function more efficiently, though the dosage must be controlled very

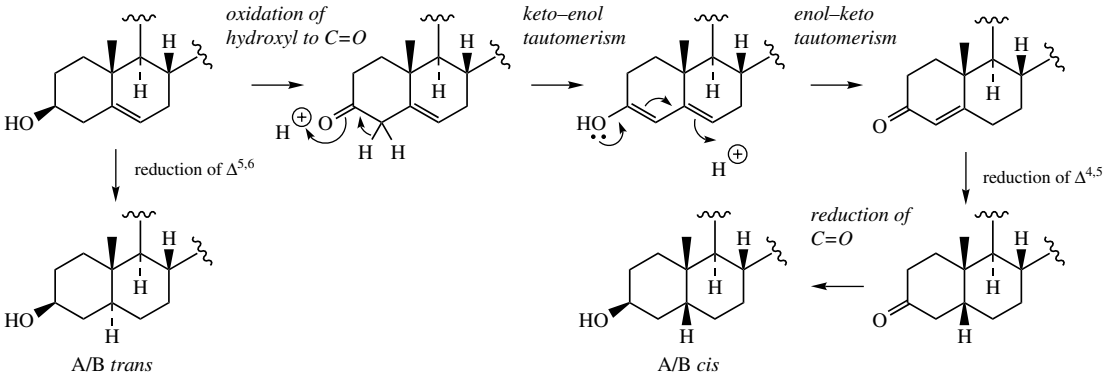


Figure 5.89

Sarsaparilla

Sarsaparilla consists of the dried roots of various *Smilax* species (Liliaceae/Smilacaceae), including *S. aristolochiaefolia*, *S. regelii*, and *S. febrifuga*, known respectively as Mexican, Honduran, and Ecuadorian sarsaparilla. The plants are woody climbers indigenous to Central America. Sarsaparilla has a history of use in the treatment of syphilis, rheumatism, and skin diseases, but is now mainly employed as a flavouring in the manufacture of non-alcoholic drinks. It has some potential as a raw material for the semi-synthesis of medicinal steroids, being a source of sarsasapogenin and smilagenin (Figure 5.87). The roots contain 1.8–2.4% steroidal saponins, including parillin (Figure 5.90).

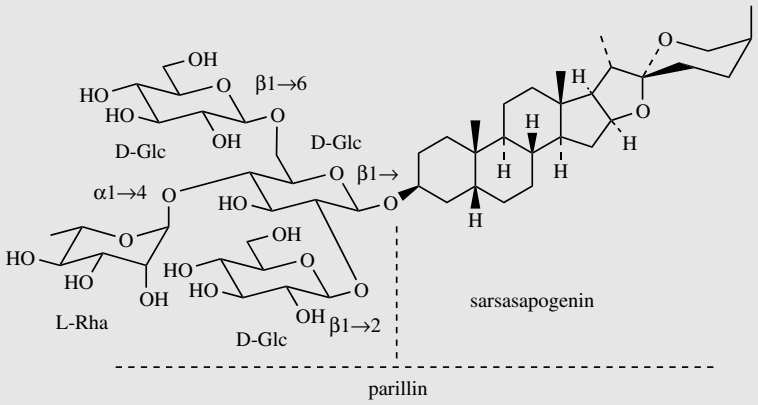


Figure 5.90

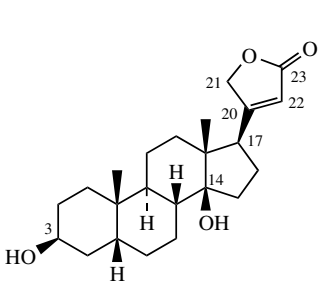
Yucca

Yucca brevifolia (Agavaceae) has been explored as a potential source of sarsasapogenin for steroid production, especially at times when market prices of diosgenin from *Dioscorea* became prohibitively expensive. The plant grows extensively in the Mojave desert in California, and high levels of sarsasapogenin (8–13%) are present in the seeds. This means the plants can be harvested regularly without damage. The subsequent stabilization of *Dioscorea* prices in the 1970s stopped further commercial utilization.

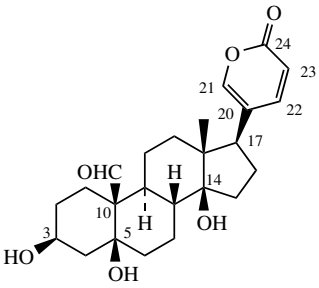
carefully since the therapeutic dose is so close to the toxic dose. The cardioactive effects of *Digitalis* were discovered as a result of its application in the treatment of dropsy, an accumulation of water in the body tissues. *Digitalis* alleviated dropsy indirectly by its effect on the heart, improving the blood supply to the kidneys and so removing excess fluid.

The therapeutic action of cardioactive glycosides depends on the structure of the aglycone, and on the type and number of sugar units attached. Two types of aglycone are recognized, **cardenolides**, e.g. **digitoxigenin** from *Digitalis purpurea*, which are C_{23} compounds, and **bufadienolides**, e.g. **hellebrigenin** from *Helleborus niger*, which are C_{24} structures (Figure 5.91). Stereochemistry is very important for activity, and these compounds have *cis* fusions for both the A/B and C/D rings, 3β- and 14β-hydroxyl groups with the glycoside function at C-3, and an α,β-unsaturated lactone grouping at C-17β. This lactone ring is five membered in the cardenolides, and six membered in the bufadienolides. The hellebrigenin structure shows two other modifications not found in the basic steroid skeleton, namely a hydroxyl at the bridgehead carbon C-5, and a formyl group at C-10, being an oxidized form of the normal methyl. The three-dimensional shape of digitoxigenin is shown in Figure 5.91. These basic structures arise biosynthetically by metabolism of cholesterol, in which the side-chain is cleaved to a two-carbon acetyl group, followed by incorporation of either two or three carbons for cardenolides or bufadienolides respectively (Figure 5.92).

Shortening of the cholesterol side-chain is accomplished by stepwise hydroxylation at C-22 and then C-20, then cleavage of the C-20/22 bond giving **pregnenolone**, which is then oxidized in ring A giving **progesterone** (Figure 5.92). This can be reduced to give the *cis*-fused A/B system as in 3β-hydroxy-5β-pregnan-20-one (compare Figure 5.89) which is the substrate for 14β-hydroxylation, i.e. inverting the stereochemistry at this centre. Inversion is atypical for hydroxylation by mono-oxygenases, which are found to hydroxylate with retention of configuration. Whatever the mechanism of this hydroxylation, no Δ^8 or Δ^{15} double bond intermediates are involved. Hydroxylation in the side-chain at C-21 follows. The lactone ring is created at this stage. An intermediate malonate ester is involved, and ring formation probably occurs via the aldol addition process shown in Figure 5.93 giving the cardenolide **digitoxigenin**, the carboxyl carbon of the malonate ester being lost by decarboxylation during the process (compare malonate in the acetate pathway). Alternatively, three carbons from oxaloacetate can be incorporated by a similar esterification/aldol reaction sequence. This would produce **bufalin** (Figure 5.92), a bufadienolide structure found in the skin of toads (*Bufo* spp.), from which this class of compound was originally isolated and



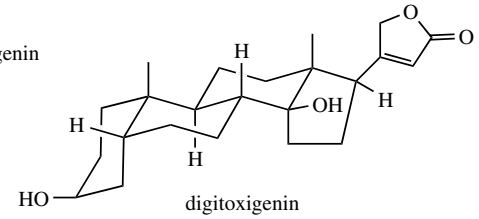
cardenolide - digitoxigenin



bufadienolide - hellebrigenin

Characteristic features of cardiac glycosides:

- *cis*-fused A/B and C/D rings
- 14β-hydroxyl
- unsaturated lactone at C-17β
- sugar residues on 3β-hydroxyl



digitoxigenin

Figure 5.91

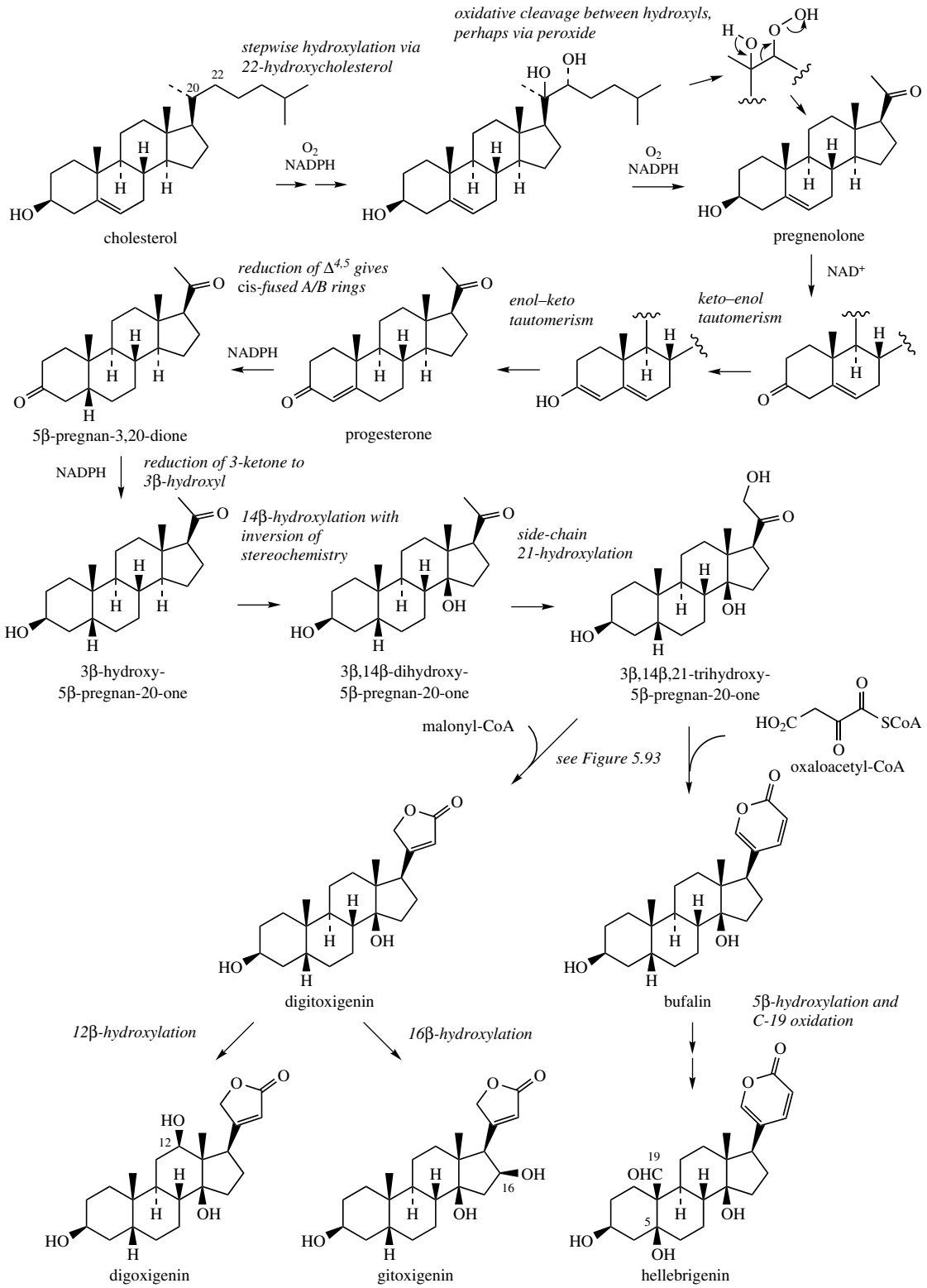

stepwise hydroxylation via 22-hydroxycholesterol
oxidative cleavage between hydroxyls, perhaps via peroxide
cholesterol
pregnenolone
reduction of Δ4,5 gives cis-fused A/B rings
enol–keto tautomerism
keto–enol tautomerism
5β-pregnan-3,20-dione
progesterone
reduction of 3-ketone to 3β-hydroxyl
14β-hydroxylation with inversion of stereochemistry
side-chain 21-hydroxylation
3β-hydroxy-5β-pregnan-20-one
3β,14β-dihydroxy-5β-pregnan-20-one
3β,14β,21-trihydroxy-5β-pregnan-20-one
malonyl-CoA
see Figure 5.93
oxaloacetyl-CoA
digitoxigenin
bufalin
5β-hydroxylation and C-19 oxidation
12β-hydroxylation
16β-hydroxylation
digoxigenin
gitoxigenin
hellebrigenin

Figure 5.92

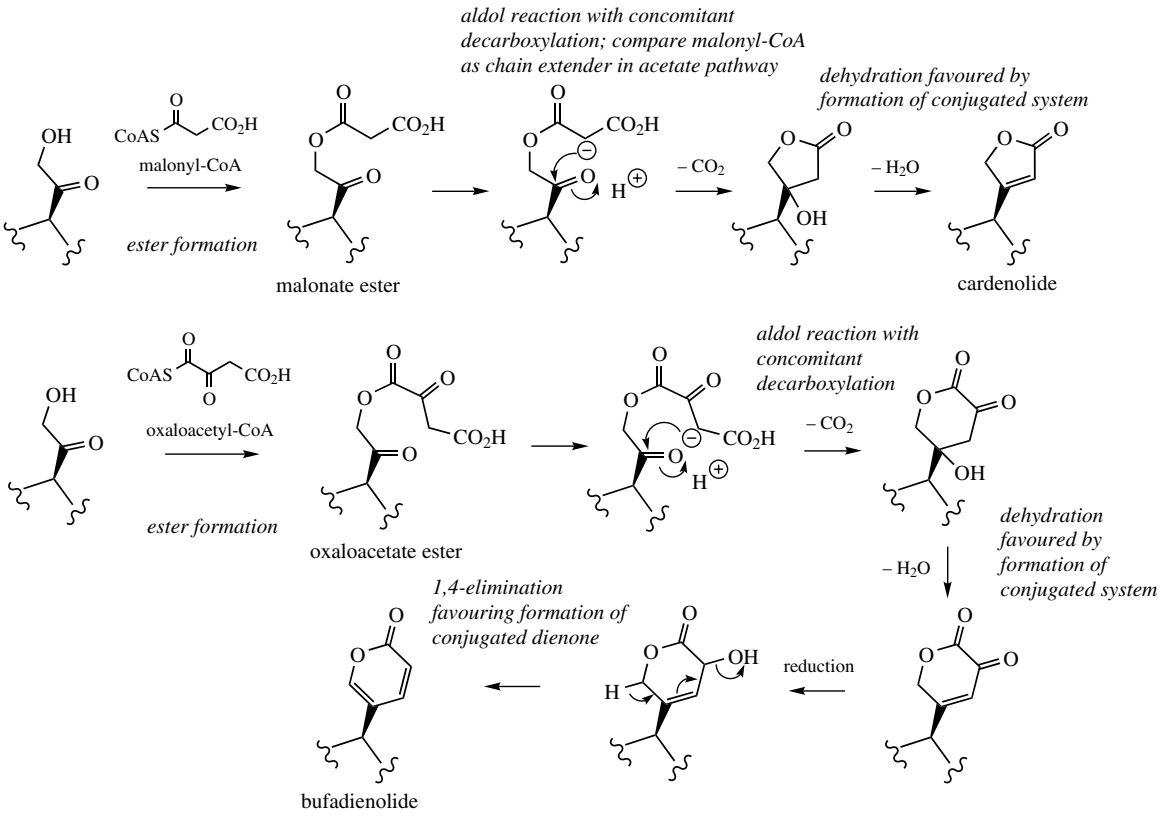


Figure 5.93

has subsequently taken the general name. Note that in the subsequent formation of **hellebrigenin** (Figure 5.92), hydroxylation at C-5 occurs with the expected retention of stereochemistry, and not with inversion as seen at C-14.

The fundamental pharmacological activity resides in the aglycone portion, but is considerably modified by the nature of the sugar at C-3. This increases water solubility and binding to heart muscle. The sugar unit may have one to four monosaccharides; many, e.g. D-**digitoxose** and D-**digitalose** (Figure 5.94), are unique to this group of compounds. About 20 different sugars have been characterized, and with the exception of D-glucose, they are 6-deoxy- (e.g. L-rhamnose) or 2,6-dideoxy- (e.g. D-digitoxose) hexoses, some of which are 3-methyl ethers (e.g. D-digitalose and D-**cymarose** (Figure 5.94)). In plants, cardiac glycosides are confined to the Angiosperms, but are found in both monocotyledons and dicotyledons. The cardenolides are more common, and the plant

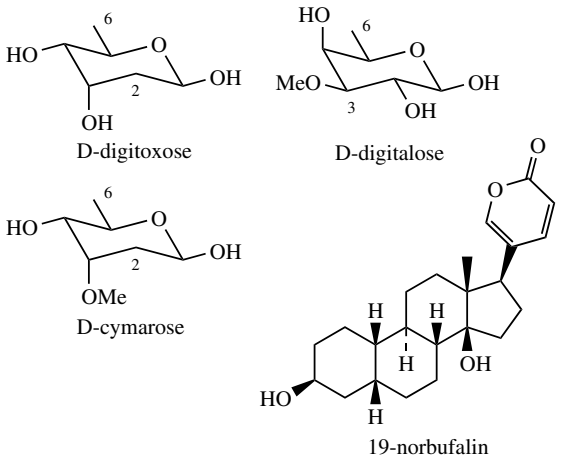


Figure 5.94

families the Apocynaceae (e.g. *Strophanthus*)*, Liliaceae (e.g. *Convallaria*)*, and Scrophulariaceae (e.g. *Digitalis*)* yield medicinal agents. The rarer bufadienolides are found in some members of

the Liliaceae (e.g. *Urginea*)* and Ranunculaceae (e.g. *Helleborus*), as well as toads. Monarch butterflies and their larvae are known to accumulate in their bodies a range of cardenolides, which they ingest from their food plant, the common milkweed (*Asclepias syriaca*; Asclepiadaceae). This makes them unpalatable to predators such as birds. Endogenous *Digitalis*-like compounds have also been detected, albeit in very small quantities, in mammalian tissues. **19-Norbufalin** (Figure 5.94) is found in human eye lenses, at higher levels if these are cataract afflicted, and it is believed to regulate ATPase activity under some physiological and pathological conditions.

Digitalis purpurea

Digitalis leaf consists of the dried leaf of the red foxglove *Digitalis purpurea* (Scrophulariaceae). The plant is a biennial herb, common in Europe and North America, which forms a low rosette of leaves in the first year, and its characteristic spike of purple (occasionally white) bell-shaped flowers in the second year. It is potentially very toxic, but is unlikely to be ingested by humans. *Digitalis purpurea* is cultivated for drug production, principally in Europe, the first year leaves being harvested then rapidly dried at 60°C as soon as possible after collection. This procedure is necessary to inactivate hydrolytic enzymes which would hydrolyse glycoside linkages in the cardioactive glycosides giving rise to less active derivatives. Even so, some partial hydrolysis does occur. Excess heat may also cause dehydration in the aglycone to Δ^{14}-anhydro compounds, which are inactive.

Because of the pronounced cardiac effects of digitalis, the variability in the cardiac glycoside content, and also differences in the range of structures present due to the effects of enzymic hydrolysis, the crude leaf drug is usually assayed biologically rather than chemically. Prepared digitalis is a biologically standardized preparation of powdered leaf, its activity being assessed on cardiac muscle of guinea pig or pigeon and compared against a standard preparation. It may be diluted to the required activity by mixing in powdered digitalis of lower potency, or inactive materials such as lucerne (*Medicago sativa*) or grass. The crude drug is hardly ever used now, having been replaced by the pure isolated glycosides.

The cardioactive glycoside content of *Digitalis purpurea* leaf is 0.15–0.4%, consisting of about 30 different structures. The major components are based on the aglycones digitoxigenin, gitoxigenin, and gitaloxigenin (Figure 5.95), the latter being a formate ester. The glycosides comprise two series of compounds, those with a tetrasaccharide *glucose*–(*digitoxose*)$_s$–unit and those with a trisaccharide (*digitoxose*)$_3$–unit. The latter group (the secondary glycosides) are produced by partial hydrolysis from the former group (the primary glycosides) during drying by the enzymic action of a β-glucosidase, which removes the terminal glucose. Thus the principal glycosides in the fresh leaves, namely purpureaglycoside A and purpureaglycoside B (Figure 5.95), are partially converted into digitoxin and gitoxin respectively (Figure 5.95), which normally predominate in the dried leaf. These transformations are indicated schematically in Figure 5.96. In the fresh leaf, purpureaglycoside A can constitute about 50% of the glycoside mixture, whilst in the dried leaf, the amounts could be negligible if the plant material is old or poorly stored. The gitaloxigenin-based glycosides are relatively unstable, and the formyl group on the aglycone is readily lost by hydrolysis. Other minor glycosides are present, but neither the fresh nor dried leaf contain any significant quantities of the free aglycones.

Glycosides of the gitoxigenin series are less active than the corresponding members of the digitoxigenin-derived series. **Digitoxin** is the only compound routinely used as a drug, and it is employed in congestive heart failure and treatment of cardiac arrhythmias, particularly atrial fibrillation.

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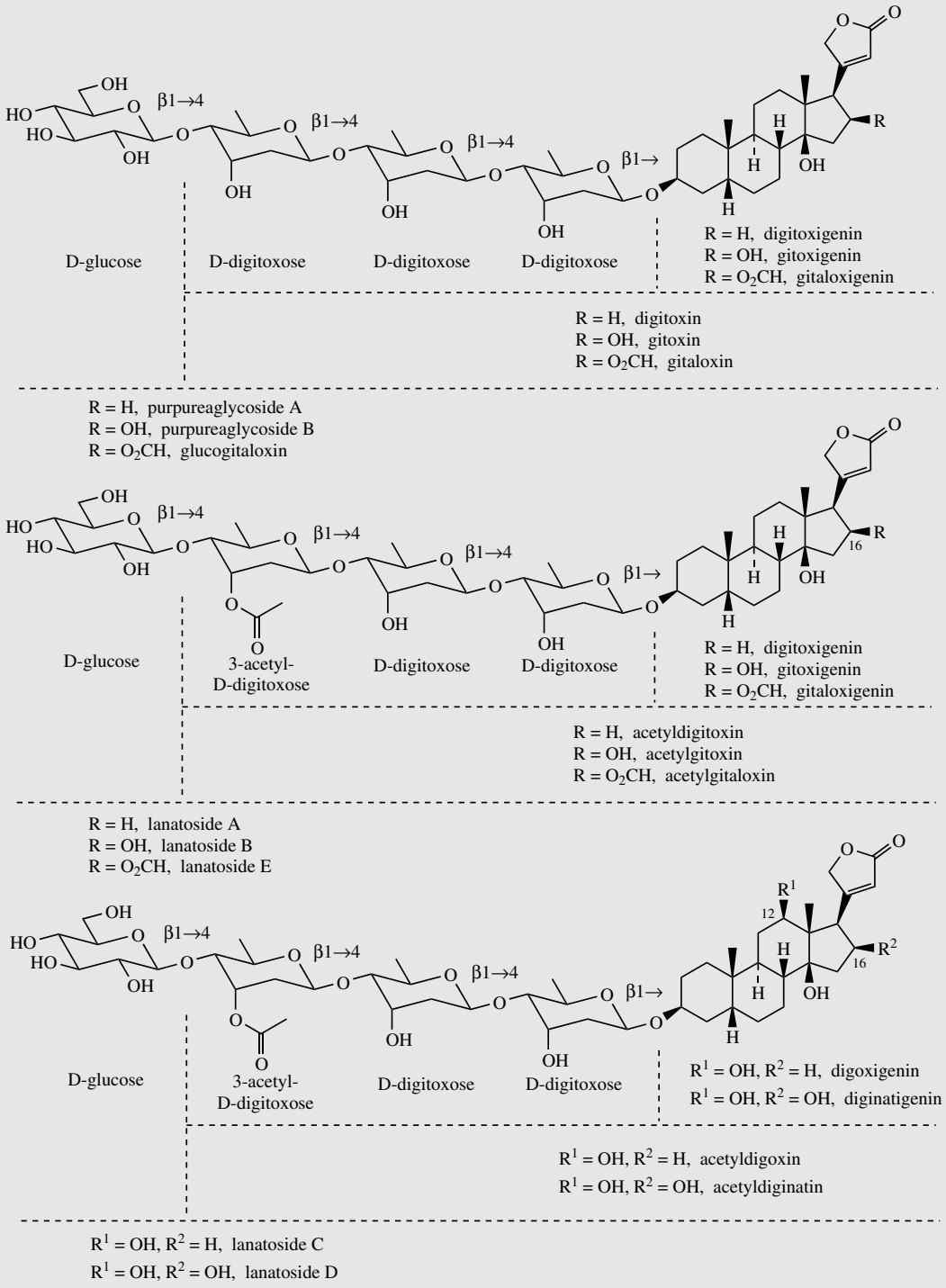

Figure 5.95

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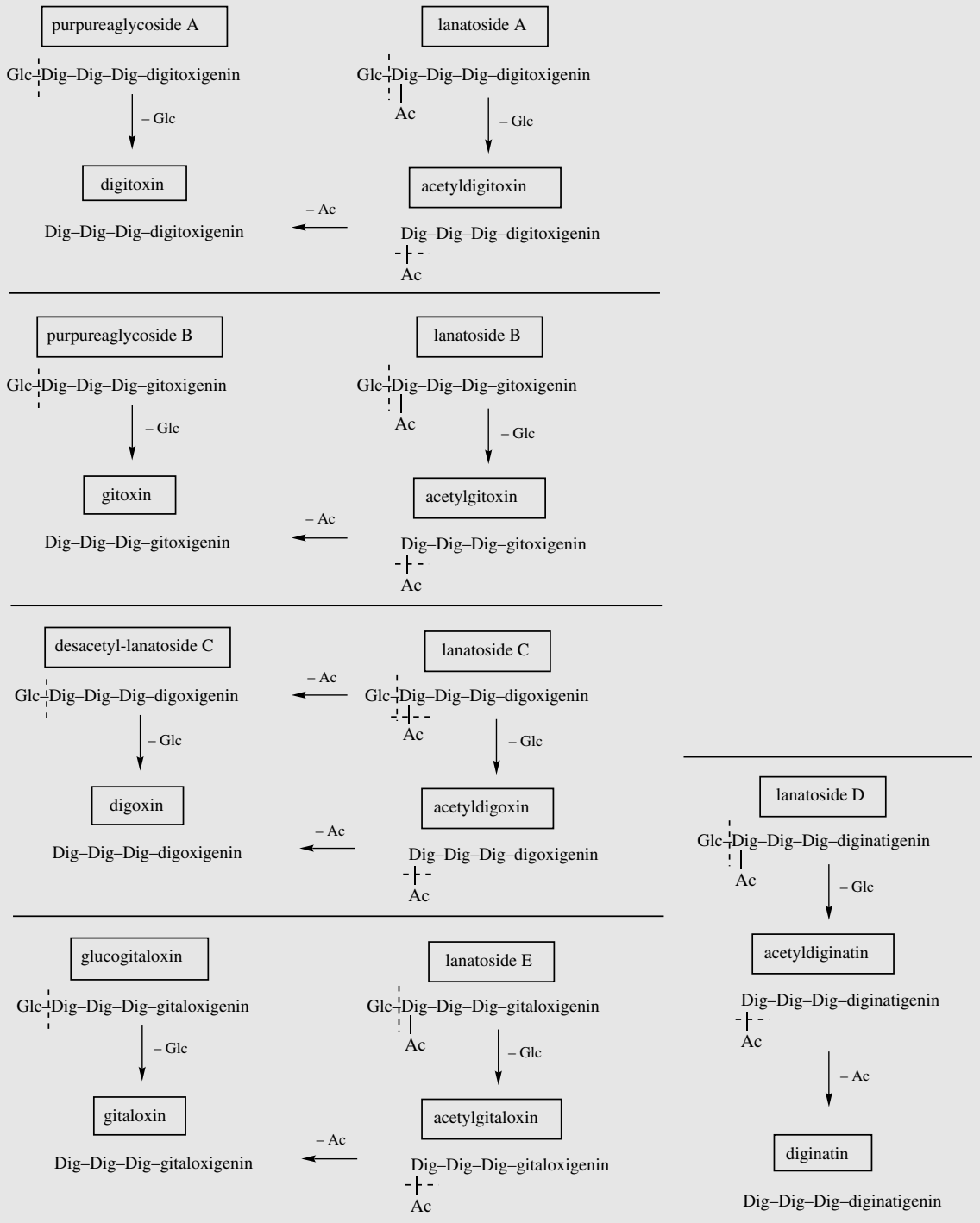

Figure 5.96

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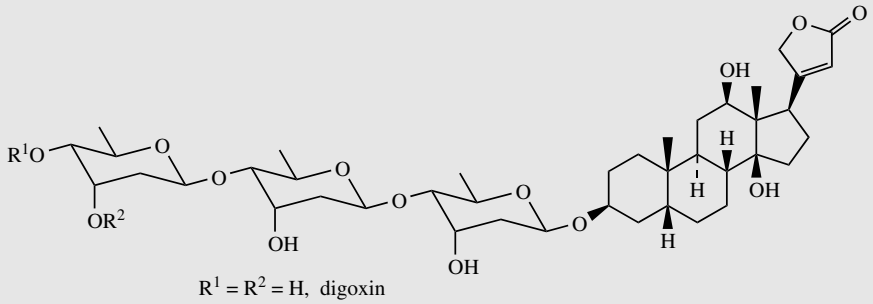
Digitalis lanata

Digitalis lanata (Scrophulariaceae), the Grecian foxglove, is a perennial or biennial herb from Southern and Central Europe, and differs in appearance from the red foxglove by its long narrow smoother leaves, and its smaller flowers of a yellow-brown colour. It is cultivated in Europe, the United States and South America, and is harvested and dried in a similar manner to *D. purpurea*. It has not featured as a crude drug, but is used exclusively for the isolation of individual cardiac glycosides, principally digoxin and lanatoside C (Figure 5.97).

The total cardenolide content of up to 1% is two to three times that found in *D. purpurea*. The main constituents resemble those of *D. purpurea*, but contain an acetyl ester function on the third digitoxose, that furthest from the aglycone. This acetyl group makes the compounds easier to isolate from the plant material and makes crystallization easier. Drying of the leaf is similarly accompanied by some partial hydrolysis of the original fresh leaf constituents through enzymic action, and both the terminal glucose and the acetyl group may be hydrolysed off, extending the range of compounds isolated. The *D. lanata* cardiac glycosides are based on five aglycones, digitoxigenin, gitoxigenin, and gitaloxigenin, as found in *D. purpurea*, plus digoxigenin and diginatigenin (Figure 5.95), which do not occur in *D. purpurea*. The primary glycosides containing the acetylated tetrasaccharide unit *glucose–acetyldigitoxose–*(*digitoxose*)$_2$–are called lanatosides. Lanatosides A and C (Figure 5.95) constitute the major components in the fresh leaf (about 50–70%) and are based on the aglycones digitoxigenin and digoxigenin respectively. Lanatosides B, D, and E (Figure 5.95) are minor components derived from gitoxigenin, diginatigenin, and gitaloxigenin respectively. Enzymic hydrolysis of the lanatosides generally involves loss of the terminal glucose prior to removal of the acetyl function, so that compounds like acetyldigitoxin and acetyldigoxin as well as digitoxin and digoxin are present in the dried leaf as decomposition products from lanatosides A and C respectively. These transformations are also indicated in simplified form in Figure 5.96.

Digoxin (Figure 5.97) has a rapid action and is more quickly eliminated from the body than digitoxin, and is therefore the most widely used of the cardioactive glycosides. Digoxin is more hydrophilic than digitoxin, binds less strongly to plasma proteins and is mainly eliminated by the kidneys, whereas digitoxin is metabolized more slowly by the liver. It is used in congestive



$R^1 = R^2 = H$, digoxin
$R^1 = Me$, $R^2 = H$, medigoxin (metildigoxin)
$R^1 = Glc$, $R^2 = Ac$, lanatoside C
$R^1 = Glc$, $R^2 = H$, deslanoside (desacetyl-lanatoside C)

Figure 5.97

(*Continues*)

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heart failure and atrial fibrillation. **Lanatoside C** and **deslanoside** (**desacetyl-lanatoside C**) (Figure 5.97) have also been employed, though not to the same extent. They have very rapid action and are suited for treatment of cardiac emergencies by injection. A semi-synthetic derivative **medigoxin** or **metildigoxin** (methyl replacing the glucose in lanatoside C) (Figure 5.97) has also been available, being more active via better bioavailability.

The cardioactive glycosides increase the force of contractions in the heart, thus increasing cardiac output and allowing more rest between contractions. The primary effect on the heart appears to be inhibition of Na^+/K^+-ATPase in the cell membranes of heart muscle, specifically inhibiting the Na^+ pump, thereby raising the intracellular Na^+ concentration. The resultant decrease in the Na^+ gradient across the cell membrane reduces the energy available for transport of Ca^{2+} out of the cell, leads to an increase in intracellular Ca^{2+} concentration, and provides the positive ionotropic effect and increased force of contractions. The improved blood circulation also tends to improve kidney function leading to diuresis and loss of oedema fluid often associated with heart disease. However, the diuretic effect, historically important in the treatment of dropsy, is more safely controlled by other diuretic drugs.

To treat congestive heart failure, an initial loading dose of the cardioactive glycoside is followed by regular maintenance doses, the amounts administered depending on drug bioavailability and subsequent metabolism or excretion. Because of the extreme toxicity associated with these compounds (the therapeutic level is 50–60% of the toxic dose; a typical daily dose is only about 1 mg) dosage must be controlled very carefully. Bioavailability has sometimes proved erratic and can vary between different manufacturers' formulations, so patients should not be provided with different preparations during their treatment. Individual patients also excrete the glycosides or metabolize them by hydrolysis to the aglycone at different rates, and ideally these processes should be monitored. Levels of the drug in blood plasma can be measured quite rapidly by radioimmunoassay using a specific antibody. A **digoxin-specific antibody** is available both for assay and also as a means of reversing life-threatening digoxin overdose. It has also successfully reversed digitoxin overdose, thus demonstrating a somewhat broader specificity. The value of digoxin treatment for heart failure where the heartbeat remains regular has recently been called into question. It still remains a recognized treatment for atrial fibrillation.

Many other species of *Digitalis*, e.g. *D. dubia, D. ferruginea, D. grandiflora, D. lutea, D. mertonensis, D. nervosa, D. subalpina*, and *D. thaspi* contain cardioactive glycosides in their leaves, and some have been evaluated and cultivated for drug use.

Strophanthus

Strophanthus is the dried ripe seeds of *Strophanthus kombé* or *S. gratus* (Apocynaceae), which are tall vines from equatorial Africa. *Strophanthus kombé* has a history of use by African tribes as an arrow poison, and the seeds contain 5–10% cardenolides, a mixture known as K-strophanthin. This has little drug use today, though it was formerly used medicinally as a cardiac stimulant. The main glycoside (about 80%) is K-strophanthoside (Figure 5.98) with smaller amounts of K-strophanthin-β and cymarin, related to K-strophanthoside as shown. These are derivatives of the aglycone strophanthidin. *Strophanthus gratus* contains 4–8% of **ouabain** (G-strophanthin) (Figure 5.98), the rhamnoside of ouabigenin. Ouabigenin is rather

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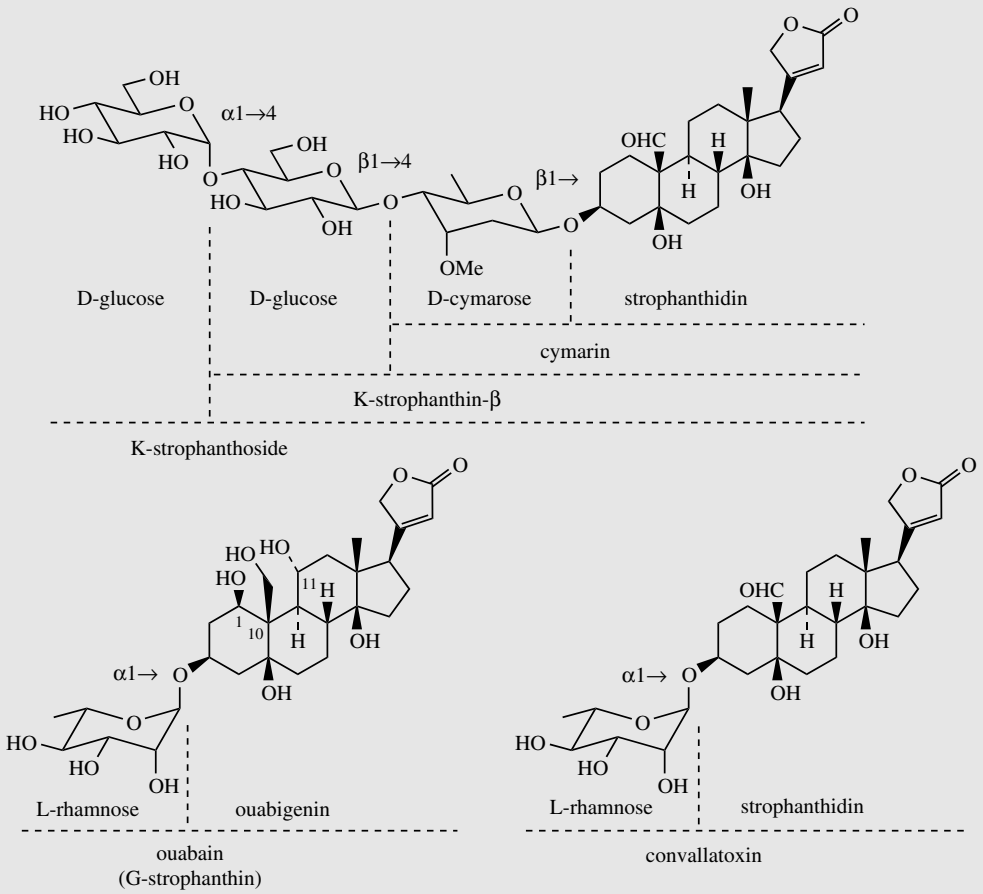

Figure 5.98

unusual in having additional hydroxylation at 1β and 11α, as well as a hydroxymethyl at C-10. Ouabain is a stable, crystalline material, which is often employed as the biological standard in assays for cardiac activity. It is a potent cardiac glycoside and acts quickly, but wears off rapidly. It is very polar with rapid renal elimination and must be injected because it is so poorly absorbed orally. It has been used for emergency treatment in cases of acute heart failure. It is still official in many pharmacopoeias.

Convallaria

The dried roots and tops of lily of the valley, *Convallaria majalis* (Liliaceae/Convallariaceae), contain cardioactive glycosides (0.2–0.3%) and are used in some European countries rather than digitalis. The effects are similar, but the drug is less cumulative. This plant is widely cultivated as an ornamental, particularly for its intensely perfumed small white flowers, and must be considered potentially toxic. The major glycoside (40–50%) is convallatoxin (Figure 5.98), the rhamnoside of strophanthidin.

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Squill

Squill (white squill) consists of the dried sliced bulbs of the white variety of *Urginea maritima* (formerly *Scilla maritima*; also known as *Drimia maritima*) (Liliaceae/Hyacinthaceae) which grows on seashores around the Mediterranean. The plant contains bufadienolides (up to 4%), principally scillaren A and proscillaridin A (Figure 5.99). The aglycone of scillaren A is scillarenin, which is unusual in containing a Δ^4 double bond and thus lacks the *cis* A/B ring fusion found in the majority of cardiac glycosides. Squill is not usually used for its cardiac properties, as the glycosides have a short duration of action. Instead, squill is employed for its expectorant action in preparations such as Gee's linctus. Large doses cause vomiting and a digitalis-like action on the heart.

Red squill is a variety of *Urginea maritima* that contains an anthocyanin pigment (see page 150) and bufadienolides that are different from those of the white squill. The main glycosides are glucoscilliroside and scilliroside (Figure 5.99), glucosides of scillirosidin. This chemical variety should not be present in medicinal squill, and has mainly been employed as a rodenticide. Rodents lack a vomiting reflex and are poisoned by the cardiac effects, whilst in other animals and humans vomiting will occur due to the emetic properties of the drug. The use of red squill as a rodenticide is now considered inhumane.

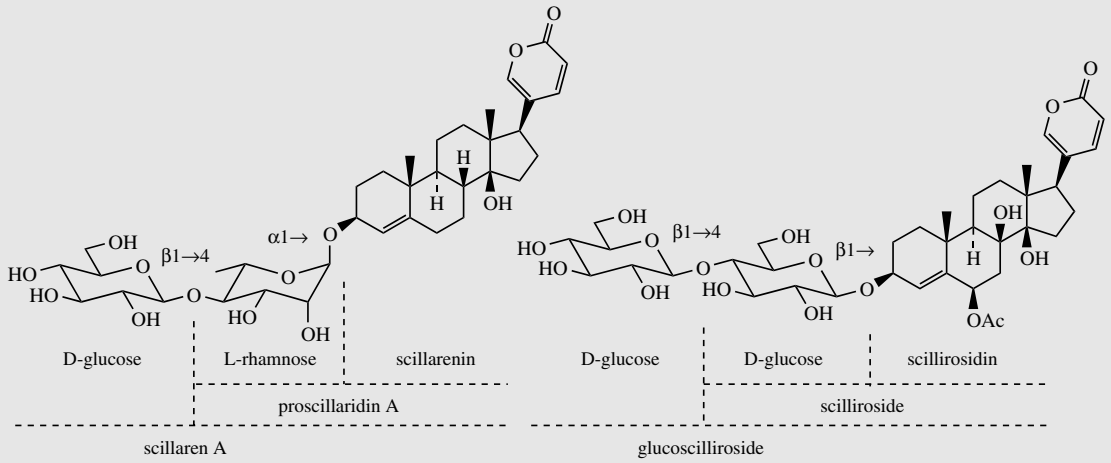


Figure 5.99

Toxic Plants

Many plants containing cardioactive glycosides are widely grown as ornamentals and must be considered toxic and treated with due care and respect. These include *Digitalis* species, *Convallaria majalis*, *Helleborus* species, and oleander (*Nerium oleander*; Apocynaceae).

Phytosterols

The major sterol found in mammals is the C_{27} compound cholesterol, which acts as a precursor for other steroid structures such as sex hormones and corticosteroids. The main sterols in plants, fungi, and algae are characterized by extra one-carbon or two-carbon substituents on the side-chain, attached at C-24. These substituent carbons are numbered 24^1 and 24^2 (Figure 5.77); some older publications

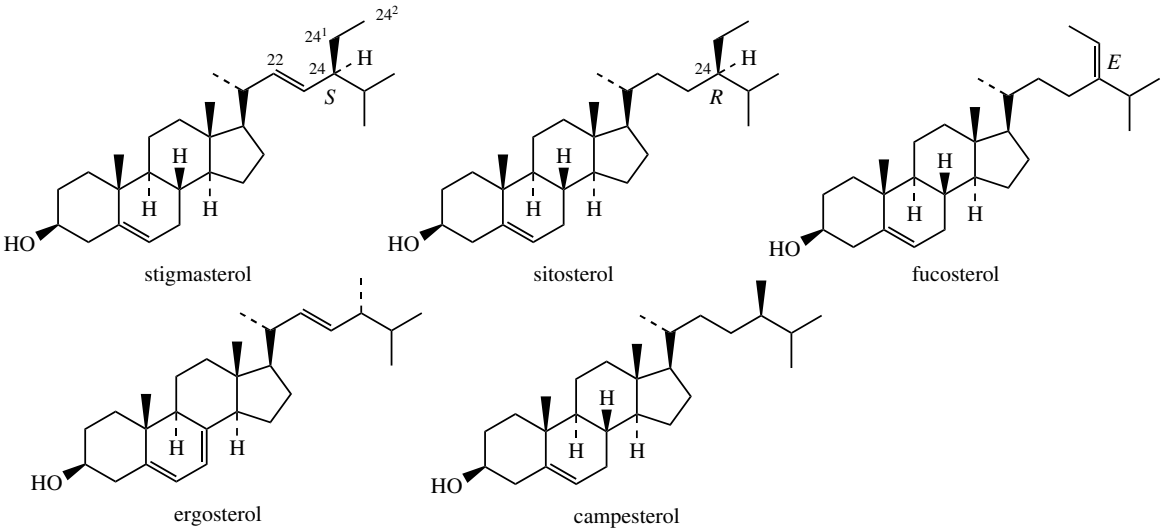


Figure 5.100

may use 28 and 29. The widespread plant sterols **campesterol** and **sitosterol** (Figure 5.100) are respectively 24-methyl and 24-ethyl analogues of cholesterol. **Stigmasterol** contains additional unsaturation in the side-chain, a *trans*-Δ^{22} double bond, a feature seen in many plant sterols, but never in mammalian ones. The introduction of methyl and ethyl groups at C-24 generates a new chiral centre, and the 24-alkyl groups in campesterol, sitosterol, and stigmasterol are designated α. The predominant sterol found in fungi is **ergosterol** (Figure 5.100), which has a β-oriented 24-methyl, as well as a *trans*-Δ^{22} double bond and additional Δ^{7} unsaturation. The descriptors α and β unfortunately do not relate to similar terms for the steroid ring system, but are derived from consideration of Fischer projections for the side-chain, substituents to the left being designated α and those to the right as β. Systematic *RS* nomenclature is preferred, but note that this defines sitosterol as 24*R* whilst stigmasterol, because of its extra double bond, is 24*S*. The majority of plant sterols have a 24α-methyl or 24α-ethyl substituent, whilst algal sterols tend to have 24β-ethyls, and fungi 24β-methyls. The most abundant sterol in brown algae (*Fucus* spp.; Fucaceae) is **fucosterol** (Figure 5.100), which demonstrates a further variant, a 24-ethylidene substituent. Such groups can have *E*-configurations as in fucosterol, or the alternative *Z*-configuration. Sterols are found predominantly in free alcohol form, but also as esters with long chain fatty acids (e.g. palmitic, oleic, linoleic, and α-linolenic acids), as glycosides, and as fatty acylated glycosides. These sterols, termed phytosterols, are structural components of membranes in plants, algae, and fungi, and affect the permeability of these membranes. They also appear to play a role in cell proliferation.

The source of the extra methyl or ethyl side-chain carbons in both cases is *S*-adenosylmethionine (SAM), and to achieve alkylation the side-chain must have a Δ^{24} double bond, i.e. the side-chains seen in lanosterol and cycloartenol. The precise mechanisms involved have been found to vary according to organism, but some of the demonstrated sequences are given in Figure 5.101. Methylation of the Δ^{24} double bond at C-24 via SAM yields a carbocation which undergoes a hydride shift and loss of a proton from C-24^{1} to generate the 24-methylene side-chain. This can be reduced to a 24-methyl either directly, or after allylic isomerization. Alternatively, the 24-methylene derivative acts as substrate for a second methylation step with SAM, producing a carbocation. Discharge of this cation by proton loss produces a 24-ethylidene side-chain, and reduction or isomerization/reduction gives a 24-ethyl group. The *trans*-Δ^{22} double bond is introduced only after alkylation at C-24 is completed. No stereochemistry is intended in

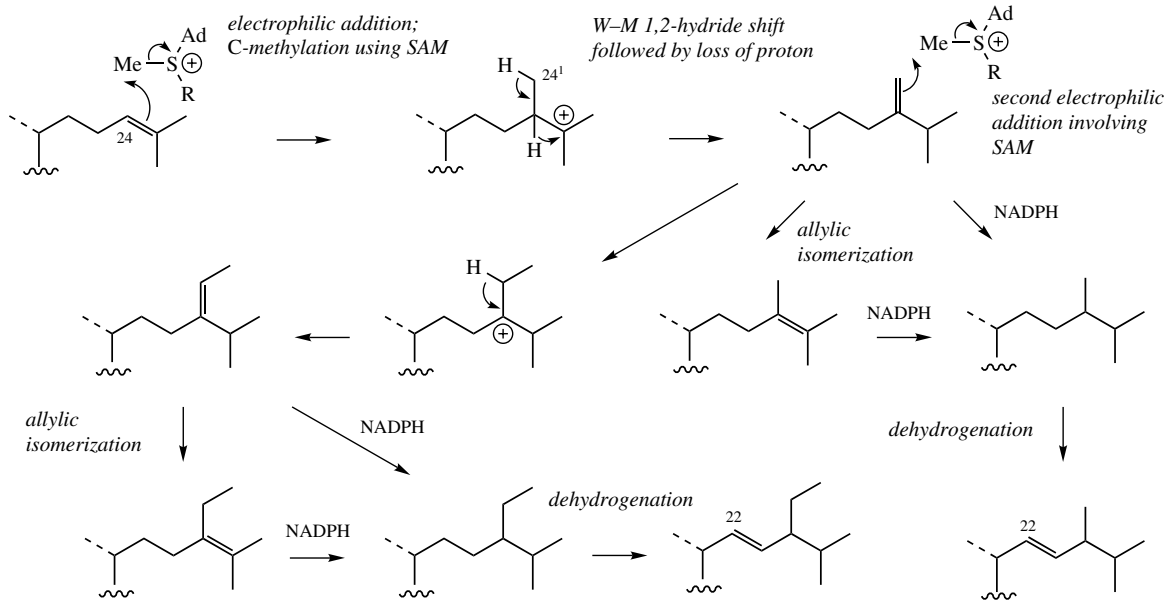


Figure 5.101

Figure 5.101. It is apparent that stereochemistries in the 24-methyl, 24-ethyl, and 24-ethylidene derivatives could be controlled by the reduction processes or by proton loss as appropriate. It is more plausible for different stereochemistries in the 24-methyl and 24-ethyl side-chains to arise from reduction of different double bonds, rather than reduction of the same double bond in two different ways. In practice, other mechanisms involving a 25(26)-double bond are also found to operate.

The substrates for alkylation are found to be cycloartenol in plants and algae, and lanosterol in fungi. The second methylation step in plants and algae usually involves **gramisterol** (24-methylenelophenol) (Figure 5.102). This indicates that the processes of side-chain alkylation and the steroid skeleton modifications, i.e. loss of methyls, opening of the cyclopropane ring, and migration of the double bond, tend to run concurrently rather than sequentially. Accordingly, the range of plant and algal sterol derivatives includes products containing side-chain alkylation, retention of one or more skeletal methyls, and possession of a cyclopropane ring, as well as those more abundant examples such as sitosterol and stigmasterol based on a cholesterol-type skeleton. Most fungal sterols originate from lanosterol, so less variety is encountered. The most common pathway from lanosterol to **ergosterol** in fungi involves initial side-chain alkylation to **eburicol** (24-methylenedihydrolanosterol), which is the substrate for 14-demethylation (Figure 5.103). Loss of the 4-methyls then gives **fecosterol**, from which ergosterol arises by further side-chain and ring B modifications. Although the transformations are similar to those occurring in the mammalian pathway for lanosterol → cholesterol, the initial side-chain alkylation means the intermediates formed are different. Some useful anti-fungal agents, e.g. ketoconazole and miconazole, are specific inhibitors of the 14-demethylation reaction in fungi, but do not affect cholesterol biosynthesis in humans. Inability to synthesize the essential sterol components of their membranes proves fatal for the fungi. Similarly, 14-demethylation in plants proceeds via **obtusifoliol** (Figure 5.102) and plants are unaffected by azole derivatives developed as agricultural fungicides. The antifungal effect of polyene antibiotics such as amphotericin and nystatin depends on their ability to bind strongly to ergosterol in fungal membranes and not to cholesterol in mammalian cells (see page 102).

Sitosterol and **stigmasterol** (Figure 5.100) are produced commercially from soya beans* (*Glycine max*; Leguminosae/Fabaceae) as raw materials

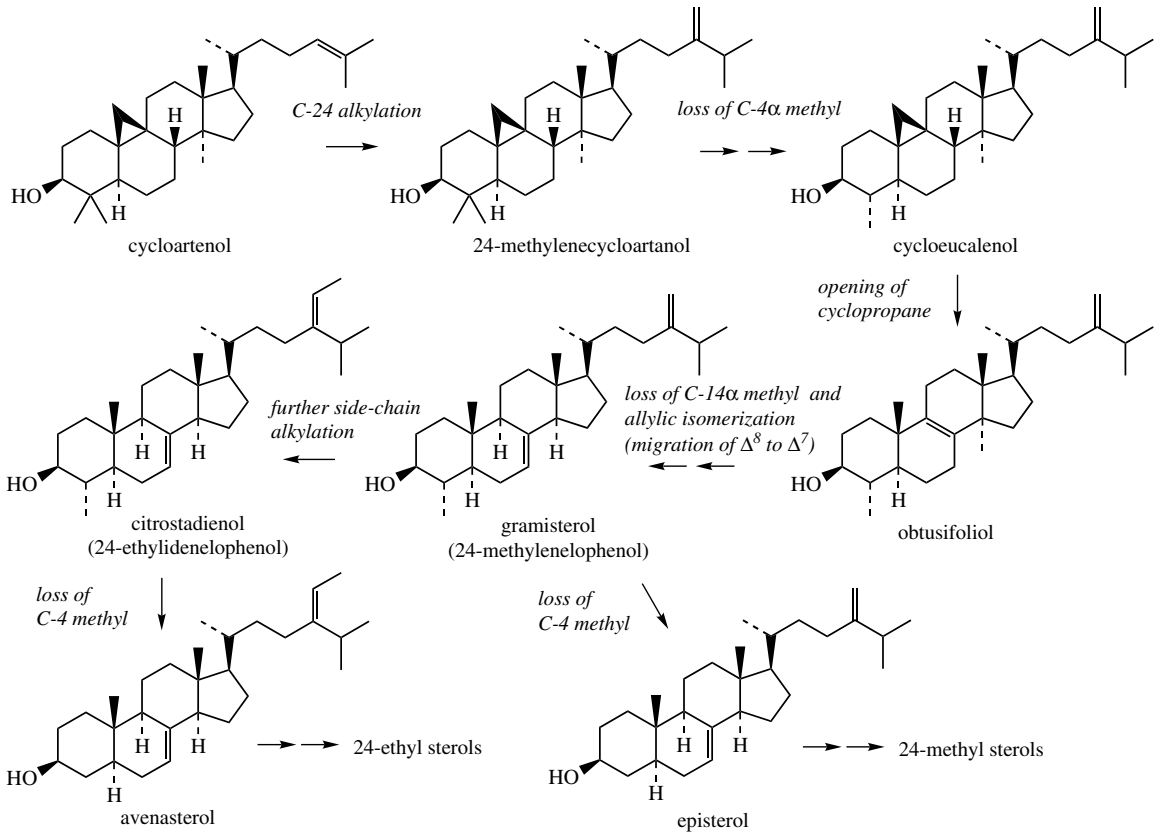


Figure 5.102

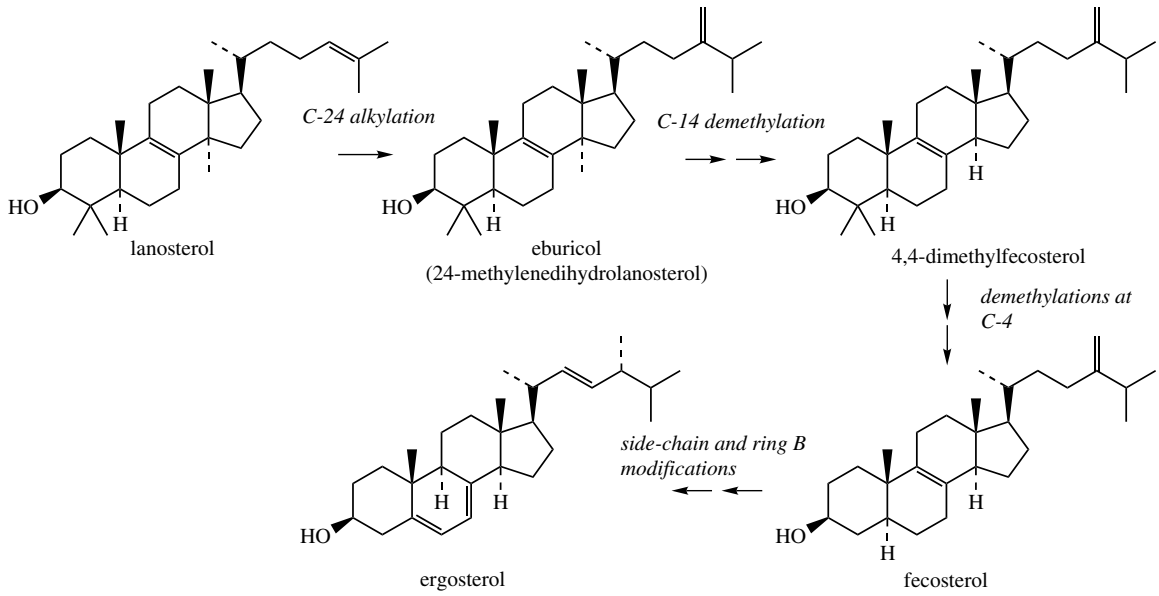
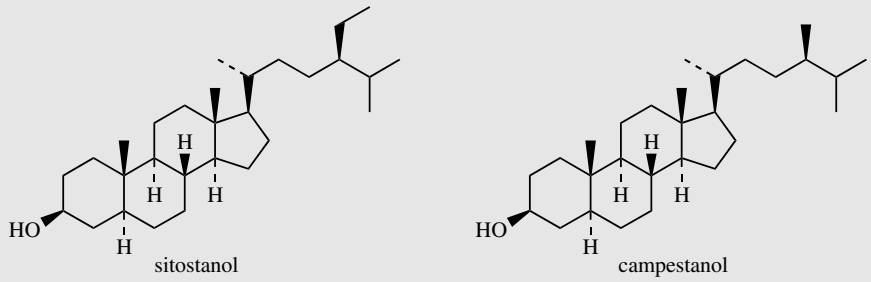


Figure 5.103

Soya Bean Sterols

Soya beans or soybeans (*Glycine max*; Leguminosae/Fabaceae) are grown extensively in the United States, China, Japan, and Malaysia as a food plant. They are used as a vegetable, and provide a high protein flour, an important edible oil (Table 3.2), and an acceptable non-dairy soybean milk. The flour is increasingly used as a meat substitute. Soy sauce is obtained from fermented soybeans and is an indispensable ingredient in Chinese cookery. The seeds also contain substantial amounts (about 0.2%) of sterols. These include stigmasterol (about 20%), sitosterol (about 50%) and campesterol (about 20%) (Figure 5.100), the first two of which are used for the semi-synthesis of medicinal steroids. In the seed, about 40% of the sterol content is in the free form, the remainder being combined in the form of glycosides, or as esters with fatty acids. The oil is usually solvent extracted from the dried flaked seed using hexane. The sterols can be isolated from the oil after basic hydrolysis as a by-product of soap manufacture, and form part of the unsaponifiable matter.

The efficacy of dietary plant sterols in reducing cholesterol levels in laboratory animals has been known for many years. This has more recently led to the introduction of **plant sterol esters** as food additives, particularly in margarines, as an aid to reducing blood levels of low density lipoprotein (LDL) cholesterol, known to be a contributory factor in atherosclerosis and the incidence of heart attacks (see page 236). Plant sterol esters are usually obtained by esterifying sitosterol from soya beans with fatty acids to produce a fat-soluble product. Regular consumption of this material (recommended 1.3 g per day) is shown to reduce blood LDL cholesterol levels by 10–15%. The plant sterols are more hydrophobic than cholesterol and have a higher affinity for micelles involved in fat digestion, effectively decreasing intestinal cholesterol absorption. The plant sterols themselves are not absorbed from the GI tract. Of course, the average diet will normally include small amounts of plant sterol esters. Related materials used in a similar way are **plant stanol esters**. Stanols are obtained by hydrogenation of plant sterols, and will consist mainly of sitostanol (from sitosterol and stigmasterol) and campestanol (from campesterol) (Figure 5.104); these are then esterified with fatty acids. Regular consumption of plant stanol esters (recommended 3.4 g per day) is shown to reduce blood LDL cholesterol levels by an average of 14%. Much of the material used in preparation of plant stanol esters originates from tall oil, a by-product of the wood pulping industry. This contains campesterol, sitosterol, and also sitostanol. The stanols are usually transesterified with rapeseed oil, which is rich in unsaturated fatty acids (see page 43).



sitostanol campestanol

Figure 5.104

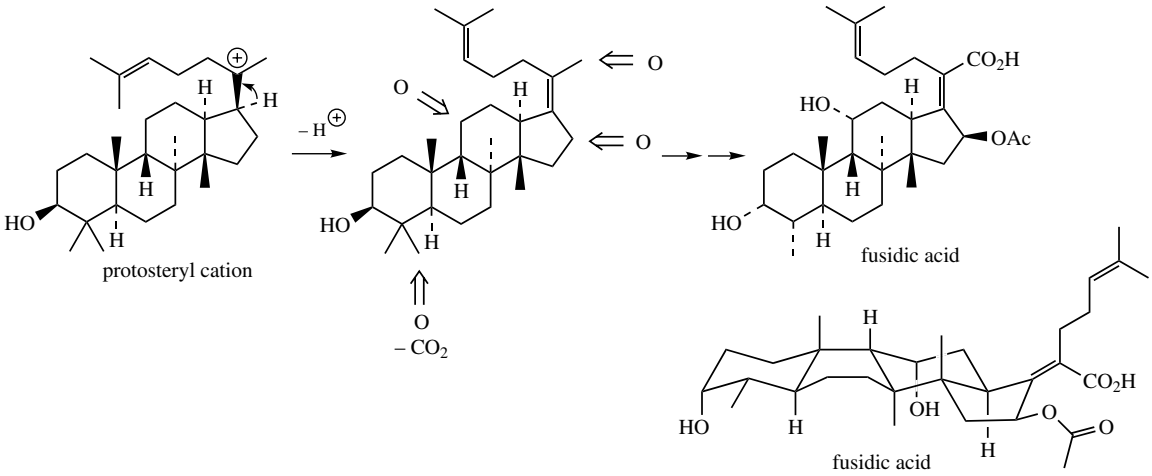


Figure 5.105

for the semi-synthesis of medicinal steroids (see pages 266, 279). For many years, only stigmasterol was utilized, since the Δ^{22} double bond allowed chemical degradation of the side-chain to be effected with ease. The utilization of sitosterol was not realistic until microbiological processes for removal of the saturated side-chain became available.

Fusidic acid* (Figure 5.105), an antibacterial agent from *Acremonium fusidioides*, has no additional side-chain alkylation, but has lost one C-4 methyl and undergone hydroxylation and oxidation of a side-chain methyl. Its relationship to the protosteryl cation is shown in Figure 5.105. The stereochemistry in fusidic acid is not typical of most steroids, and ring B adopts a boat conformation; the molecular shape is comparable to the protosteryl cation (Figure 5.57, page 216).

Vitamin D

Vitamin D_3 (**colecalciferol**, **cholecalciferol**)* is a sterol metabolite formed photochemically in animals from **7-dehydrocholesterol** by the sun's irradiation of the skin (Figure 5.106). 7-Dehydrocholesterol is the immediate $\Delta^{5,7}$ diene precursor of cholesterol (see page 234), and a photochemical reaction allows ring opening to precholecalciferol. A thermal 1,7-hydrogen shift follows to give colecalciferol (vitamin D_3). Vitamin D_3 is also manufactured photosynthetically by the same route. **Vitamin D_2** (**ergocalciferol**)* may be obtained from ergosterol in exactly the same way, and, although found in plants and yeasts, large amounts are obtained semi-synthetically by the sequence shown in Figure 5.106, using ergosterol from yeast

Fusidic Acid

Fusidic acid (Figure 5.105) is a steroidal antibiotic produced by cultures of the fungus *Acremonium fusidioides* (formerly *Fusidium coccineum*). It has also been isolated from several *Cephalosporium* species. Fusidic acid and its salts are narrow-spectrum antibiotics active against Gram-positive bacteria. It is primarily used, as its sodium salt, in infections caused by penicillin-resistant *Staphylococcus* species, especially osteomyelitis since fusidic acid concentrates in bone. It is usually administered in combination with another antibiotic to minimize development of resistance. Fusidic acid reversibly inhibits protein biosynthesis at the translocation step by binding to the larger subunit of the ribosome (see page 407).

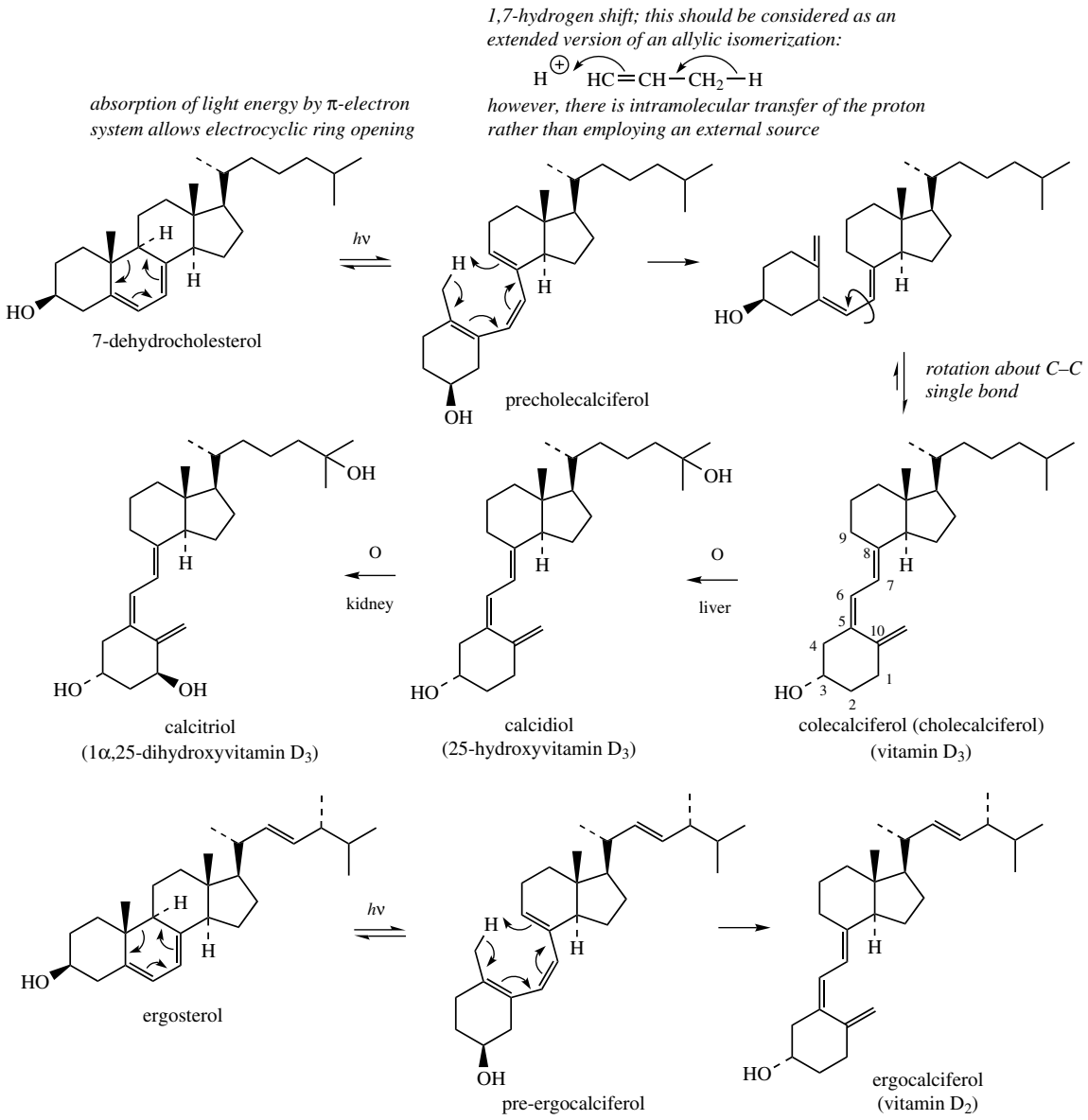


Figure 5.106

(*Saccharomyces cerevisiae*). Vitamin D_3 is not itself the active form of the vitamin, and in the body it is hydroxylated first to **calcidiol** and then to **calcitriol** (Figure 5.106). Colecalciferol and calcitriol have also been found in several plant species.

Systematic nomenclature of vitamin D derivatives utilizes the obvious relationship to steroids, and the term *seco* (ring opened) is incorporated into the root name (compare secologanin as a ring-opened analogue of loganin, page 189). The numbering system for steroids is also retained, and vitamin D_3 becomes a derivative of 9,10-seco-cholestane, namely (5*Z*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol, '9,10' indicating the site of ring cleavage. Note that it is necessary to indicate the configuration of two of the double bonds, and the somewhat confusing β-configuration for the 3-hydroxy shows it is actually the same as in cholesterol.

Vitamin D

Vitamin D_3 (**colecalciferol**, **cholecalciferol**) (Figure 5.106) is the main form of the fat-soluble vitamin D found in animals, though **vitamin D_2** (**ergocalciferol**) (Figure 5.106) is a constituent of plants and yeasts. Vitamin D_3 is obtained in the diet from liver and dairy products such as butter, cream, and milk, whilst large amounts can be found in fish liver oils, e.g. cod liver oil and halibut liver oil (Table 3.2). Further requirements are produced naturally when the sterol 7-dehydrocholesterol is converted into colecalciferol by the effects of UV light on the skin. With a proper diet, and sufficient exposure to sunshine, vitamin D deficiency should not occur. Vitamin D deficiency leads to rickets, an inability to calcify the collagen matrix of growing bone, and is characterized by a lack of rigidity in the bones, particularly in children. In adults, osteoporosis may occur. In most countries, foods such as milk and cereals are usually fortified with vitamin D_3, obtained commercially by UV irradiation of 7-dehydrocholesterol which is produced in quantity by semi-synthesis from cholesterol. Vitamin D_2 has a similar activity in humans and is manufactured by UV irradiation of yeast, thereby transforming the ergosterol content. Other compounds with vitamin D activity have also been produced: vitamin D_4 from 22,23-dihydroergosterol, vitamin D_5 from 7-dehydrositosterol, vitamin D_6 from 7-dehydrostigmasterol, and vitamin D_7 from 7-dehydrocampesterol. Vitamin D_1 was an early preparation, later shown to be a mixture of vitamin D_2 and a photochemical by-product lumisterol (9β,10α-ergosterol). Vitamin D is unstable to heat, light, and air.

Vitamin D_3 is not itself the active form of the vitamin, and in the body it is hydroxylated firstly to 25-hydroxyvitamin D_3 (calcidiol) (Figure 5.106) by an enzyme in the liver, and then to 1α,25-dihydroxyvitamin D_3 (calcitriol) by a kidney enzyme. Calcitriol is then transported to the bones, intestine, and other organs. It stimulates the absorption of calcium and phosphate in the intestine and the mobilization of calcium from bone. **Calcitriol** and other analogues, e.g. **alfacalcidol** and **dihydrotachysterol** (Figure 5.107) are available for use where chronic vitamin D deficiency is due to liver or kidney malfunction. The long term use of calcitriol and alfacalcidol (1α-hydroxyvitamin D_3) in the treatment of osteoporosis may lead to toxic effects arising from elevated serum calcium levels. Toxicity is much reduced in the related 1α-hydroxyvitamin D_2, and this agent is being investigated further.

Vitamin D is also known to have other physiological functions, including a role in immune suppression, hormone secretion, and the differentiation of both normal and malignant cells.

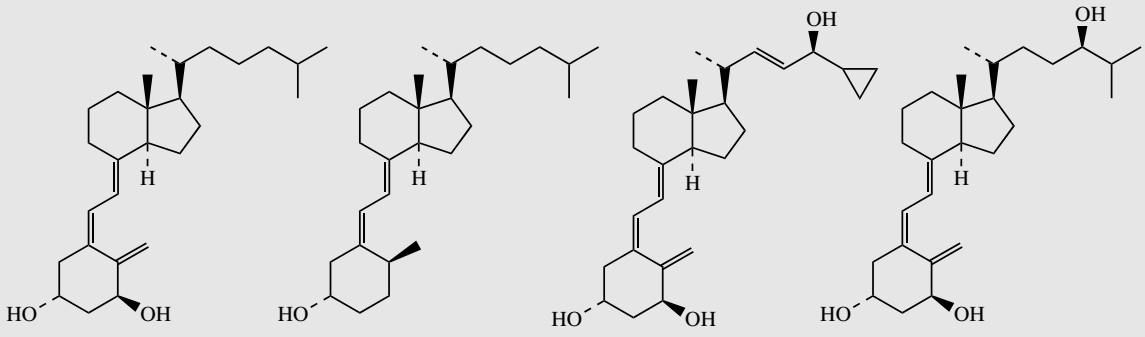


Figure 5.107

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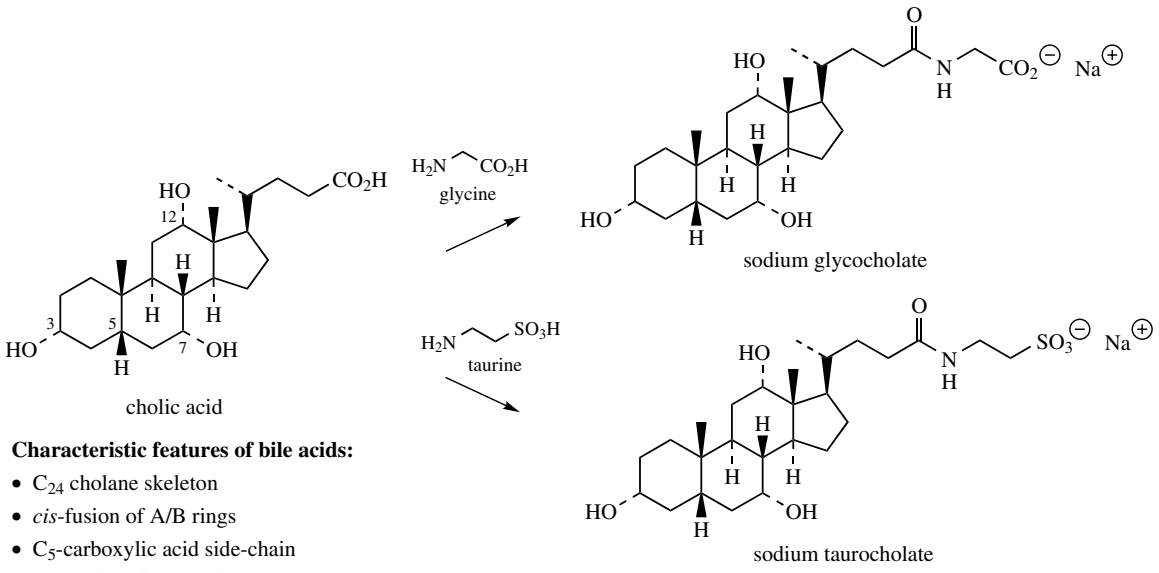
Two vitamin D derivatives, **calcipotriol** and **tacalcitol** (Figure 5.107) are widely used in the topical treatment of psoriasis, to inhibit the cell proliferation characteristic of this condition.

Vitamin D_2 is also employed as a rodenticide. High doses are toxic to rats and mice, the vitamin causing fatal hypercalcaemia.

Bile Acids

The **bile acids*** are C_{24} steroidal acids that occur in salt form in bile, secreted into the gut to emulsify fats and encourage digestion. They act as detergents by virtue of their relatively non-polar steroid nucleus and the polar side-chain, which contains a carboxylic acid group, that is typically bound via an amide linkage to glycine or taurine. Thus, for example, **cholic acid** (Figure 5.108) is found as **sodium glycocholate** and **sodium taurocholate**. Metabolism to bile acids is also the principal way in which mammals degrade cholesterol absorbed from the diet. These structures are formed in the liver from cholesterol by a sequence which oxidizes off three carbons from the side-chain (Figure 5.109). This is achieved by initial oxidation of one of the side-chain methyls to an acid, followed by a β-oxidation sequence as seen with fatty acids (see Figure 2.11), removing the three-carbon unit as propionyl-CoA. Other essential features of the molecule are introduced earlier. The A/B ring system is *cis*-fused, and this is achieved by reduction of a Δ^4 rather than a Δ^5 double bond (see page 241). Migration of the double bond is accomplished via the 3-ketone, and when this is reduced back to a hydroxyl the configuration at C-3 is changed to 3α. Both **cholic acid** and **chenodeoxycholic acid** (Figure 5.110) are formed in the liver, though the 7α-hydroxyl functions of these compounds can be removed by intestinal microflora, so that mammalian bile also contains **deoxycholic acid** and **lithocholic acid** (Figure 5.110). The bile salts are then usually reabsorbed and stored in the gall bladder, although they are also excreted as the body's main means of eliminating excess cholesterol. Inability to remove cholesterol by bile acid synthesis and excretion may contribute to atherosclerosis and gallstone disease; gallstones often contain more than 70% of cholesterol.



Characteristic features of bile acids:

- C_{24} cholane skeleton
- *cis*-fusion of A/B rings
- C_5-carboxylic acid side-chain
- 3α- and 7α-hydroxyls

Figure 5.108

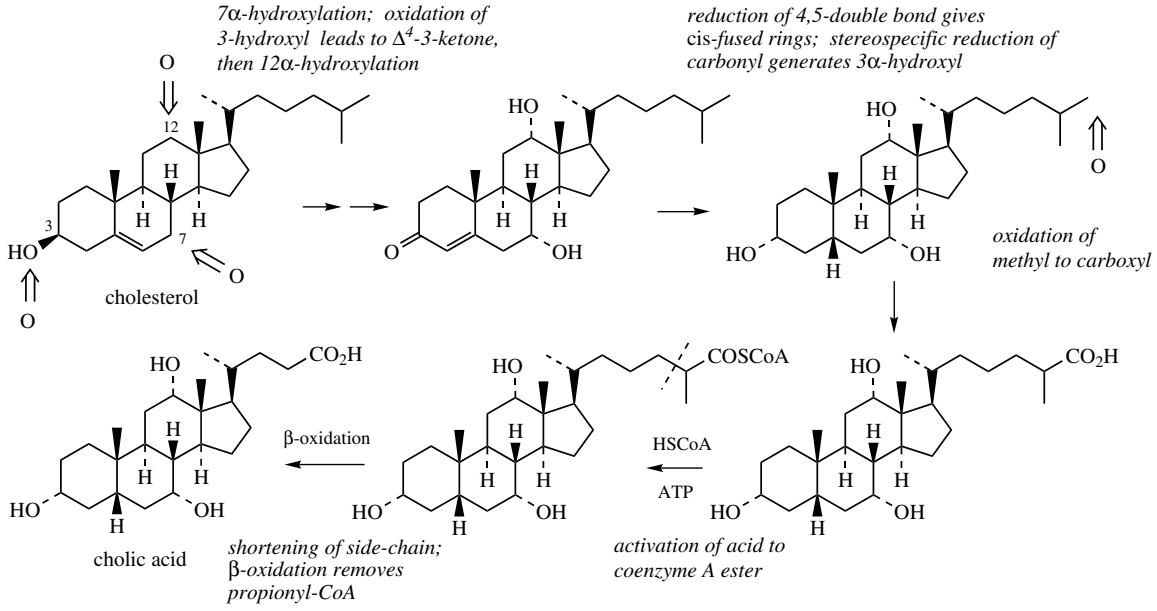


Figure 5.109

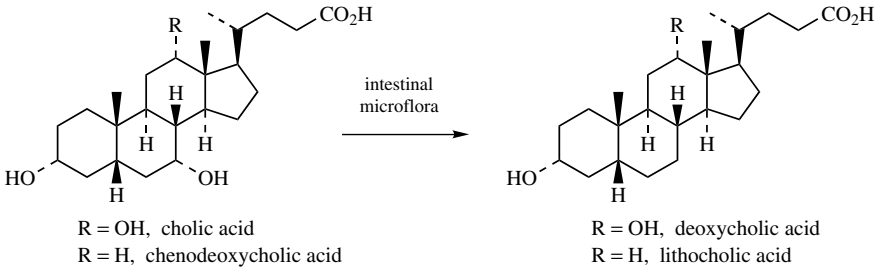


Figure 5.110

Bile Acids

Bile acids are obtained by purification from fresh ox bile taken from carcasses as a by-product of the meat trade. **Chenodeoxycholic acid** (Figure 5.110) and **ursodeoxycholic acid** (Figure 5.111) are used to dissolve cholesterol gallstones as an alternative to surgery. By suppressing synthesis of both cholesterol and cholic acid, they contribute to removal of biliary cholesterol and consequently a gradual dissolution of gallstones which may have formed due to supersaturation. Partial or complete dissolution requires treatment over a period of many months, and is not effective for radio-opaque gallstones, which contain appreciable levels of calcium salts. **Dehydrocholic acid** (Figure 5.111) may be used, after surgery, to improve biliary drainage. Anion-exchange resins such as **colestyramine** (**cholestyramine**) and **colestipol** are used as cholesterol-lowering drugs to bind bile acids and prevent their reabsorption. This promotes hepatic conversion of cholesterol into bile acids, thus increasing breakdown of low density lipoprotein cholesterol, and is of value in treating high risk coronary patients.

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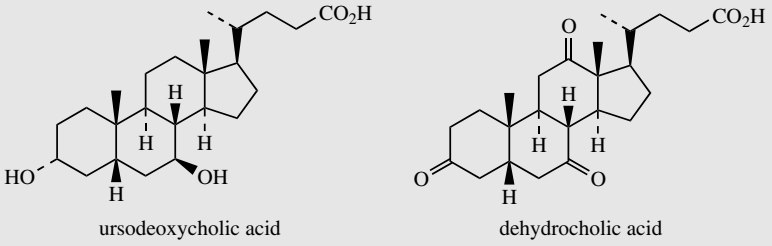


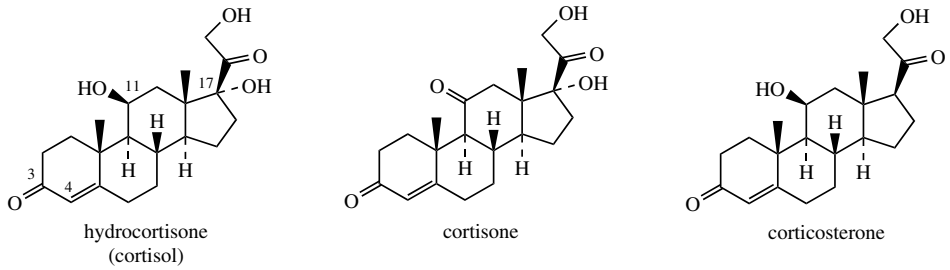
Figure 5.111

Bile acids are still important as starting materials for the semi-synthesis of other medicinal steroids, being a cheap and readily accessible raw material.

Adrenocortical Hormones/Corticosteroids

A large number of steroid hormones have been isolated and characterized from the adrenal glands. Since they are produced by the adrenal cortex, the outer part of the adrenal glands near the kidneys, they are termed **adrenocortical hormones** or **corticosteroids***. They contain a pregnane C_{21} skeleton and fall into two main activity groups, the **glucocorticoids** and the **mineralocorticoids**, although it is difficult to separate entirely the two types of activity in one molecule. Glucocorticoids are concerned with the synthesis of carbohydrate from protein, and deposition of glycogen in the liver. They also play an important role in inflammatory processes. Mineralocorticoids are concerned with the control of electrolyte balance, active compounds promoting the retention of Na^+ and Cl^-, and the excretion of K^+.

Examples of natural glucocorticoids include **hydrocortisone** (**cortisol**) and **corticosterone** (Figure 5.112), whilst **aldosterone** and **desoxycorticosterone** (**cortexone**) (Figure 5.113) typify mineralocorticoids. Desoxycorticosterone has also been found in plants. Some common features of these molecules are the β-$CO.CH_2OH$ side-chain at C-17, and frequently an α-hydroxy also at this position. Ring A usually contains a Δ^4-3-keto functionality. The 11β-hydroxy is essential for glucocorticoid activity. In aldosterone, the principal mineralocorticoid hormone, the methyl

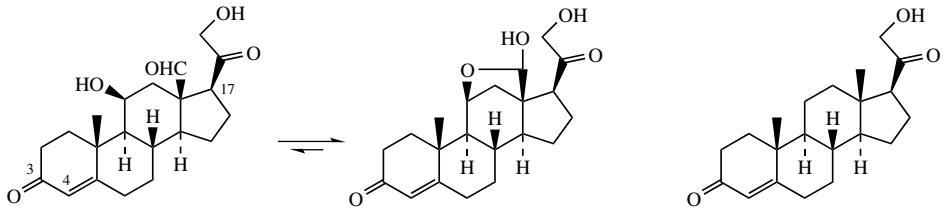


Characteristic features of glucocorticoids:
- C_{21} pregnane skeleton
- 17β-$CO.CH_2OH$ side-chain
- 11β-hydroxyl
- Δ^4-3-keto (usually)
- 17α-hydroxyl (usually)

Figure 5.112

group (C-18) has been oxidized to an aldehyde, and this is able to react with the 11β-hydroxyl, so that aldosterone exists predominantly in the hemiacetal form (Figure 5.113). This essentially eliminates the glucocorticoid activity.

The corticosteroids are produced from cholesterol via **pregnenolone** and **progesterone**. This involves side-chain cleavage as seen in the biosynthesis of cardioactive glycosides (see page 243) and the same sequence of reactions is operative. From progesterone, the formation of **desoxycorticosterone**, **corticosterone**, and **hydrocortisone** (**cortisol**) (Figure 5.114) requires only a series of hydroxylation steps, catalysed by cytochrome



Characteristic features of mineralocorticoids:

- C_{21} pregnane skeleton
- 17β-CO.CH_2OH side-chain
- Δ^4-3-keto (usually)

Figure 5.113

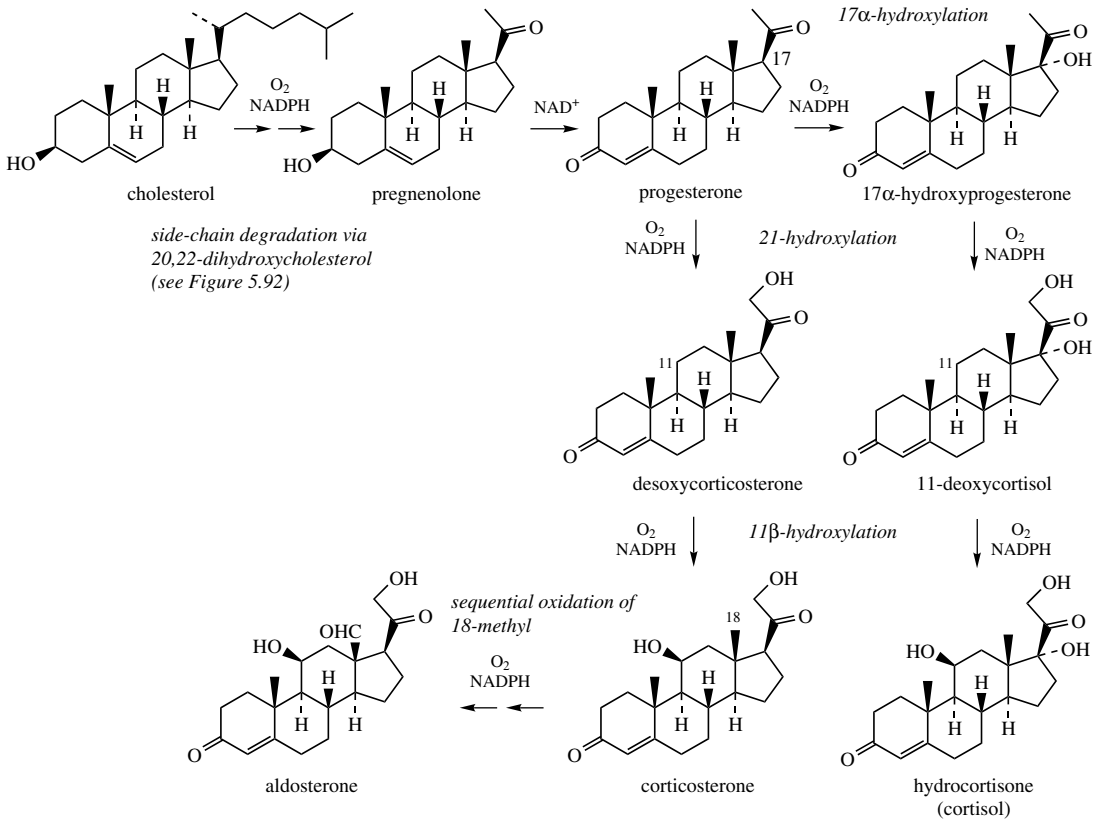


Figure 5.114

P-450-dependent hydroxylases with NADPH and O_2 cofactors. Thus, positions 17, 21 and 11 may be hydroxylated, and the exact order can in fact vary from that shown in Figure 5.114, according to species. It can be seen that production of hydrocortisone from cholesterol actually utilizes cytochrome P-450-dependent enzymes in four of the five steps. The further oxidation of C-18 to an aldehyde via the alcohol allows formation of **aldosterone** from corticosterone, again involving a P-450 system.

Semi-Synthesis of Corticosteroids

The medicinal use of corticosteroids was stimulated by reports of the dramatic effects of **cortisone** on patients suffering from rheumatoid arthritis in the late 1940s and early 1950s. The cortisone employed was isolated from the adrenal glands of cattle, and later was produced semi-synthetically by a laborious process from **deoxycholic acid** (see page 260) isolated from ox bile and necessitating over 30 chemical steps. Increased demand for cortisone and **hydrocortisone** (**cortisol**) (it had been shown that cortisone was reduced in the liver to hydrocortisone as the active agent) led to exploitation of alternative raw materials, particularly plant sterols and saponins. A major difficulty in any semi-synthetic conversion was the need to provide the 11β-hydroxyl group which was essential for glucocorticoid activity.

Sarmentogenin (Figure 5.115) had been identified as a natural 11-hydroxy cardenolide in *Strophanthus sarmentosus* but it was soon appreciated that the amounts present in the seeds, and the limited quantity of plant material available, would not allow commercial exploitation of this compound. As an alternative to using a natural 11-oxygenated substrate, compounds containing a 12-oxygen substituent might be used instead, in that this group activates position 11 and allows chemical modification at the adjacent site. Indeed, this was a feature of the semi-synthesis of cortisone from deoxycholic acid, which contains a 12α-hydroxyl. **Hecogenin** (Figure 5.115) from sisal (*Agave sisalana*; Agavaceae) (see page 240), a steroidal sapogenin with a 12-keto function, made possible the economic production of cortisone on a commercial scale. This material is still used in the semi-synthesis of steroidal drugs, and the critical

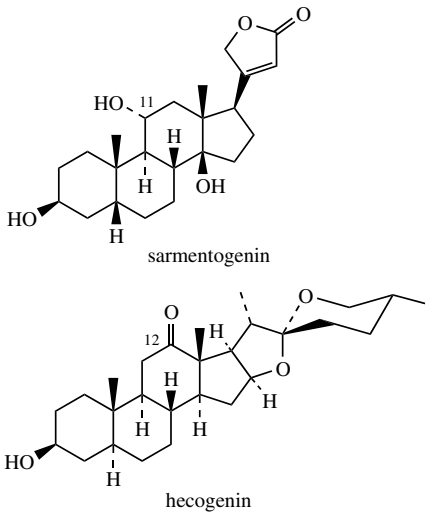


Figure 5.115

modifications in ring C are shown in Figure 5.116. Bromination α to the 12-keto function generates the 11α-bromo derivative, which on treatment with base gives the 12-hydroxy-11-ketone by a base-catalysed keto–enol tautomerism mechanism. The 12-hydroxyl is then removed by hydride displacement of the acetate using calcium in liquid ammonia. The new 11-keto sapogenin is subjected to the side-chain degradation used with other sapogenins, e.g. diosgenin (see Figure 5.119), to the 11-ketopregnane (Figure 5.117) which can then be used for conversion into cortisone, hydrocortisone, and other steroid drugs.

Of much greater importance was the discovery in the mid-1950s that hydroxylation at C-11 could be achieved via a microbial fermentation. **Progesterone** was transformed by *Rhizopus arrhizus* into **11α-hydroxyprogesterone** (Figure 5.118) in yields of up to 85%. More recently, *Rhizopus nigricans* has been employed, giving even higher yields. 11α-Hydroxyprogesterone is then converted into hydrocortisone by chemical means, the 11β configuration being introduced via oxidation to the 11-keto and then a stereospecific reduction step. Progesterone could be obtained in good yields (about 50%) from **diosgenin** extracted from Mexican yams (*Dioscorea* species; Dioscoreaceae) (see page 239) or **stigmasterol** from soya beans (*Glycine max*; Leguminosae/Fabaceae) (see page 256). Steroidal sapogenins such as diosgenin may be degraded by the **Marker degradation**

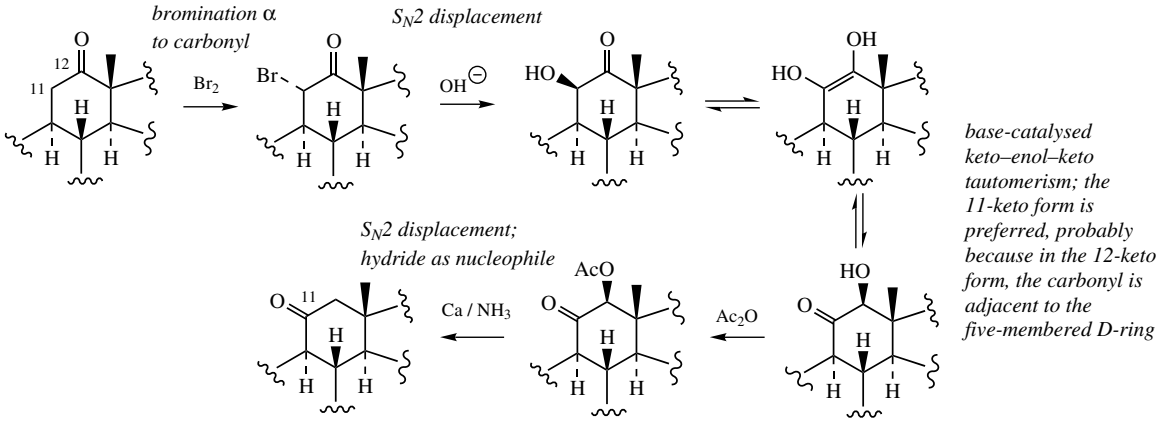


Figure 5.116

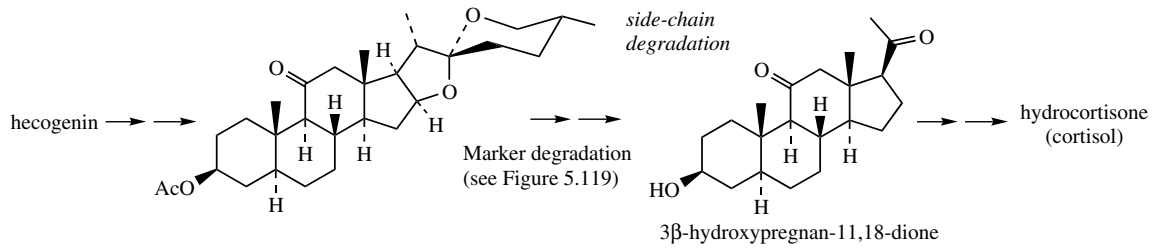


Figure 5.117

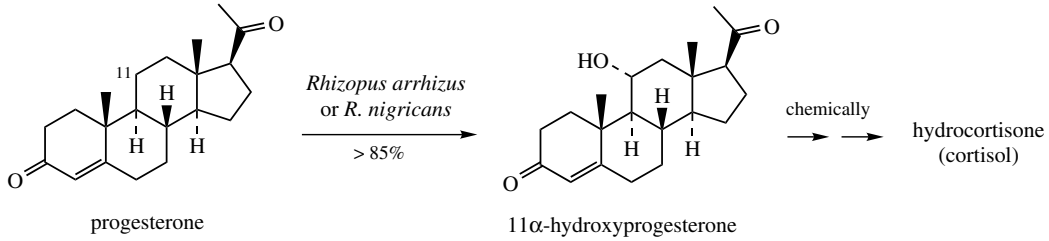


Figure 5.118

(Figure 5.119), which removes the spiroketal portion, leaving carbons C-20 and C-21 still attached to contribute to the pregnane system. Initial treatment with acetic anhydride produces the diacetate, by opening the ketal, dehydrating in ring E, and acetylating the remaining hydroxyls. The double bond in ring E is then selectively oxidized to give a product, which now contains the unwanted side-chain carbons as an ester function, easily removed by hydrolysis. Under the conditions used, the product is the α,β-unsaturated ketone. Hydrogenation of the double bond is achieved in a regioselective and stereoselective manner, addition of hydrogen being from the less-hindered α-face to give pregnenolone acetate. **Progesterone** is obtained by hydrolysis of the ester function and Oppenauer oxidation to give the preferred α,β-unsaturated ketone (see page 241). It is immediately obvious from Figure 5.119 that, since the objective is to remove the unwanted ring F part of the sapogenin, features like the stereochemistry at C-25 are irrelevant, and the same general degradation procedure can be used for other sapogenins. It is equally applicable to the nitrogen-containing analogues of

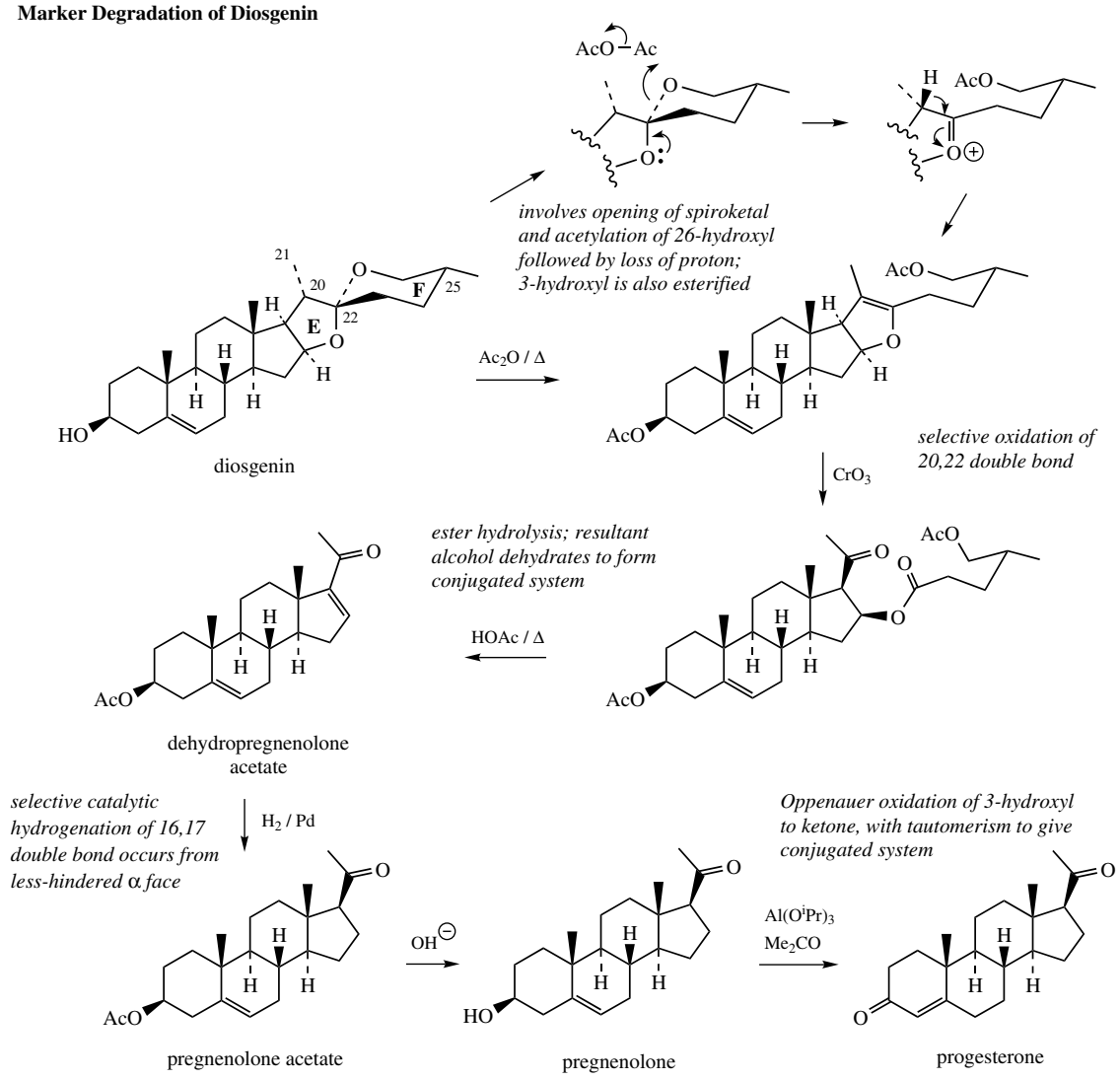


Figure 5.119

sapogenins, e.g. **solasodine** (Figure 5.88). In such compounds, the stereochemistry at C-22 is also quite immaterial.

Degradation of the sterol **stigmasterol** to progesterone is achieved by the sequence shown in Figure 5.120. The double bond in the side-chain allows cleavage by ozonolysis, and the resultant aldehyde is chain shortened via formation of an enamine with piperidine. This can be selectively oxidized to progesterone. In this sequence, the ring A transformations are carried out as the first reaction. A similar route can be used for the fungal sterol **ergosterol**, though an additional step is required for reduction of the Δ^7 double bond.

An alternative sequence from diosgenin to hydrocortisone has been devised, making use of another microbiological hydroxylation, this time a direct 11β-hydroxylation of the steroid ring system (Figure 5.121). The fungus *Curvularia lunata* is able to 11β-hydroxylate **cortexolone** to **hydrocortisone** in yields of about 60%.

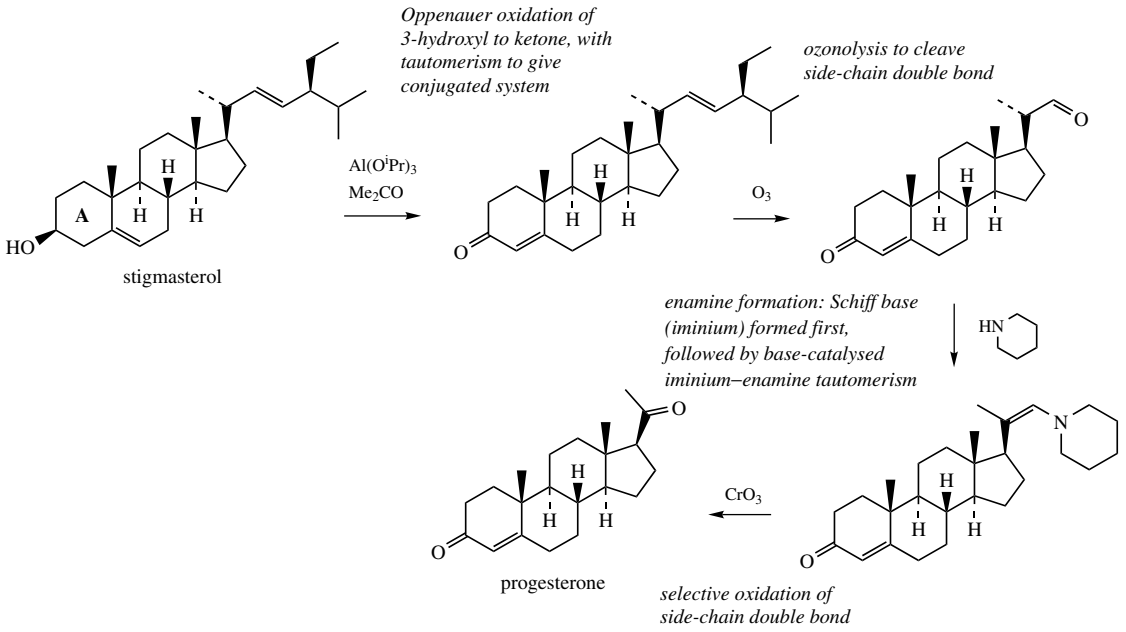


Figure 5.120

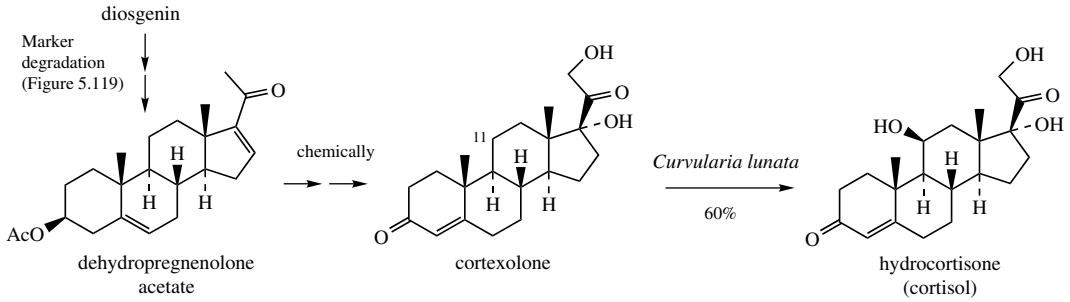
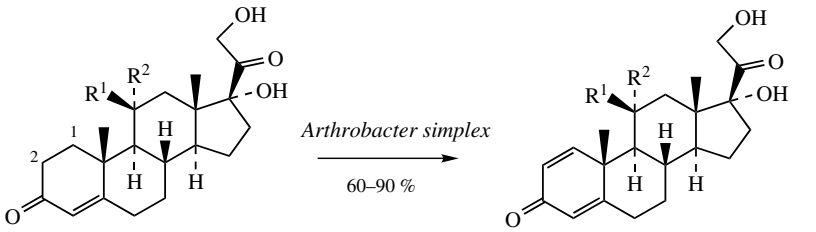


Figure 5.121

Although a natural corticosteroid, cortexolone may be obtained in large amounts by chemical transformation from 16-dehydropregnenolone acetate, an intermediate in the Marker degradation of diosgenin (Figure 5.119).

Some steroid drugs are produced by total synthesis, but, in general, the ability of microorganisms to biotransform steroid substrates has proved invaluable in exploiting inexpensive natural steroids as sources of drug materials. It is now possible via microbial fermentation to hydroxylate the steroid nucleus at virtually any position and with defined stereochemistry. These processes are in general more expensive than chemical transformations, and are only used commercially when some significant advantage is achieved, e.g. replacement of several chemical steps. The therapeutic properties of cortisone and hydrocortisone can be further improved by the microbial introduction of a 1,2-double bond, giving **prednisone** and **prednisolone** respectively (Figure 5.122). These agents surpass the parent hormones in antirheumatic and antiallergic activity with fewer side effects. As with cortisone, prednisone is converted in the body into the active agent, in this case prednisolone.



R^1R^2 = O, cortisone
R^1 = OH, R^2 = H, hydrocortisone (cortisol)

R^1R^2 = O, prednisone
R^1 = OH, R^2 = H, prednisolone

Figure 5.122

Corticosteroid Drugs

Glucocorticoids are primarily used for their antirheumatic and anti-inflammatory activities. They give valuable relief to sufferers of rheumatoid arthritis and osteoarthritis, and find considerable use for the treatment of inflammatory conditions by suppressing the characteristic development of swelling, redness, heat, and tenderness. They exert their action by interfering with prostaglandin biosynthesis, via production of a peptide that inhibits the phospholipase enzyme responsible for release of arachidonic acid from phospholipids (see page 55). However, these agents merely suppress symptoms and they do not provide a cure for the disease. Long term usage may result in serious side-effects, including adrenal suppression, osteoporosis, ulcers, fluid retention, and increased susceptibility to infections. Because of these problems, steroid drugs are rarely the first choice for inflammatory treatment, and other therapies are usually tried first. Nevertheless corticosteroids are widely used for inflammatory conditions affecting the ears, eyes, and skin, and in the treatment of burns. Some have valuable antiallergic properties helping in reducing the effects of hay fever and asthma. In some disease states, e.g. Addison's disease, the adrenal cortex is no longer able to produce these hormones, and replacement therapy becomes necessary. The most common genetic deficiency is lack of the 21-hydroxylase enzyme in the biosynthetic pathway, necessary for both hydrocortisone and aldosterone biosynthesis (Figure 5.114). This can then lead to increased synthesis of androgens (see Figure 5.133).

Mineralocorticoids are primarily of value in maintaining electrolyte balance where there is adrenal insufficiency.

Natural corticosteroid drugs **cortisone** (as **cortisone acetate**) and **hydrocortisone** (**cortisol**) (Figure 5.112) are valuable in replacement therapies, and hydrocortisone is one of the most widely used agents for topical application in the treatment of inflammatory skin conditions. The early use of natural corticosteroids for anti-inflammatory activity tended to show up some serious side-effects on water, mineral, carbohydrate, protein, and fat metabolism. In particular, the mineralocorticoid activity is usually considered an undesirable effect. In an effort to optimize anti-inflammatory activity, many thousands of chemical modifications to the basic structure were tried. Introduction of a Δ^1 double bond modifies the shape of ring A and was found to increase glucocorticoid over mineralocorticoid activity, e.g. **prednisone** and **prednisolone** (Figure 5.122). A 9α-fluoro substituent increased all activities, whereas 16α- or 16β-methyl groups reduced the mineralocorticoid activity without affecting

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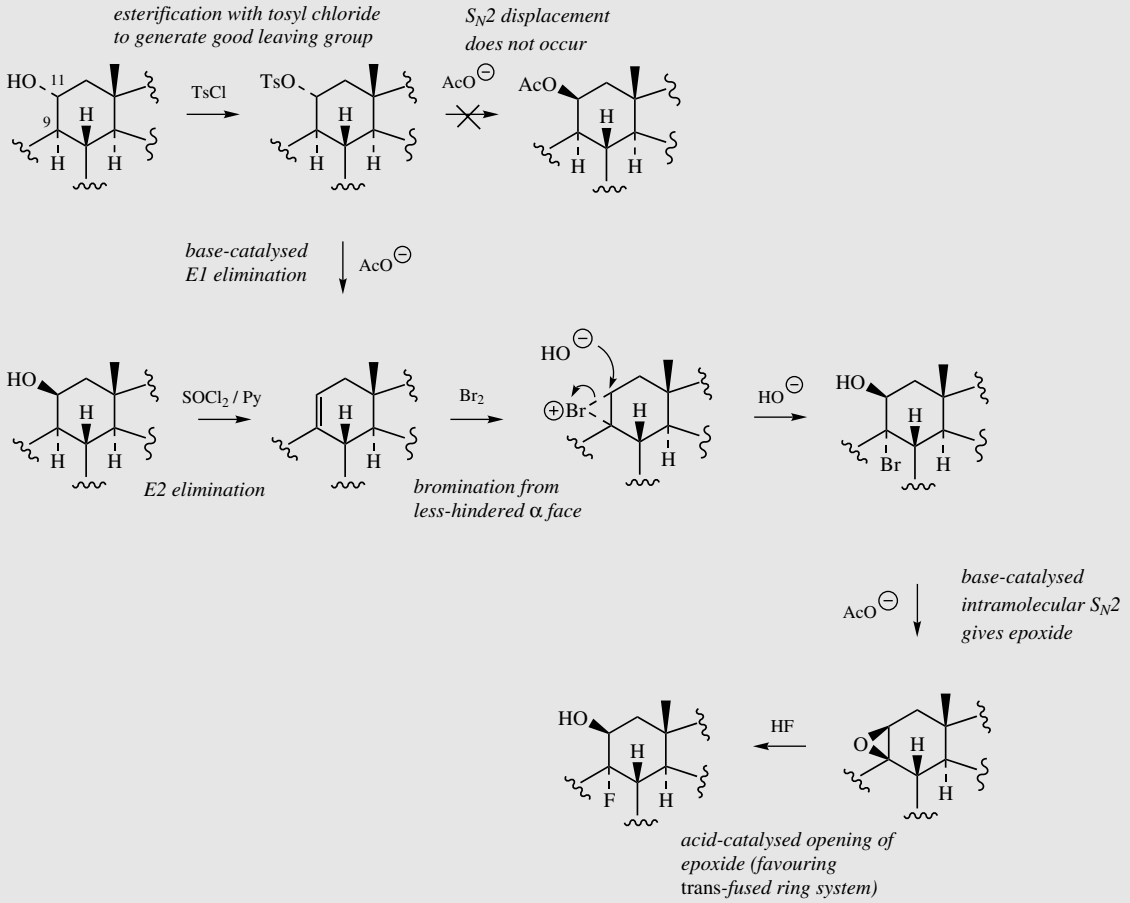

Figure 5.123

the glucocorticoid activity. The discovery that 9α-fluoro analogues had increased activity arose indirectly from attempts to epimerize 11α-hydroxy compounds into the active 11β-hydroxy derivatives (Figure 5.123). Thus, when an 11α-tosylate ester was treated with acetate, a base-catalysed elimination was observed rather than the hoped-for substitution, which is hindered by the methyl groups (Figure 5.123). This *syn* elimination suggests an E1 mechanism is involved. The same $\Delta^{9(11)}$-ene can also be obtained by dehydration of the 11β-alcohol by using thionyl chloride. Addition of HOBr to the 9(11)-double bond proceeds via electrophilic attack from the less-hindered α-face, giving the cyclic bromonium ion, and then ring opening by β-attack of hydroxide at C-11. Attack at C-9 is sterically hindered by the methyl at C-10. 9α-Bromocortisol 21-acetate produced in this way was less active as an anti-inflammatory than cortisol 21-acetate by a factor of three, and 9α-iodocortisol acetate was also less active by a factor of ten. Fluorine must be introduced indirectly by the β-epoxide formed by base treatment of the 9α-bromo-10β-hydroxy analogue (Figure 5.123). The resultant 9α-fluorocortisol 21-acetate (**flu**orohy**drocortisone** acetate; **fludrocortisone acetate**) (Figure 5.124) was found to be about 11 times more active than cortisol acetate. However, its mineralocorticoid activity was

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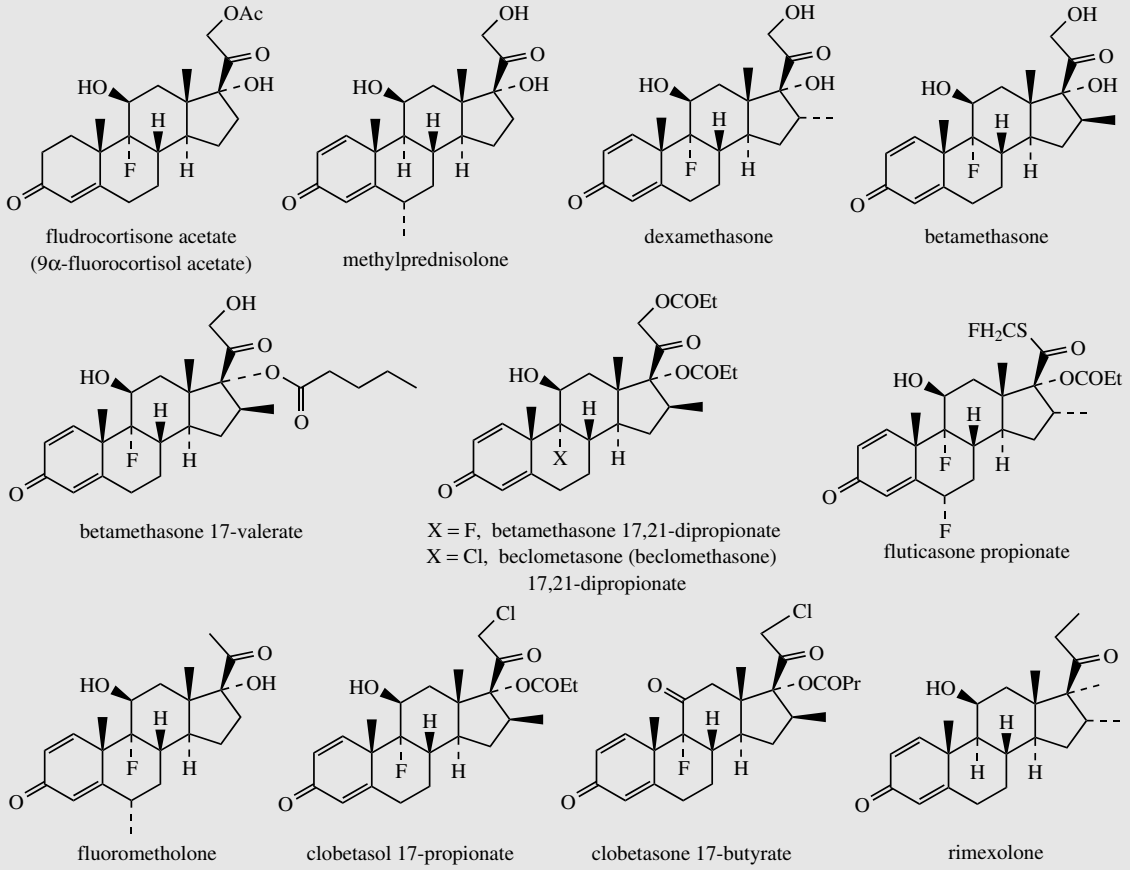

Figure 5.124

also increased some 300-fold, so its anti-inflammatory activity has no clinical relevance, and it is only employed for its mineralocorticoid activity. The introduction of a 9α-fluoro substituent into prednisolone causes powerful Na^+ retention. These effects can be reduced (though usually not eliminated entirely) by introducing a substituent at C-16, either a 16α-hydroxy or a 16α/16β-methyl. The 16α-hydroxyl can be introduced microbiologically, e.g. as in the conversion of 9α-fluoroprednisolone into **triamcinolone** (Figure 5.125). The ketal formed from triamcinolone and acetone, **triamcinolone acetonide** (Figure 5.125) provides a satisfactory means of administering this anti-inflammatory by topical application in the treatment of skin disorders such as psoriasis. **Methylprednisolone** (Figure 5.124) is a 6α-methyl derivative of prednisolone showing a modest increase in activity over the parent compound. A 6-methyl group can be supplied by reaction of the Grignard reagent MeMgBr with a suitable 5,6-epoxide derivative. **Dexamethasone** and **betamethasone** (Figure 5.124) exemplify respectively 16α- and 16β-methyl derivatives in drugs with little, if any, mineralocorticoid activity. The 16-methyl group is easily introduced by a similar Grignard reaction with an appropriate α,β-unsaturated Δ^{16}-20-ketone. Betamethasone, for topical application, is typically formulated as a C-17 ester with valeric acid (**betamethasone 17-valerate**), or as

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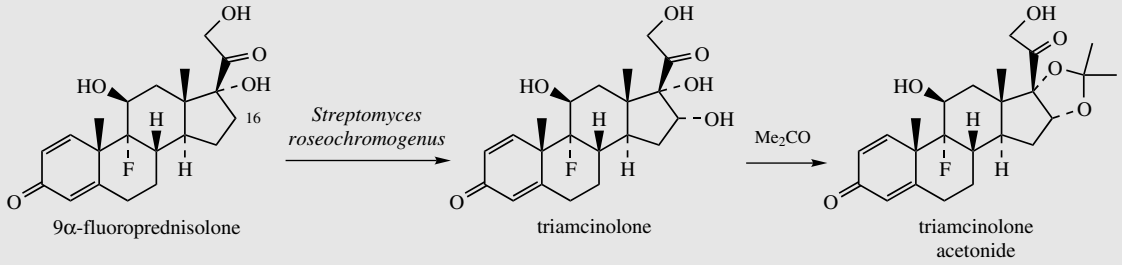

Figure 5.125

the 17,21-diester with propionic acid (**betamethasone 17,21-dipropionate**) (Figure 5.124). The 9α-chloro compound **beclometasone 17,21-dipropionate** (**beclomethasone 17,21-dipropionate**) is also an important topical agent for eczema and psoriasis, and as an inhalant for the control of asthma. **Fluticasone propionate** (Figure 5.124) is also used in asthma treatment, and is representative of compounds where the 17-side-chain has been modified to a carbothiate (sulphur ester).

Although the anti-inflammatory activity of hydrocortisone is lost if the 21-hydroxyl group is not present, considerable activity is restored when a 9α-fluoro substituent is introduced. **Fluorometholone** (Figure 5.124) is a corticosteroid that exploits this relationship and is of value in eye conditions. Other agents are derived by replacing the 21-hydroxyl with a halogen, e.g. **clobetasol 17-propionate** and **clobetasone 17-butyrate** (Figure 5.124), which are effective topical drugs for severe skin disorders. In **rixemolone**, a recently introduced anti-inflammatory for ophthalmic use, neither a 21-hydroxy nor a 9α-fluoro substituent is present, but instead there are 17α- and 16α-methyl substituents. Rimexolone has significant advantages in eye conditions over drugs such as dexamethasone, in that it does not significantly raise intraocular pressure.

Many other corticosteroids are currently available for drug use. Structures of some of these are given in Figure 5.126, grouped according to the most characteristic structural features, namely 16-methyl, 16-hydroxy, and 21-chloro derivatives. The recently introduced **deflazacort** (Figure 5.127) is a drug with high glucocorticoid activity, but does not conveniently fit into any of these groups in that it contains an oxazole ring spanning C-16 and C-17.

Trilostane (Figure 5.127) is an adrenocortical suppressant, which inhibits synthesis of glucocorticoids and mineralocorticoids and has value in treating Cushing's syndrome, characterized by a moon-shaped face and caused by excessive glucocorticoids. This drug is an inhibitor of the dehydrogenase–isomerase that transforms pregnenolone into progesterone (Figures 5.92 and 5.114).

Spironolactone (Figure 5.127) is an antagonist of the endogenous mineralocorticoid aldosterone and inhibits the sodium-retaining action of aldosterone whilst also decreasing the potassium-secreting effect. Classified as a potassium-sparing diuretic, it is employed in combination with other diuretic drugs to prevent excessive potassium loss. Progesterone (page 273) is also an aldosterone antagonist; the spironolactone structure differs from progesterone in its 7α-thioester substituent, and replacement of the 17β side-chain with a 17α-spirolactone.

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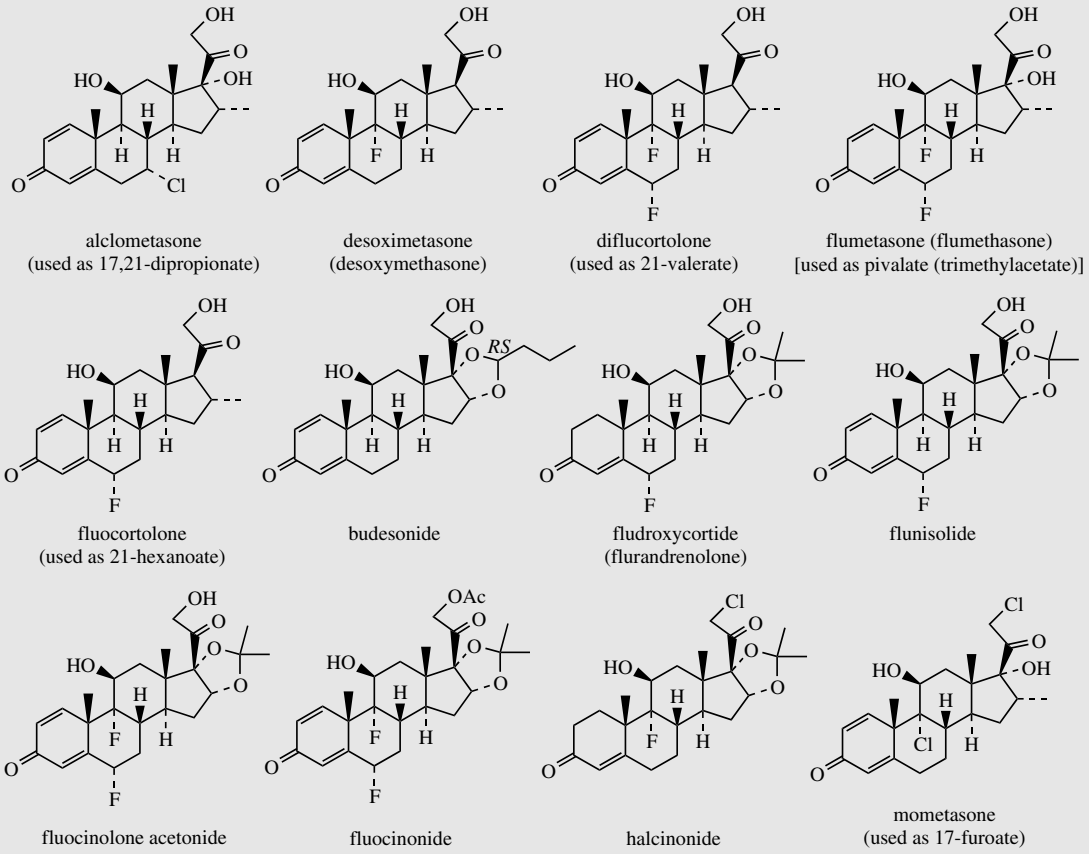


Figure 5.126

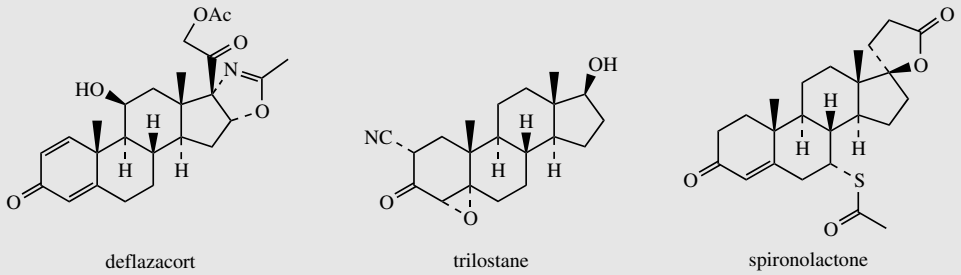
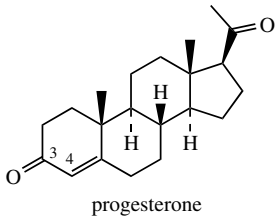


Figure 5.127

Progestogens

Progestogens* (progestins; gestogens) are female sex hormones, concerned with preparing the uterus for pregnancy, and then maintaining the necessary conditions. There is only one naturally occurring progestational steroid and that is **progesterone** (Figure 5.128), which is secreted by the corpus luteum following release of an ovum. Progesterone is also an intermediate in the biosynthesis of the corticosteroids, e.g. hydrocortisone and aldosterone (see page 263), and its derivation from cholesterol via pregnenolone has already been seen in the formation of cardioactive glycosides (see page 243).



progesterone

Characteristic features of progestogens:

- C_{21} pregnane skeleton
- Δ^4-3-keto

Figure 5.128

Oestrogens

The **oestrogens** (US spelling: **estrogens**) are female sex hormones produced in the ovaries, and also in the placenta during pregnancy. They are responsible for the female sex characteristics, and together with progesterone control the menstrual cycle. Oestrogens were first isolated from the urine of pregnant women, in which levels increase some 50-fold during the pregnancy. In horses, levels rise by as much as 500 times during pregancy. Oestrogens occur both in free form, and as glucuronides

Progestogen Drugs

Quantities of **progesterone** (Figure 5.128) for drug use are readily available by semi-synthesis using the Marker degradation (see page 264). However, progesterone is poorly absorbed, and it is not suitable for oral use, being rapidly metabolized in the liver. Many semi-synthetic analogues have been produced, and it was thus appreciated that the α,β-unsaturated ketone system in ring A was essential for activity. The side-chain function at C-17 could be modified, and ethisterone (17α-ethynyltestosterone) (Figure 5.129), developed as a potential androgen, was found to be active orally as a progestational agent. This incorporates an ethynyl side-chain at C-17, a feature of several semi-synthetic steroidal hormones used as drugs. This group, referred to as 'ethinyl' in drug molecules, is introduced by nucleophilic

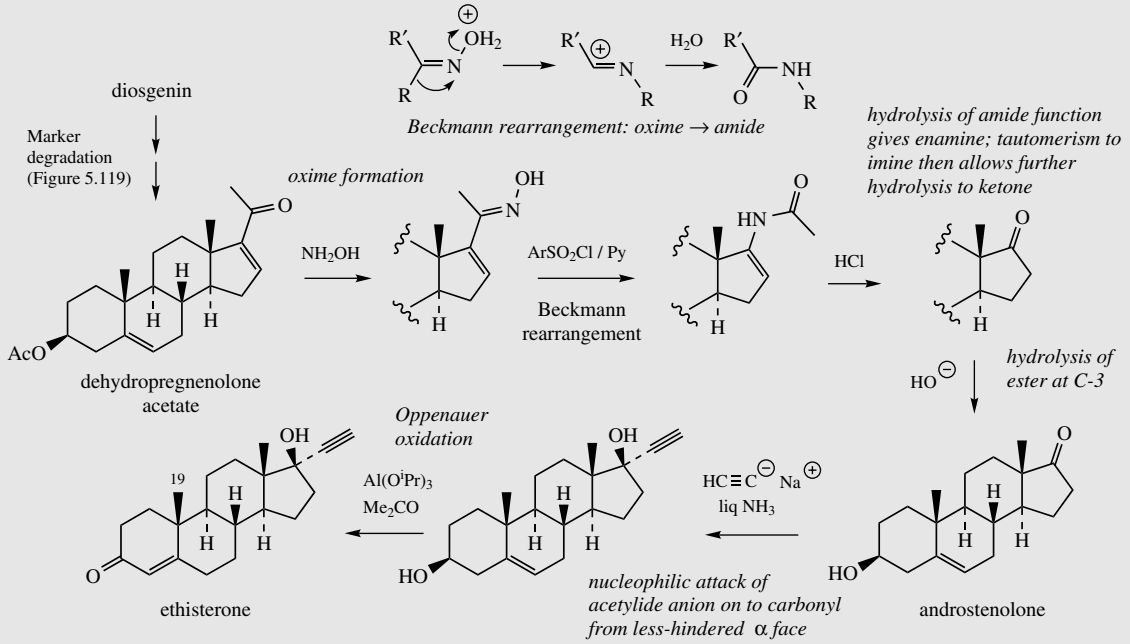


Figure 5.129

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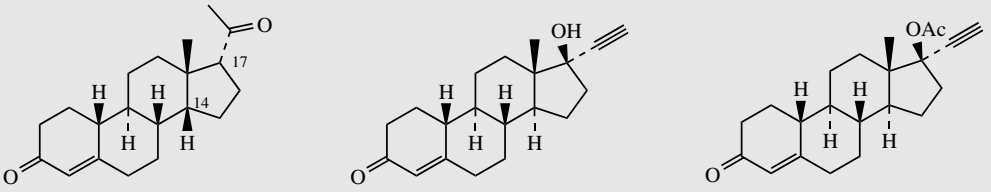

Figure 5.130

attack of acetylide anion on to a C-17 carbonyl (Figure 5.129), attack coming from the α-face, the methyl C-18 hindering approach from the β-face. The substrate androstenolone is readily obtained from the Marker degradation intermediate dehydropregnenolone acetate (Figure 5.119). The oxime (Figure 5.129) is treated with a sulphonyl chloride in pyridine and undergoes a Beckmann rearrangement in which C-17 migrates to the nitrogen giving the amide. This amide is also an enamine and can be hydrolysed to the 17-ketone. Acetylation or other esterification of the 17-hydroxyl in progestogens increases lipid solubility and extends the duration of action by inhibiting metabolic degradation. Examples include norethisterone acetate, medroxyprogesterone acetate, and hydroxyprogesterone caproate, discussed below.

Though considerably better than progesterone, the oral activity of ethisterone is still relatively low, and better agents were required. An important modification from ethisterone was the 19-*nor* analogue, **norethisterone** and its ester **norethisterone acetate** (Figure 5.130). Attention was directed to the 19-norsteroids by the observation that 19-nor-14β,17α-progesterone (Figure 5.130), obtained by degradation of the cardioactive glycoside strophanthidin (see page 250), displayed eight times higher progestational activity than progesterone, despite lacking the methyl C-19, and having the unnatural configurations at the two centres C-14 (C/D rings *cis*-fused) and C-17. Norethisterone can be synthesized from the oestrogen estrone (see page 279) which already lacks the C-9 methyl, or from androstenolone (Figure 5.129) by a sequence which allows oxidation of C-19 to a carboxyl, which is readily lost by decarboxylation when adjacent to the α,β-unsaturated ketone system.

Although ethisterone and norethisterone are structurally C_{21} pregnane derivatives, they may also be regarded as 17-ethynyl derivatives of testosterone (see page 282), the male sex hormone, and 19-nortestosterone respectively. Many of the commonly used progestogens fall into these two classes. Semi-synthetic analogues of progesterone, still containing the 17-acetyl side-chain, tend to be derivatives of 17α-hydroxyprogesterone, another biosynthetic intermediate on the way to hydrocortisone (Figure 5.114) that also has progesterone-like activity. Examples include **hydroxyprogesterone caproate**, and **gestonorone** (**gestronol**) **caproate** (Figure 5.131). **Medroxyprogesterone acetate** (Figure 5.131) contains an additional 6α-methyl, introduced to block potential deactivation by metabolic hydroxylation, and is 100–300 times as potent as ethisterone on oral administration. **Megestrol acetate** (Figure 5.131) contains a 6-methyl group and an additional Δ^6 double bond. **Norgestrel** (Figure 5.131) is representative of progestogens with an ethyl group replacing the 13-methyl. Although these can be obtained by semi-synthesis from natural 13-methyl compounds, norgestrel is produced by total synthesis as the racemic compound.

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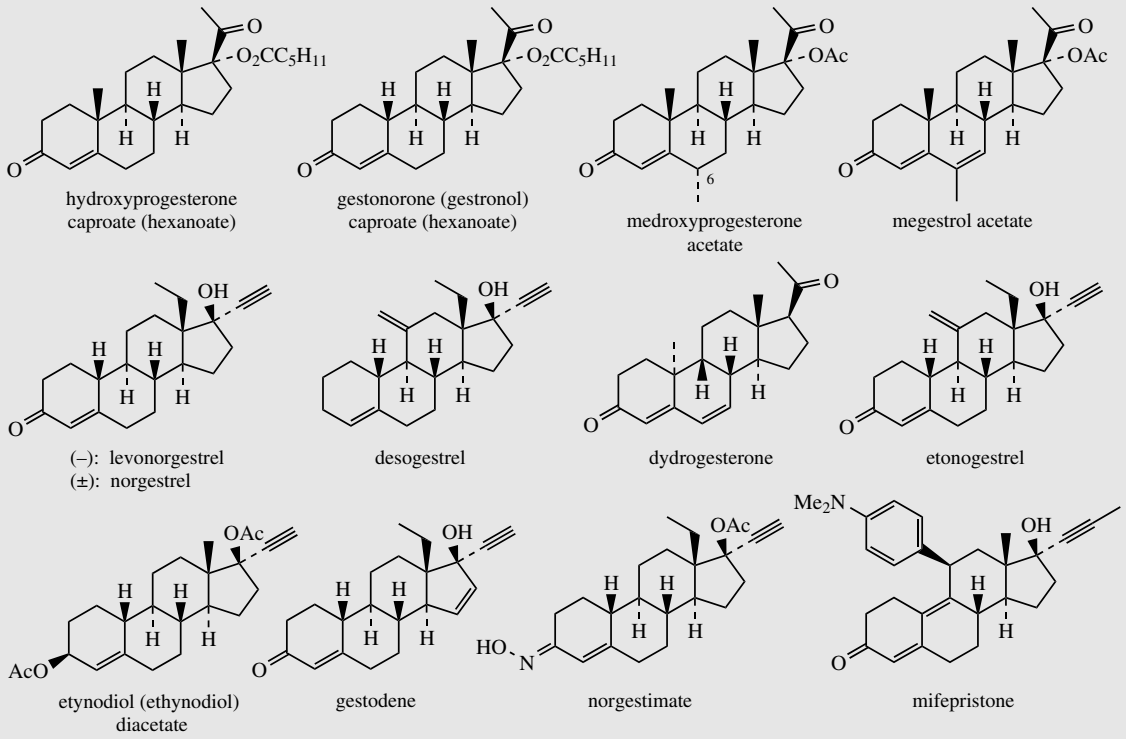

Figure 5.131

Since only the laevorotatory enantiomer which has the natural configuration is biologically active, this enantiomer, **levonorgestrel**, is now replacing the racemic form for drug use. In **desogestrel** (Figure 5.131), further features are the modification of an 11-oxo function to an 11-methylene, and removal of the 3-ketone. Structures of some other currently available progestogen drugs are shown in Figure 5.131.

During pregnancy, the corpus luteum continues to secrete progesterone for the first three months, after which the placenta becomes the supplier of both progesterone and oestrogen. Progesterone prevents further ovulation and relaxes the uterus to prevent the fertilized egg being dislodged. In the absence of pregnancy, a decline in progesterone levels results in shedding of the uterine endometrium and menstruation. Progestogens are useful in many menstrual disorders, and as **oral contraceptives** either alone at low dosage (progestogen-only contraceptives, e.g. norethisterone, levonorgestrel) or in combination with oestrogens (combined oral contraceptives, e.g. ethinylestradiol + norethisterone, ethinylestradiol + levonorgestrel). The combined oestrogen–progestogen preparation inhibits ovulation, but normal menstruation occurs when the drug is withdrawn for several days each month. The low dosage progestogen-only pill appears to interfere with the endometrial lining to inhibit fertilized egg implantation, and thickens cervical mucus making a barrier to sperm movement. The progestogen-only formulation is less likely to cause thrombosis, a serious side-effect sometimes experienced from the use of oral contraceptives. There appears to be a slightly higher risk of thrombosis in patients using the so-called 'third generation' oral

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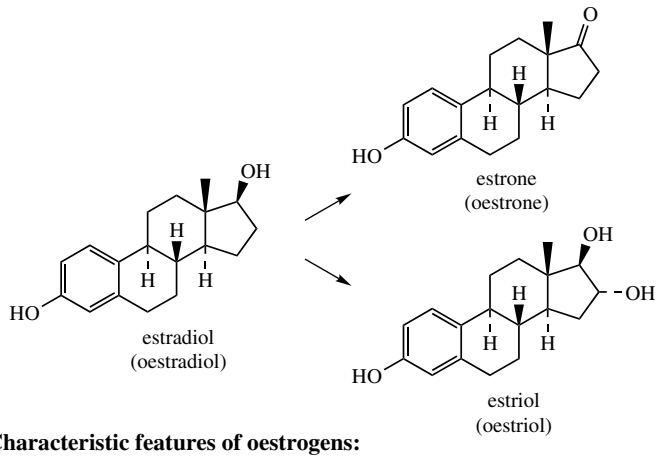
contraceptive pills containing the newer progestogens **desogestrel** and **gestodene**. Current oral contraceptives have a much lower hormone content than the early formulations of the 1960s and 1970s, typically about 10% of the progestogen and 50% of the oestrogen content. Deep muscular injections of medroxyprogesterone or norethisterone esters, and implants of levonorgestrel can be administered to provide long-acting contraception. A high dose of levonorgestrel, alone or in combination with ethinylestradiol, is the drug of choice for emergency contraception after unprotected intercourse, i.e. the 'morning-after' pill. Hormone replacement therapy (HRT) in non-hysterectomized women also uses progestogen–oestrogen combinations (see page 279), whilst progestogens such as norethisterone, megestrol acetate, medroxyprogesterone acetate, and gestonorone caproate also find application in the treatment of breast cancers.

Mifepristone (Figure 5.131) is a progestogen antagonist used orally as an abortifacient to terminate pregnancy. This drug has a higher affinity for the progesterone receptor than does the natural hormone, and prevents normal responses. This leads to loss of integrity of the uterine endometrial lining, and detachment of the implanted fertilized egg.

at position 3; they are not restricted to females since small amounts are produced in the male testis. The principal and most potent example is **estradiol** (also **oestradiol**, but US spelling has been generally adopted), though only low levels are found in urine, and larger amounts of the less active metabolites **estrone** (**oestrone**) and the 16α-hydroxylated derivative **estriol** (**oestriol**) are present (Figure 5.132). Estrone has also been found in significant quantities in some plant seeds, e.g. pomegranate and date palm. These compounds have an aromatic A ring, a consequence of which is that C-19, the methyl on C-10, is absent. There is now no carbon side-chain at C-17, and the basic C_{18} skeleton is termed estrane.

The biosynthetic pathway to estradiol and estrone (Figure 5.133) proceeds from cholesterol via pregnenolone and bears a resemblance to the hydrocortisone pathway (Figure 5.114) in the early 17-hydroxylation step. Indeed, the same cytochrome P-450-dependent enzyme catalyses 17-hydroxylation of both pregnenolone and



Characteristic features of oestrogens:

- C_{18} estrane skeleton
- aromatic A ring (consequent loss of C-10 methyl)
- no side-chain

Figure 5.132

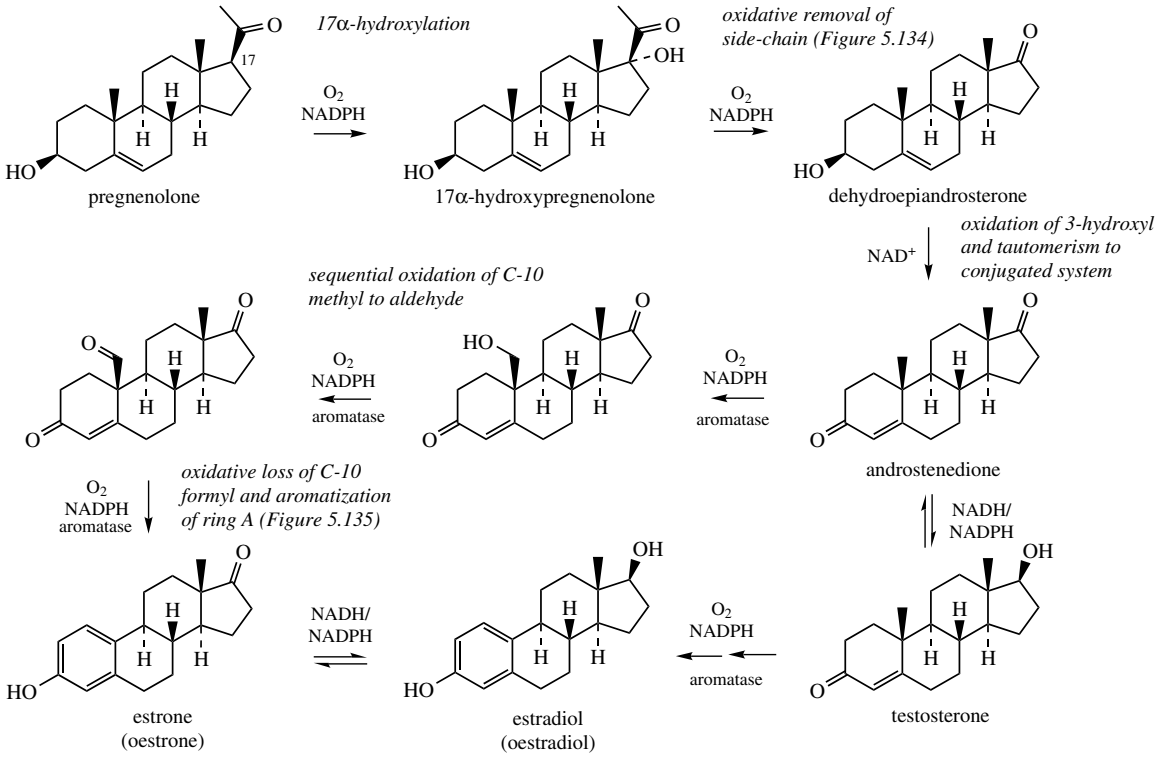


Figure 5.133

progesterone, as well as the next step in oestrogen biosynthesis, and it plays a significant role in controlling the direction of steroid synthesis. Whilst 17α-hydroxyprogesterone is transformed by 21-hydroxylation for hydrocortisone biosynthesis, in oestrogen biosynthesis the α-hydroxyketone function is oxidized from 17α-hydroxypregnenolone, cleaving off the two-carbon side-chain as acetic acid. The product is the 17-ketone **dehydroepiandrosterone**, which is the most abundant steroid in the blood of young adult humans, with levels peaking at about 20 years of age, then declining as the person ages. Apart from its role as a precursor of hormones, it presumably has other physiological functions, though these still remain to be clarified. A mechanism for the side-chain cleavage reaction, initiated by attack of an enzyme-linked peroxide, is shown in Figure 5.134, and is analogous to that proposed for loss of the 14-methyl group during cholesterol biosynthesis (see page 235). Oxidation and tautomerism in rings A/B then give **androstenedione** (Figure 5.133). Either androstenedione, or its reduction product **testosterone**, is a substrate for aromatization in ring A, with loss of C-19. This sequence is also catalysed by a single cytochrome P-450-dependent enzyme, called **aromatase**, and the reaction proceeds via sequential oxidation of the methyl, with its final elimination as formic acid (Figure 5.135). The mechanism suggested is analogous to that of the side-chain cleavage reaction. Formation of the aromatic ring is then a result of enolization. As with other steroid hormones, the exact order of some of the steps, including formation of the Δ^4-3-keto function, 17-hydroxylation, reduction of the 17-keto, and aromatization in ring A, can vary according to organism, or site of synthesis in the body. Since breast tumours require oestrogens for growth, the design of **aromatase inhibitors*** has become an important target for anticancer drug research.

The aromatic ring makes the oestrogen molecule almost planar (see page 233) and is essential for activity. Changes which remove the aromaticity, e.g. partial reduction, or alter stereochemistry, give analogues with reduced or no activity.

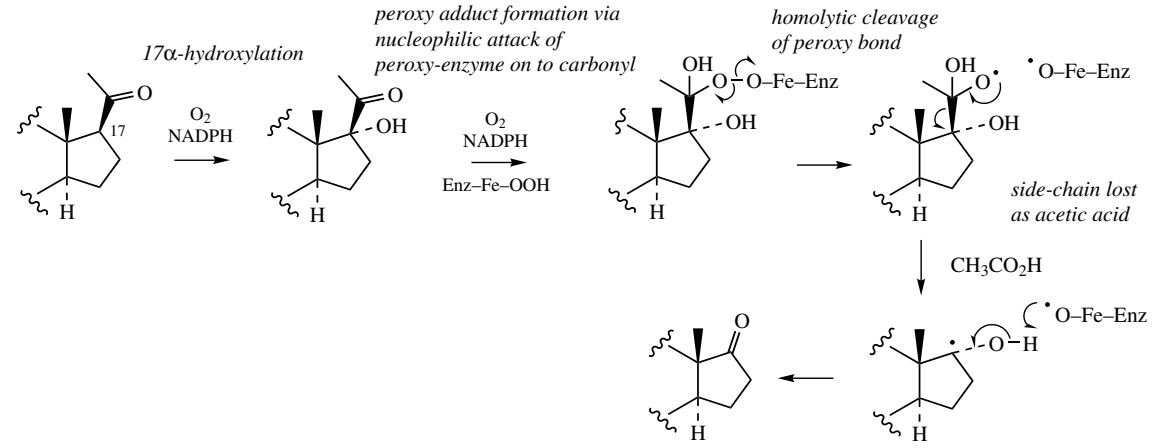


Figure 5.134

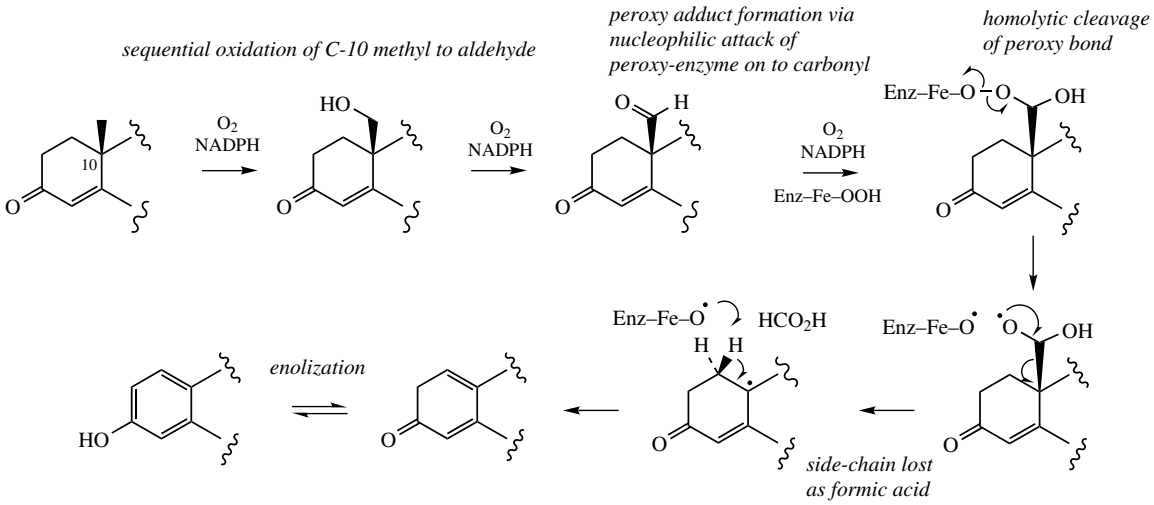


Figure 5.135

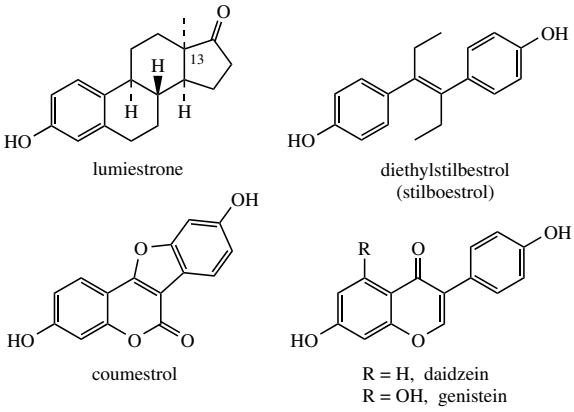


Figure 5.136

Thus, exposure of estrone to UV light leads to inversion of configuration at C-13 adjacent to the carbonyl function, and consequently formation of a *cis*-fused C/D ring system. The product, **lumiestrone** (Figure 5.136) is no longer biologically active. Some planar non-steroidal structures can also demonstrate oestrogenic activity as a result of a similar shape and relative spacing of oxygen functions. Thus, the synthetic **diethylstilbestrol** (**stilboestrol**) (Figure 5.136) has been widely used as an oestrogenic drug, and **coumestrol**, **daidzein**, and **genistein** (Figure 5.136) are naturally occurring isoflavonoids with oestrogenic properties from lucerne, clovers, and soya beans, and are termed

Oestrogen Drugs

Oestrogens suppress ovulation and with progestogens form the basis of combined oral contraceptives and hormone replacement therapy (HRT). They are also used to supplement natural oestrogen levels where these are insufficient as in some menstrual disorders, and to suppress androgen formation and thus tumour growth of cancers dependent on androgens, e.g. prostate cancers. Oestrogens appear to offer a number of beneficial effects to women, including protection against osteoporosis, heart attacks, and possibly Alzheimer's disease. However, some cancers, e.g. breast and uterine cancers, are dependent on a supply of oestrogen for growth, especially during the early stages, so high oestrogen levels are detrimental. Steroidal oestrogens for drug use were originally obtained by processing pregnancy urines, but the dramatic increase in demand due to the introduction of oral contraceptives required development of semi-synthetic procedures. Androstenolone formed via the Marker degradation of diosgenin (Figure 5.129) may be transformed to the dione by catalytic reduction of the Δ^5 double bond and oxidation of the 3-hydroxyl (Figure 5.137). This then allows production of androstadienedione by dibromination and base-catalysed elimination of HBr. Alternatively, it is now possible to achieve the synthesis of androstadienedione in a single step by a microbiological fermentation of either sitosterol obtained from soya beans (see page 256), or of cholesterol obtained in large quantities from the woolfat of sheep, or from the spinal cord of cattle (see page 236). These materials lack unsaturation in the side-chain and were not amenable to simple chemical oxidation processes, as for example with stigmasterol (see page 266). Their exploitation required the development of suitable biotransformations, and use of *Mycobacterium phlei* has now achieved this objective (Figure 5.137). The aromatization step to estrone can be carried out in low yields by vapour-phase free-radical-initiated thermolysis, or more recently with considerably better yields using a dissolving-metal reductive thermolysis. In both processes, the methyl at C-10 is lost. This sequence gives estrone, from which **estradiol** (**oestradiol**) may be obtained by reduction of the 17-carbonyl. However, by far the most commonly used medicinal estrogen is **ethinylestradiol** (**ethinyloestradiol**) (Figure 5.137), which is 12 times as effective as estradiol when administered orally. This analogue can be synthesized from estrone by treatment with potassium acetylide in liquid ammonia, which attacks from the less-hindered α-face (see page 273). The ethynyl substituent prevents oxidation at C-17, as in the metabolism of estradiol to the less active estrone. The phenol group allows synthesis of other derivatives, e.g. the 3-methyl ether **mestranol** (Figure 5.137), which acts as a pro-drug, being oxidized in the liver to ethinylestradiol. To retain oestrogenic activity, structural modifications appear effectively limited to the addition of the 17α-ethynyl group, and to substituents on the 3-hydroxyl. The ester **estradiol valerate** (**oestradiol valerate**) facilitates prolonged action through slower absorption and metabolism.

The lower activity metabolites **estriol** (**oestriol**) (about 2% activity of estradiol) and **estrone** (**oestrone**) (about 33% activity) (Figure 5.132) are sometimes used in **hormone replacement therapy** (HRT). Oestrogen and progesterone levels decline naturally at menopause when the menstrual cycle ceases. The sudden reduction in oestrogen levels can lead to a number of unpleasant symptoms, including tiredness, hot flushes, vaginal dryness, and mood changes. HRT reduces these symptoms, and delays other long-term consequences of reduced oestrogen levels, including osteoporosis and atherosclerosis. HRT currently provides the best therapy for preventing osteoporosis, a common disease in post-menopausal women.

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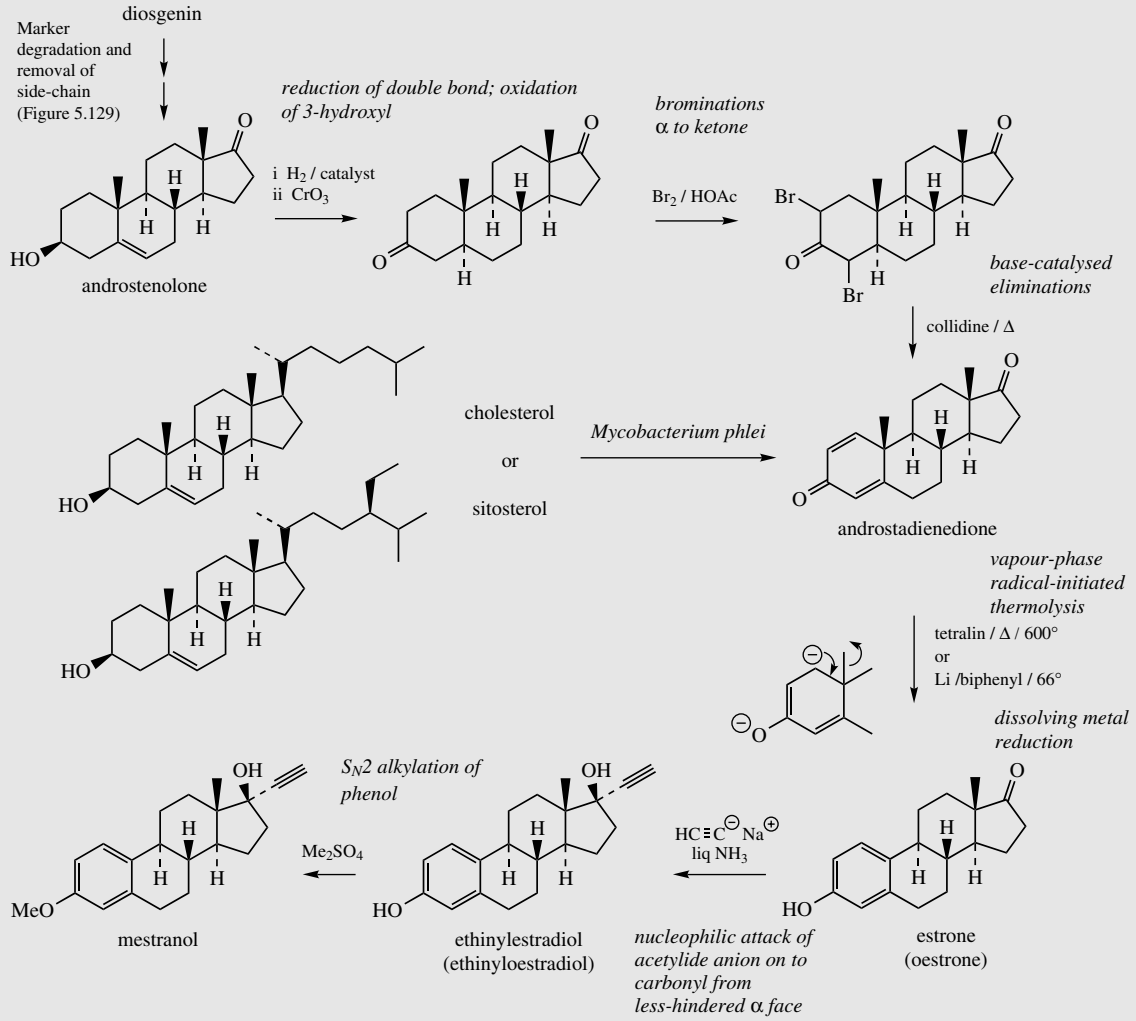

Figure 5.137

Osteoporosis is characterized by a generalized loss of bone mass leading to increased risk of fracture, and a sharp reduction in endogenous oestrogen levels is recognized as a critical factor. Oestrogen and progestogen combinations are used in HRT unless the woman has had a hysterectomy, in which case oestrogen alone is prescribed. Natural oestrogen structures are preferred to synthetic structures such as ethinylestradiol or mestranol. Before the availability of plant-derived semi-synthetic oestrogens, extraction of urine from pregnant women and pregnant horses allowed production of oestrogen mixtures for drug use. **Conjugated equine oestrogens** are still widely prescribed for HRT and are obtained by extraction from the urine of pregnant mares and subsequent purification, predominantly in Canada and the USA. Mares are about 120 days pregnant when urine collection begins, and collection continues for another 150–160 days. During this period, a mare will produce about 400–500 litres of urine.

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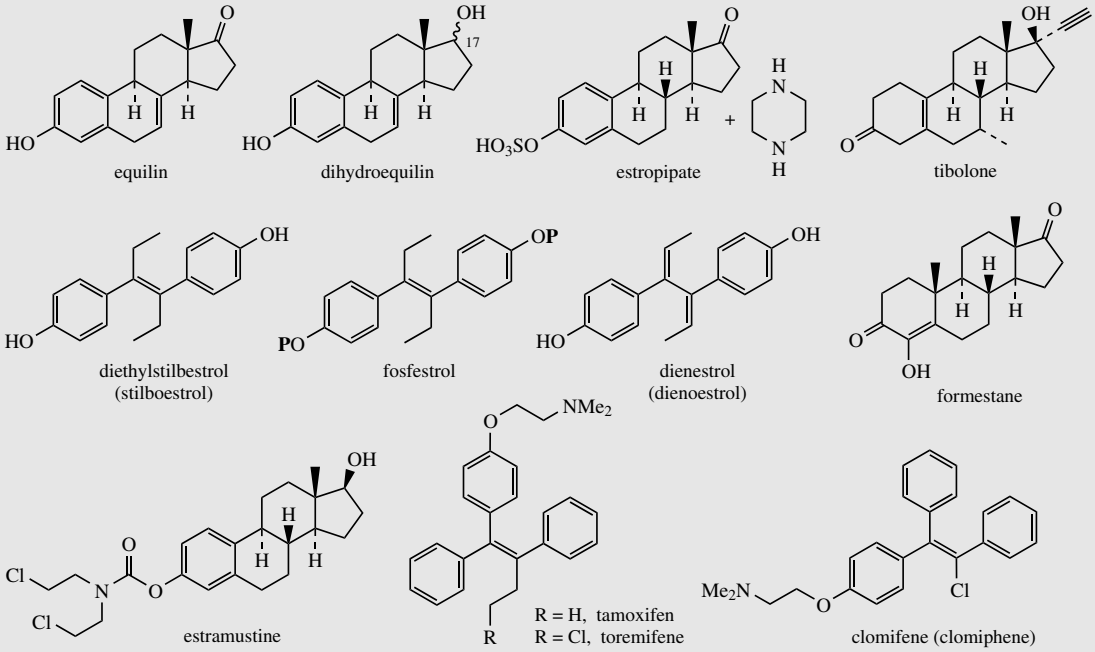

Figure 5.138

Horses are maintained in an almost continuous state of pregnancy, and need to be kept in confined stalls and fitted with a suitable urine collection device, though the use of catheters to collect urine has been discontinued. Animal welfare groups urge women to reject these drug preparations in favour of plant-derived alternatives. Conjugated equine oestrogens provide a profile of natural oestrogens based principally on estrone and equilin (Figure 5.138). It consists of a mixture of oestrogens in the form of sodium salts of their sulphate esters, comprising mainly estrone (50–60%) and equilin (20–30%), with smaller amounts of 17α-dihydroequilin, 17α-estradiol, and 17β-dihydroequilin. The semi-synthetic **estropipate** is also a conjugated oestrogen, the piperazine salt of estrone sulphate.

The structure of **tibolone** (Figure 5.138) probably resembles that of a progestogen more than it does an oestrogen. Although it does not contain an aromatic A-ring, the 5(10)-double bond ensures a degree of planarity. This agent combines both oestrogenic and progestogenic activity, and also has weak androgenic activity, and has been introduced for treatment of vasomotor symptoms of menopause.

Diethylstilbestrol (**stilboestrol**) and **dienestrol** (**dienoestrol**) (Figure 5.138) are the principal non-steroidal oestrogen drugs, used topically via the vagina. **Fosfestrol** (Figure 5.138) has value in the treatment of prostate cancer, being hydrolysed by the enzyme phosphatase to produce diethylstilbestrol as the active agent.

In **estramustine** (Figure 5.138), estradiol is combined with a cytotoxic alkylating agent of the nitrogen mustard class via a carbamate linkage. This drug has a dual function, a hormonal effect by suppressing androgen (testosterone) formation, and an antimitotic effect from the mustine residue. It is of value in treating prostate cancers.

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Phyto-oestrogens are predominantly isoflavonoid derivatives found in food plants and are used as dietary supplements to provide similar benefits to HRT, especially in countering some of the side-effects of the menopause in women. These compounds are discussed under isoflavonoids (see page 156). **Dioscorea** (**wild yam**) root or extract (see page 239) is also marketed to treat the symptoms of menopause as an alternative to HRT. Although there is a belief that this increases levels of progesterone, which is then used as a biosynthetic precursor of other hormones, there is no evidence that diosgenin is metabolized in the human body to progesterone.

Aromatase Inhibitors

Formestane (Figure 5.138), the 4-hydroxy derivative of androstenedione, represents the first steroid aromatase inhibitor to be used clinically. It reduces the synthesis of oestrogens and is of value in treating advanced breast cancer in post-menopausal patients.

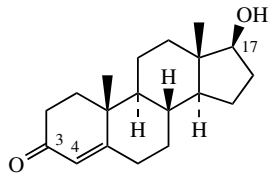
Oestrogen Receptor Antagonists

Breast cancer is dependent on a supply of oestrogen, and a major success in treating this disease has been the introduction of **tamoxifen** (Figure 5.138). This drug contains the stilbene skeleton seen in diethylstilbestrol and related oestrogens, but acts as an oestrogen-receptor antagonist rather than as an agonist in breast tissue, and deprives the cells of oestrogen. However, it is an agonist in bone and uterine tissue. The chlorinated analogue **toremifene** is also available, but is used primarily in post-menopausal women. Oestrogen antagonists can also be used as fertility drugs, occupying oestrogen receptors and interfering with feedback mechanisms and leading to ova release. **Clomifene** (**clomiphene**) (Figure 5.138), and to a lesser extent tamoxifen, are used in this way, but can lead to multiple pregnancies.

phyto-oestrogens (see page 156). Dietary natural isoflavonoids are believed to give some protection against breast cancers, and are also recommended to alleviate the symptoms of menopause.

Androgens

The primary male sex hormone, or **androgen**, is **testosterone** (Figure 5.139). This is secreted by the testes and is responsible for development and maintenance of the male sex characteristics. Androgens* also have a secondary physiological effect, an anabolic activity which stimulates growth of bone and muscle, and promotes storage of protein. The biosynthetic pathway to testosterone is outlined in Figure 5.133, where it can feature as an intermediate in the pathway to oestrogens. Low levels of testosterone are also synthesized in females in the ovary. Testosterone lacks any side-chain and has a 17β-hydroxyl as in estradiol, but still contains the methyl C-19 and the Δ^4-3-one system in ring A. This C_{19} skeleton is designated androstane.



testosterone

Characteristic features of androgens:

- C_{19} androstane skeleton
- no side-chain
- Δ^4-3-keto
- 17β-hydroxyl

Figure 5.139

It is particularly worthy of note that the routes to corticosteroids, progestogens, oestrogens, and androgens involve common precursors or partial pathways. This means that these processes need to be under very tight control if a person's normal physiological functions and characteristics are to be maintained. This balanced production is regulated primarily by gonadotrophic and hypothalamic proteins from the pituitary (see page 411).

Androgen Drugs

Testosterone can be produced from androstenolone (Figure 5.129) by chemical routes requiring reduction of the 17-carbonyl and oxidation of the 3-hydroxyl, and necessitating appropriate protecting groups. A simple high-yielding process (Figure 5.140) exploits yeast, in which fermentation firstly under aerobic conditions oxidizes the 3-hydroxyl, and then in the absence of air reduces the 17-keto group. Testosterone is not active orally since it is easily metabolized in the liver, and it has to be implanted, or injected in the form of esters. Transdermal administration from impregnated patches has also proved successful, and is now the method of choice for treating male sexual impotence caused by low levels of sex hormones (hypogonadism). Testosterone may also be prescribed for menopausal women as an adjunct to hormone replacement therapy (see page 279) to improve sex drive, and occasionally in the treatment of oestrogen-dependent breast cancer. The ester testosterone undecanoate is orally active, as is **mesterolone** (Figure 5.141), which features introduction of a 1α-methyl group and reduction of the Δ^4 double bond. Oral activity may also be attained by adding a 17α-alkyl group to reduce metabolism, e.g. 17α-methyltestosterone (Figure 5.140), such groups being introduced using appropriate Grignard reagents. However, these types of 17α-alkyl derivative are being replaced since they can sometimes cause jaundice as a side-effect.

The ratio of androgenic to anabolic activity can vary in different molecules. There have been attempts to produce steroids with low androgenic but high anabolic activity to use for various

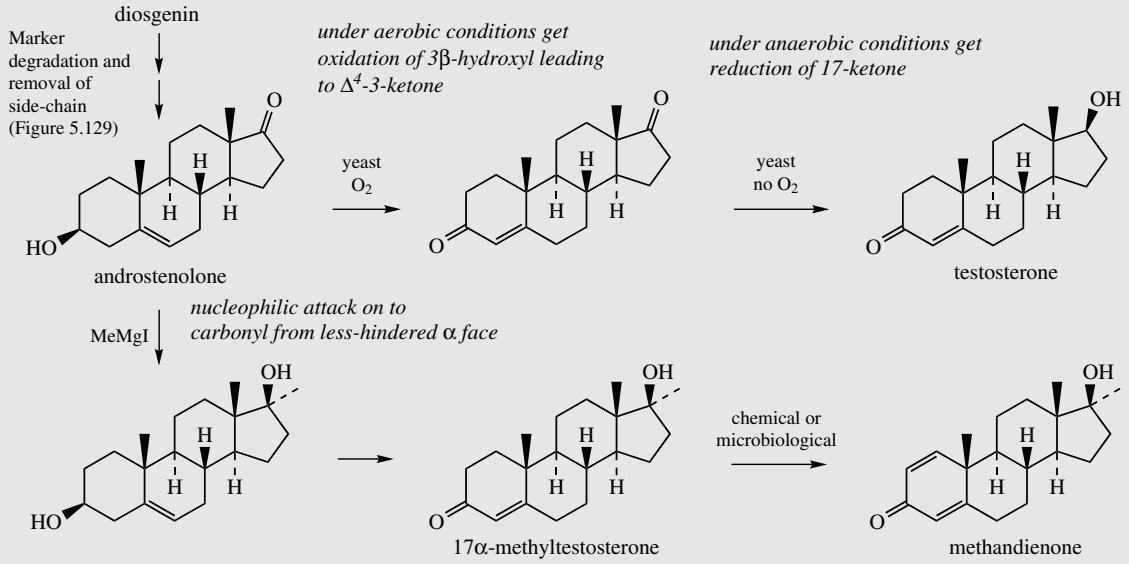


Figure 5.140

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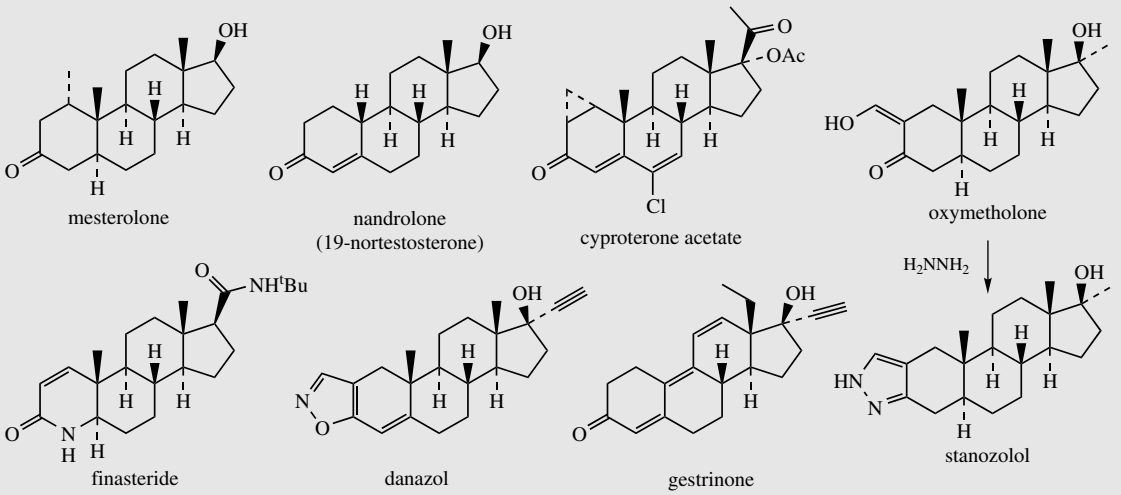

Figure 5.141

metabolic and endocrine disorders. **Methandrostenolone** (**methandienone**) (Figure 5.140), **oxymetholone**, and **nandrolone** (19-nortestosterone) (Figure 5.141) are modifications used in this way. Treatment of oxymetholone with hydrazine leads to formation of a pyrazole ring fused to a saturated ring A as seen in **stanozolol** (Figure 5.141). However, it is difficult to completely remove the androgenic activity from anabolic steroids. These materials are frequently abused by athletes wishing to promote muscle development and strength. Androgenic activity can affect the sexual characteristics of women, making them more masculine, whilst prolonged use of these drugs can lower fertility in either sex and endanger long term health by increasing the risks of heart and liver disease or cancer.

The progestogen **cyproterone acetate** (Figure 5.141) is a competitive androgen antagonist or anti-androgen, that reduces male libido and fertility, and finds use in the treatment of severe hypersexuality and sexual deviation in the male, as well as in prostate cancer. **Finasteride** (Figure 5.141) is another anti-androgen, which is of value in prostate conditions. It is a 4-aza-steroid and a specific inhibitor of the 5α-reductase involved in testosterone metabolism. This enzyme converts testosterone into dihydrotestosterone, which is actually a more potent androgen, so that its inhibition helps to reduce prostate tissue growth. Finasteride has also been noted to prevent hair loss in men, and is marketed to treat male-pattern baldness.

Dehydroepiandrosterone (DHEA) (Figure 5.133) is a precursor of androgens and oestrogens, and is the most abundant steroid in the blood of young adult humans, with levels peaking at about 20 years of age, then declining as the person ages. Whilst this hormone has a number of demonstrated biological activities, its precise physiological functions remain to be clarified. This material has become popular in the hope that it will maintain youthful vigour and health, countering the normal symptoms of age. These claims are as yet unsubstantiated, but taking large amounts of this androgen and oestrogen precursor can lead to side-effects associated with high levels of these hormones, e.g. increased risk of prostate cancer in men, or of breast cancer in women, who may also develop acne and facial hair. DHEA is not a precursor of glucocorticoids, mineralocorticoids, or of progestogens.

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Danazol and **gestrinone** (Figure 5.141) are inhibitors of pituitary gonadotrophin release, combining weak androgenic activity with antioestrogenic and antiprogestogenic activity. These highly modified structures bear one or more of the features we have already noted in discussions of androgens, progestogens and oestrogens, possibly accounting for their complex activity. These compounds are used particularly to treat endometriosis, where endometrial tissue grows outside the uterus.

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