Medicinal Natural Products. Paul M Dewick

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THE ACETATE PATHWAY: FATTY ACIDS AND POLYKETIDES

Polyketides, metabolites built primarily from combinations of acetate units, are described. The biosynthesis of saturated and unsaturated fatty acids is covered, together with prostaglandins, thromboxanes, and leukotrienes. Cyclization of polyketides to give aromatic structures is then rationalized in terms of aldol and Claisen reactions. More complex structures formed via pathways involving alkylation reactions, phenolic oxidative coupling, oxidative cleavage of aromatic rings, and employing starter groups other than acetate are developed. The use of extender groups other than malonate gives rise to macrolides and polyethers, whilst further cyclization of polyketide structures may be achieved through Diels–Alder reactions. The application of genetic engineering to modify products from the acetate pathway is discussed. Monograph topics giving more detailed information on medicinal agents include fixed oils and fats, evening primrose oil, echinacea, prostaglandins and isoprostanes, thromboxanes, leukotrienes, senna, cascara, frangula and allied drugs, St John's wort, mycophenolic acid, khellin and cromoglicate, griseofulvin, poison ivy and poison oak, aflatoxins, cannabis, tetracyclines, anthracycline antibiotics, macrolide antibiotics, avermectins, polyene antifungals, tacrolimus and sirolimus, ansa macrolides, mevastatin and other statins.

Polyketides constitute a large class of natural products grouped together on purely biosynthetic grounds. Their diverse structures can be explained as being derived from poly-β-keto chains, formed by coupling of acetic acid (C_2) units via condensation reactions,

i.e. $n CH_3CO_2H \longrightarrow -[CH_2CO]_n-$

Included in such compounds are the fatty acids, polyacetylenes, prostaglandins, macrolide antibiotics and many aromatic compounds, e.g. anthraquinones and tetracyclines.

The formation of the poly-β-keto chain could be envisaged as a series of Claisen reactions, the reverse of which are involved in the β-oxidation sequence for the metabolism of fatty acids (see page 18). Thus, two molecules of acetyl-CoA could participate in a Claisen condensation giving acetoacetyl-CoA, and this reaction could be repeated to generate a poly-β-keto ester of appropriate chain length (Figure 3.1). However, a study of the enzymes involved in fatty acid biosynthesis showed this simple rationalization could not be correct, and a more complex series of reactions was operating. It is now known that fatty acid biosynthesis involves initial carboxylation of acetyl-CoA to malonyl-CoA, a reaction involving ATP, CO_2 (as bicarbonate, HCO_3^-), and the coenzyme biotin as the carrier of CO_2 (see page 17).

The conversion of acetyl-CoA into malonyl-CoA increases the acidity of the α-hydrogens, and thus provides a better nucleophile for the Claisen condensation. In the biosynthetic sequence, no acylated malonic acid derivatives are produced, and no label from [^{14}C]bicarbonate is incorporated, so the carboxyl group introduced into malonyl-CoA is simultaneously lost by a decarboxylation reaction during the Claisen condensation (Figure 3.1). Accordingly, the carboxylation step helps to activate the α-carbon and facilitate Claisen condensation, and the carboxyl is immediately removed on completion of this task. An alternative rationalization is that decarboxylation of the malonyl ester is used to generate the acetyl enolate anion without any requirement for a strong base.

The pathways to fatty acids and aromatic polyketides branch early. The chain extension process of Figure 3.1 continues for aromatics,

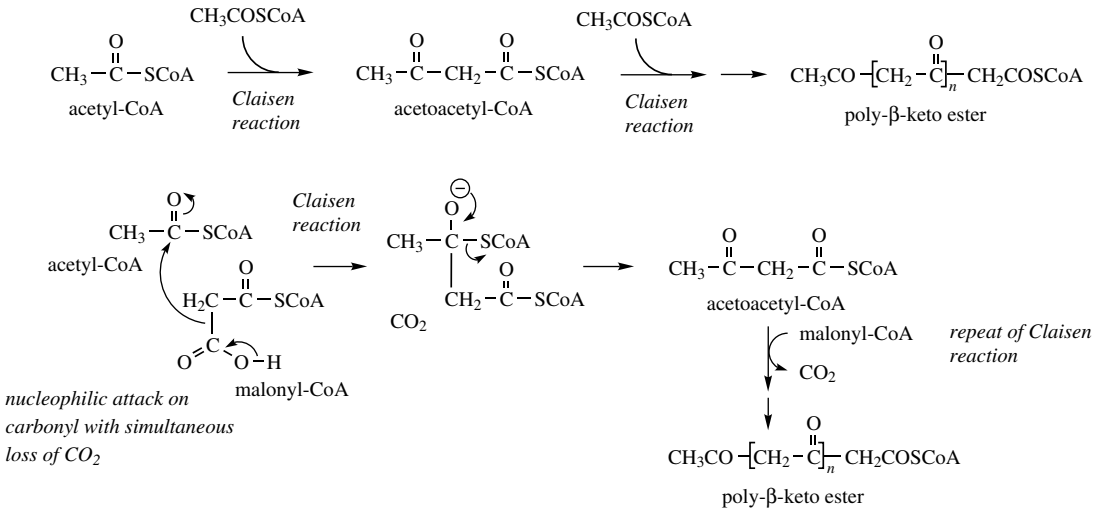


Figure 3.1

generating a highly reactive poly-β-keto chain, which has to be stabilized by association with groups on the enzyme surface until chain assembly is complete and cyclization reactions occur. However, for fatty acids, the carbonyl groups are reduced before attachment of the next malonate group. Partial reduction processes, leading to a mixture of methylenes, hydroxyls, and carbonyls, are characteristic of macrolides (see page 92).

SATURATED FATTY ACIDS

The processes of fatty acid biosynthesis are well studied and are known to be catalysed by the enzyme **fatty acid synthase**. In animals, this is a multifunctional protein containing all of the catalytic activities required, whilst bacteria and plants utilize an assembly of separable enzymes. Acetyl-CoA and malonyl-CoA themselves are not involved in the condensation step: they are converted into enzyme-bound thioesters, the malonyl ester by means of an acyl carrier protein (ACP) (Figure 3.2). The Claisen reaction follows giving acetoacetyl-ACP (β-keto acyl-ACP; R=H), which is reduced stereospecifically to the corresponding β-hydroxy ester, consuming NADPH in the reaction. Then follows elimination of water giving the *E* (*trans*) α,β-unsaturated ester. Reduction of the double bond again utilizes NADPH and generates a saturated acyl-ACP (fatty acyl-ACP; R=H) which is two carbons longer than the starting material. This can feed back into the system, condensing again with malonyl-ACP, and going through successive reduction, dehydration, and reduction steps, gradually increasing the chain length by two carbons for each cycle, until the required chain length is obtained. At that point, the fatty acyl chain can be released as a fatty acyl-CoA or as the free acid. The chain length actually elaborated is probably controlled by the specificity of the thioesterase enzymes that subsequently catalyse release from the enzyme.

The fatty acid synthase protein is known to contain an acyl carrier protein binding site, and also an active site cysteine residue in the β-ketoacyl synthase domain. Acetyl and malonyl groups are successively transferred from coenzyme A esters and attached to the thiol groups of Cys and ACP (Figure 3.3). The Claisen condensation occurs, and the processes of reduction, dehydration, and reduction then occur whilst the growing chain is attached to ACP. The ACP carries a phosphopantatheine group exactly analogous to that in coenzyme A, and this provides a long flexible arm, enabling the growing fatty acid chain to reach the active site of each enzyme in the complex, allowing the different chemical reactions to be performed without releasing intermediates from the enzyme (compare polyketide synthesis page 62 and peptide synthesis, page 421). Then the chain is transferred to the thiol of Cys, and the process can

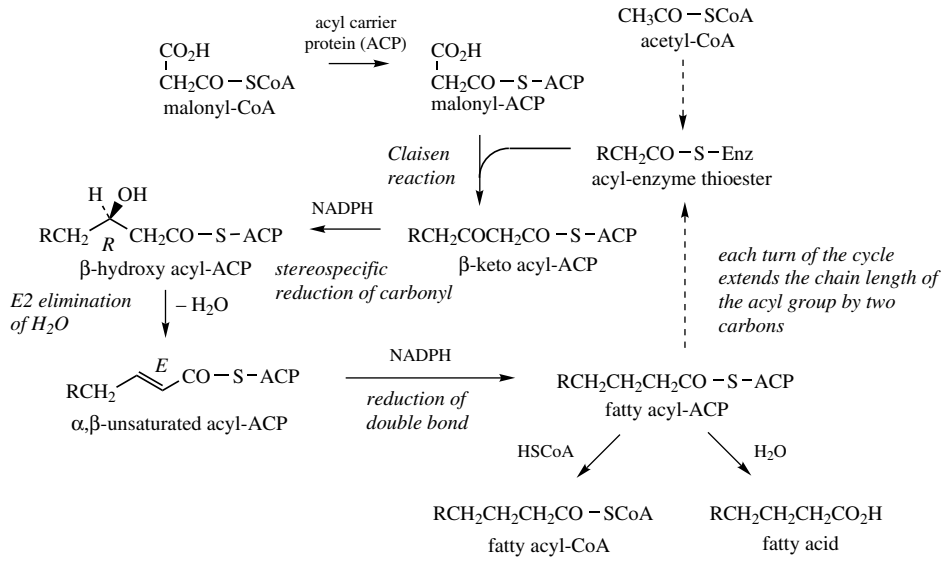


Figure 3.2

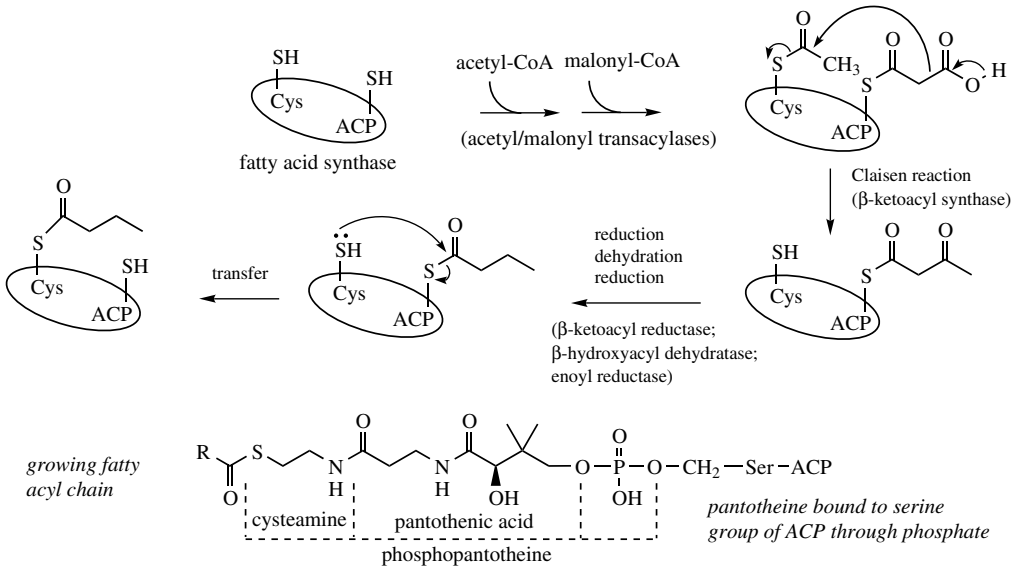
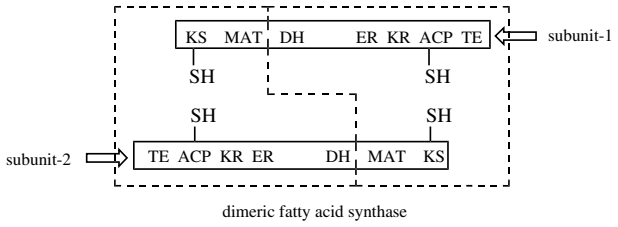


Figure 3.3

continue. Making the process even more efficient, animal fatty acid synthase is a dimeric protein containing two catalytic centres and is able to generate two growing chains. The monomeric subunits are also arranged head to tail so that the acyl group of one unit actually picks up a malonyl extender from the other unit (Figure 3.4). Note that the sequence of enzyme activities along the protein chain of the enzyme complex does not correspond with the order in which they are employed.

Thus, combination of one acetate starter unit with seven malonates would give the C_{16} fatty acid, palmitic acid, and with eight malonates the C_{18} fatty acid, stearic acid. Note that the two carbons at the head of the chain (methyl end) are provided by acetate, not malonate, whilst the remainder are derived from malonate, which itself



dotted lines indicate two sites for fatty acid synthesis, utilizing enzyme activities from both subunits

ACP: acyl carrier protein
DH: dehydratase
ER: enoylreductase
KR: β-ketoacylreductase
KS: β-ketoacylsynthase
MAT: malonyl/acetyltransferase
TE: thioesterase

Figure 3.4

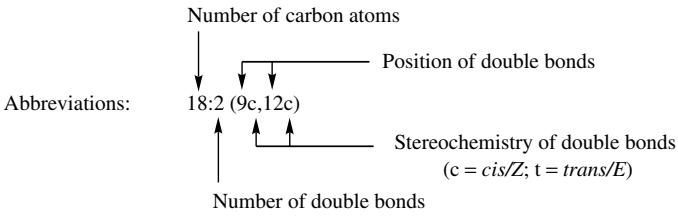
Table 3.1 Common naturally occurring fatty acids

Saturated					
butyric	$CH_3(CH_2)_2CO_2H$	4:0	stearic	$CH_3(CH_2)_{16}CO_2H$	18:0
caproic*	$CH_3(CH_2)_4CO_2H$	6:0	arachidic	$CH_3(CH_2)_{18}CO_2H$	20:0
caprylic*	$CH_3(CH_2)_6CO_2H$	8:0	behenic	$CH_3(CH_2)_{20}CO_2H$	22:0
capric*	$CH_3(CH_2)_8CO_2H$	10:0	lignoceric	$CH_3(CH_2)_{22}CO_2H$	24:0
lauric	$CH_3(CH_2)_{10}CO_2H$	12:0	cerotic	$CH_3(CH_2)_{24}CO_2H$	26:0
myristic	$CH_3(CH_2)_{12}CO_2H$	14:0	montanic	$CH_3(CH_2)_{26}CO_2H$	28:0
palmitic	$CH_3(CH_2)_{14}CO_2H$	16:0	melissic	$CH_3(CH_2)_{28}CO_2H$	30:0

*To avoid confusion, systematic nomenclature (hexanoic, octanoic, decanoic) is recommended

Unsaturated		
palmitoleic	$CH_3(CH_2)_5CH{=}CH(CH_2)_7CO_2H$	16:1 (9c)
oleic	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$	18:1 (9c)
cis-vaccenic	$CH_3(CH_2)_5CH{=}CH(CH_2)_9CO_2H$	18:1 (11c)
linoleic	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$	18:2 (9c,12c)
α-linolenic	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$	18:3 (9c,12c,15c)
γ-linolenic	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_4CO_2H$	18:3 (6c,9c,12c)
gadoleic	$CH_3(CH_2)_9CH{=}CH(CH_2)_7CO_2H$	20:1 (9c)
arachidonic	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3CO_2H$	20:4 (5c,8c,11c,14c)
eicosapentaenoic (EPA)	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3CO_2H$	20:5 (5c,8c,11c,14c,17c)
erucic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}CO_2H$	22:1 (13c)
docosapentaenoic (DPA)	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_5CO_2H$	22:5 (7c,10c,13c,16c,19c)
docosahexaenoic (DHA)	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_2CO_2H$	22:6 (4c,7c,10c,13c,16c,19c)
nervonic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{13}CO_2H$	24:1 (15c)

all double bonds are *Z* (*cis*)



is produced by carboxylation of acetate. This means that all carbons in the fatty acid originate from acetate, but malonate will only provide the C_2 chain extension units and not the C_2 starter group. The linear combination of acetate C_2 units as in Figure 3.2 explains why the common fatty acids are straight chained and possess an even number of carbon atoms. Natural fatty acids may contain from four to 30, or even more, carbon atoms, the most abundant being those with 16 or 18 carbons. Some naturally occurring fatty acids are shown in Table 3.1. The rarer fatty acids containing an odd number of carbon atoms typically originate from incorporation of a different starter unit, e.g.

propionic acid, or can arise by loss of one carbon from an even-numbered acid.

Fatty acids are mainly found in ester combination with glycerol in the form of triglycerides (Figure 3.5). These materials are called **fats** or **oils**, depending on whether they are solid or liquid at room temperature. If all three esterifying acids are the same, the triglyceride is termed simple, whereas a mixed triglyceride is produced if two or more of the fatty acids are different. Most natural fats and oils are composed largely of mixed triglycerides. In this case, isomers can exist, including potential optical isomers, since if the primary alcohols are esterified with different fatty acids the central carbon of glycerol will become chiral. In practice, only one of each pair of enantiomers is formed in nature. Triglycerides are produced from glycerol 3-phosphate by esterification with fatty acyl-CoA residues, the phosphate being removed prior to the last esterification (Figure 3.5). The diacyl ester of glycerol 3-phosphate is also known as a **phosphatidic acid**, and is the basis of phospholipid structures. In these structures, the phosphate is also esterified with an alcohol, which is usually choline, ethanolamine, serine, or *myo*-inositol, e.g. **phosphatidyl choline** (Figure 3.6). **Phospholipids** are important structural components of cell membranes, and because of the polar and non-polar regions in their structure, they have detergent-like properties. They are also able to form liposomes, which have considerable potential as drug delivery systems. A particularly important natural phospholipid is **platelet-activating factor** (**PAF**) (Figure 3.6), which resembles a phosphatidylcholine, though this compound possesses an ether linkage to a long chain fatty alcohol,

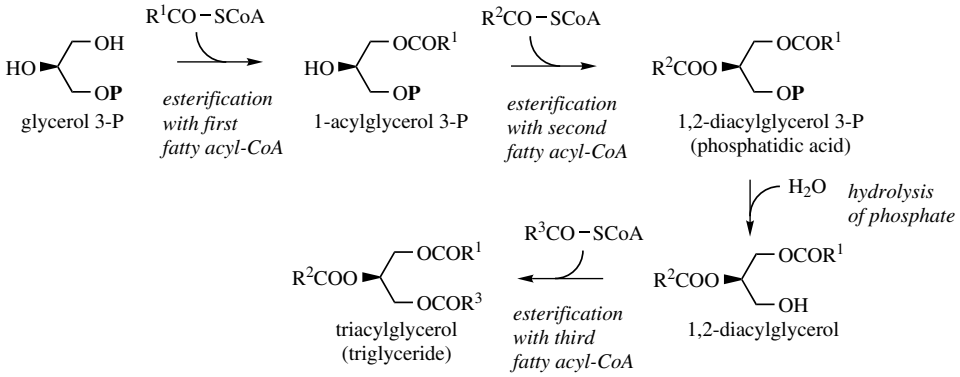


Figure 3.5

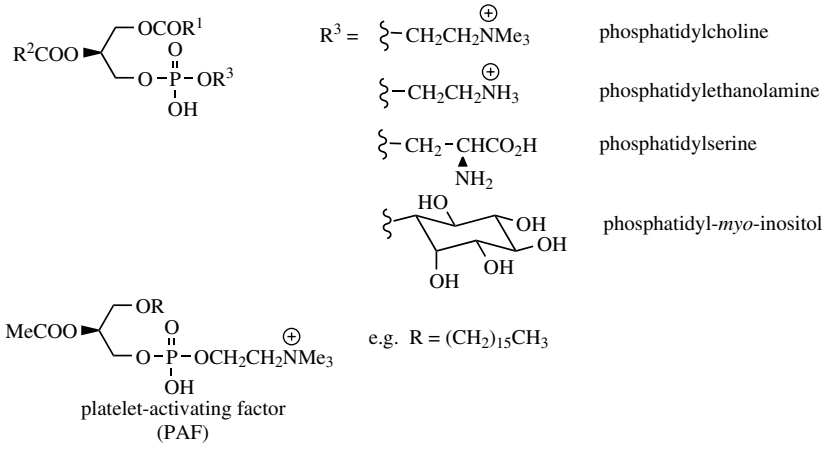


Figure 3.6

usually hexadecanol, rather than an ester linkage. The central hydroxyl of glycerol is esterified, but to acetic acid rather than to a long chain fatty acid. PAF functions at nanomolar concentrations, activates blood platelets and contributes to diverse biological effects, including thrombosis, inflammatory reactions, allergies, and tissue rejection. Long chain alcohols are reduction products from fatty acids and also feature in natural **waxes**. These are complex mixtures of esters of long chain fatty acids, usually $C_{20}-C_{24}$, with long chain monohydric alcohols or sterols.

UNSATURATED FATTY ACIDS

Animal fats contain a high proportion of glycerides of saturated fatty acids and tend to be solids, whilst those from plants and fish contain predominantly unsaturated fatty acid esters and tend to be liquids. Some of the common naturally occurring unsaturated fatty acids are also included in Table 3.1. A convenient shorthand representation for fatty acids indicating chain length with number, position and stereochemistry of double bonds is also presented in Table 3.1. A less systematic numbering starting from the methyl (the ω end) may also be encountered. Major groups of fatty acids are designated ω-3 (omega-3), ω-6 (omega-6), ω-9 (omega-9), etc (or sometimes n-3, n-6, n-9), if there is a double bond that number of carbons from the methyl terminus. This has some value in relating structures when an unsaturated fatty acid is biosynthetically elongated from the carboxyl end as during prostaglandin biosynthesis (see page 45). Double bonds at position 9 are common, but unsaturation can occur at other positions in the chain. Polyunsaturated fatty acids tend to have their double bonds in a non-conjugated array as a repeating unit $-(CH{=}CHCH_2)_n-$. In virtually all cases, the stereochemistry of the double bond is *Z* (*cis*), introducing a 'bend' into the alkyl chain. This interferes with the close association and aggregation of molecules that is possible in saturated structures, and helps to maintain the fluidity in oils and cellular membranes.

Fats and oils represent long term stores of energy for most organisms, being subjected to oxidative metabolism as required. Major oils which are produced commercially for use as foods, toiletries, medicinals, or pharmaceutical formulation aids are listed in Table 3.2. Typical fatty acid analyses are shown, though it must be appreciated that these figures can vary quite widely. For instance, plant oils show significant variation according to the climatic conditions under which the plant was grown. In colder climates, a higher proportion of polyunsaturated fatty acids is produced, so that the plant can maintain the fluidity of its storage fats and membranes. The melting points of these materials depend on the relative proportions of the various fatty acids, reflecting primarily the chain length and the amount of unsaturation in the chain. Saturation, and increasing chain length in the fatty acids gives a more solid fat at room temperature. Thus, butterfat and cocoa butter (theobroma oil) contain a relatively high proportion of saturated fatty acids and are solids. Palm kernel and coconut oils are both semi-solids having a high concentration of the saturated C_{12} acid **lauric acid**. A characteristic feature of olive oil is its very high **oleic acid** (18:1) content, whilst rapeseed oil possesses high concentrations of long chain C_{20} and C_{22} fatty acids, e.g. **erucic acid** (22:1). Typical fatty acids in fish oils have high unsaturation and also long chain lengths, e.g. **eicosapentaenoic acid (EPA)** (20:5) and **docosahexaenoic acid (DHA)** (22:6) in cod liver oil.

Unsaturated fatty acids can arise by more than one biosynthetic route, but in most organisms the common mechanism is by desaturation of the corresponding alkanoic acid, with further desaturation in subsequent steps. Most eukaryotic organisms possess a Δ^9-desaturase that introduces a *cis* double bond into a saturated fatty acid, requiring O_2 and NADPH or NADH cofactors. The mechanism of desaturation does not involve any intermediates hydroxylated at C-9 or C-10, and the requirement for O_2 is as an acceptor at the end of an electron transport chain. A stearoyl (C_{18}) thioester is the usual substrate giving an oleoyl derivative (Figure 3.7), coenzyme A esters being utilized by animal and fungal enzymes, and ACP esters by plant systems. The position of further desaturation then depends very much on the organism. Non-mammalian enzymes tend to introduce additional double bonds between the existing double bond and the methyl terminus, e.g. oleate → linoleate → α-linolenate. Animals always introduce new double bonds towards the carboxyl group. Thus **oleate** is desaturated to

Table 3.2 Fixed oils and fats

The term fat or oil has no precise significance, and merely describes whether the material is a solid (fat) or liquid (oil) at room temperature. Most commercial oils are obtained from plant sources, particularly seeds and fruits, and the oil is extracted by cold or hot expression, or less commonly by solvent extraction with hexane. The crude oil is then refined by filtration, steaming, neutralization to remove free acids, washing, and bleaching as appropriate. Many food oils are then partially hydrogenated to produce semi-solid fats. Animal fats and fish oils are usually extracted by steaming, the higher temperature deactivating enzymes that would otherwise begin to hydrolyse the glycerides.

Oils and fats feature as important food components and cooking oils, some 80% of commercial production being used as human food, whilst animal feeds account for another 6%. Most of the remaining production is used as the basis of soaps, detergents, and pharmaceutical creams and ointments. A number of oils are used as diluents (carrier or base oils) for the volatile oils employed in aromatherapy.

Oil	Source	Part used	Oil content† (%)	Typical fatty acid composition† (%)	Uses, notes
Almond	*Prunus amygdalus* var. *dulcis*, or var. *amara* (Rosaceae)	seed	40–55	oleic (62–86), linoleic (7–30), palmitic (4–9), stearic (1–2)	emollient base, toiletries, carrier oil (aromatherapy)
Arachis (groundnut, peanut)	*Arachis hypogaea* (Leguminosae/ Fabaceae)	seed	45–55	oleic (35–72), linoleic (13–43), palmitic (7–16), stearic (1–7), behenic (1–5), arachidic (1–3)	food oil, emollient base
Borage	*Borago officinalis* (Boraginaceae)	seed	28–35	linoleic (38), γ-linolenic (23–26), oleic (16), palmitic (11)	dietary supplement for γ-linolenic acid content (see page 46)
Butterfat	cow *Bos taurus* (Bovidae)	milk	2–5	palmitic (29), oleic (28), stearic (13), myristic (12), butyric (4), lauric (3), caproic (2), capric (2), palmitoleic (2)	food
Castor	*Ricinus communis* (Euphorbiaceae)	seed	35–55	ricinoleic (80–90), oleic (4–9), linoleic (2–7), palmitic (2–3), stearic (2–3)	emollient base, purgative, soap manufacture Castor seeds contain the highly toxic, but heat-labile protein ricin (see page 434)

(*Continued overleaf*)

Table 3.2 (*Continued*)

Oil	Source	Part used	Oil content† (%)	Typical fatty acid composition† (%)	Uses, notes
Coconut	*Cocos nucifera* (Palmae/ Arecaceae)	seed kernel	65–68	lauric (43–53), myristic (15–21), palmitic (7–11), caprylic (5–10), capric (5–10), oleic (6–8), stearic (2–4)	soaps, shampoos Fractionated coconut oil containing only short to medium length fatty acids (mainly caprylic and capric) is a dietary supplement
Cod-liver	cod *Gadus morrhua* (Gadidae)	fresh liver	50	oleic (24), DHA (14), palmitic (11), EPA (6), palmitoleic (7), stearic (4), myristic (3)	dietary supplement due to presence of EPA and DHA, plus vitamins A (see page 230) and D (see page 259); halibut-liver oil from halibut *Hippoglossus vulgaris* (Pleurnectideae) has similar properties and is used in the same way
Cottonseed	*Gossypium hirsutum* (Malvaceae)	seed	15–36	linoleic (33–58), palmitic (17–29), oleic (13–44), stearic (1–4)	solvent for injections, soaps Cotton seeds also contain 1.1–1.3% gossypol (see page 200) and small amounts of cyclopropenoid fatty acids, e.g. sterculic and malvalic acids (see page 50)
Evening primrose	*Oenothera biennis* (Onagraceae)	seed	24	linoleic (65–80), γ-linolenic (7–14), oleic (9), palmitic (7)	dietary supplement for γ-linolenic acid content (see page 46)
Honesty	*Lunaria annua* (Cruciferae/ Brassicaceae)	seed	30–40	erucic (43), nervonic (25), oleic (24)	nervonic acid is being investigated for the treatment of multiple sclerosis; the disease is characterized by a deficiency in nervonic acid

Lard	pig *Sus scrofa* (Suidae)	abdominal fat		oleic (45), palmitic (25), stearic (12), linoleic (10), palmitoleic (3)	foods
Linseed (flaxseed)	*Linum usitatissimum* (Linaceae)	seed	35–44	α-linolenic (30–60), oleic (39), linoleic (15), palmitic (7), stearic (4)	liniments, dietary supplement for α-linolenic acid content Formerly the basis of paints, reacting with oxygen, polymerizing, and drying to a hard film
Maize (corn)	*Zea mays* (Graminae/ Poaceae)	embryo	33–39	linoleic (34–62), oleic (19–50), palmitic (8–19), stearic (0–4)	food oil, dietary supplement, solvent for injections
Olive	*Olea europaea* (Oleaceae)	fruits	15–40	oleic (56–85), palmitic (8–20), linoleic (4–20), stearic (1–4)	food oil, emollient base
Palm kernel	*Elaeis guineensis* (Palmae/ Arecaceae)	kernel	45–50	lauric (40–52), myristic (14–18), oleic (9–16), palmitoleic (6–10), caprylic (3–6), capric (3–5), stearic (1–4), linoleic (1–3)	soaps Fractionated palm oil is a solid obtained by fractionation and hydrogenation and is used as a suppository base
Rapeseed	*Brassica napus* (Cruciferae/ Brassicaceae)	seed	40–50	erucic (30–60), oleic (9–25), linoleic (11–25), gadoleic (5–15), α-linolenic (5–12), palmitic (0–5)	food oil, using varieties producing lower levels of erucic acid where the main components are now oleic (48–60%), linoleic (18–30%), α-linolenic (6–14%), and palmitic (3–6%) acids Erucic acid is used as a plasticizer in PVC clingfilm

(*Continued overleaf*)

Table 3.2 (*Continued*)

Oil	Source	Part used	Oil content† (%)	Typical fatty acid composition† (%)	Uses, notes
Sesame	*Sesamum indicum* (Pedaliaceae)	seed	44–54	oleic (35–50), linoleic (35–50), palmitic (7–12), stearic (4–6)	food oil, soaps, solvent for injections, carrier oil (aromatherapy)
Soya (soybean)	*Glycine max* (Leguminosae/ Fabaceae)	seed	18–20	linoleic (44–62%), oleic (19–30), palmitic (7–14), α-linolenic (4–11), stearic (1–5)	food oil, dietary supplement, carrier oil (aromatherapy) Soya oil contains substantial amounts of the sterols sitosterol and stigmasterol (see page 256)
Suet (mutton tallow)	sheep *Ovis aries* (Bovidae)	abdominal fat		stearic (32), oleic (31), palmitic (27), myristic (6)	foods
Suet (beef tallow)	cow *Bos taurus* (Bovidae)	abdominal fat		oleic (48), palmitic (27), palmitoleic (11), stearic (7), myristic (3)	foods
Sunflower	*Helianthus annuus* (Compositae/ Asteraceae)	seed	22–36	linoleic (50–70), oleic (20–40), palmitic (3–10), stearic (1–10)	food oil, carrier oil (aromatherapy)
Theobroma	*Theobroma cacao* (Sterculiaceae)	kernel	35–50	oleic (35), stearic (35), palmitic (26), linoleic (3)	suppository base, chocolate manufacture Theobroma oil (cocoa butter) is a solid

†The oil yields and fatty acid compositions given in the table are typical values, and can vary widely. The quality of an oil is determined principally by its fatty acid analysis. Structures of the fatty acids are shown in Table 3.1 (see page 38).

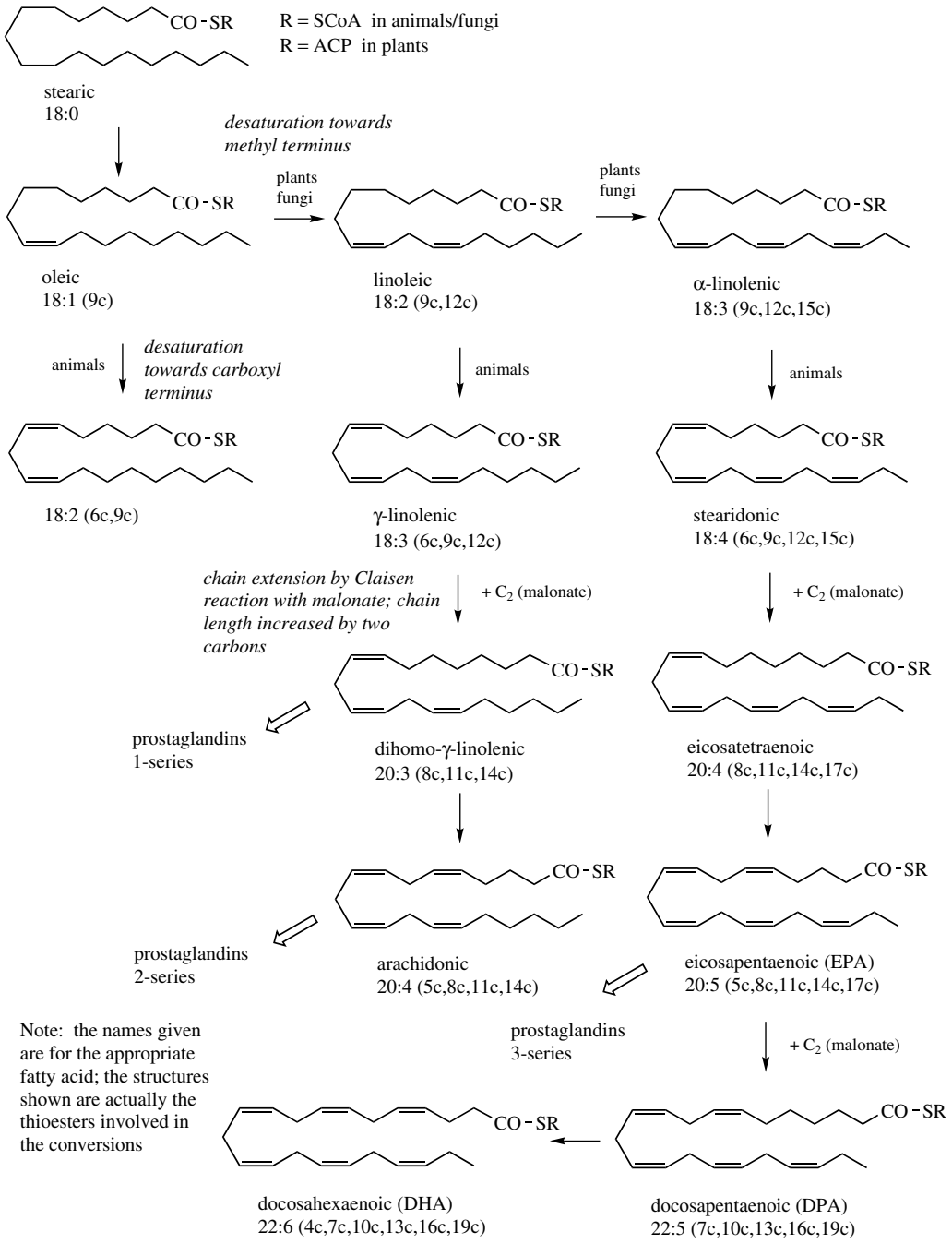


Figure 3.7

$\Delta^{6,9}$-octadecadienoate rather than linoleate. However, animals need **linoleate** for the biosynthesis of **dihomo-γ-linolenate** ($\Delta^{8,11,14}$-eicosatrienoate) and **arachidonate** ($\Delta^{5,8,11,14}$-eicosatetraenoate), C_{20} polyunsaturated fatty acid precursors of prostaglandins in the 'one' and 'two' series respectively (see page 52). Accordingly, linoleic acid must be obtained from plant material in the diet, and it is desaturated towards the carboxyl to yield **γ-linolenate**, which is then used as the

substrate for further chain extension, adding a C_2 unit from malonate, and producing dihomo-γ-linolenate. Arachidonate derives from this by additional desaturation, again towards the carboxyl end of the chain (Figure 3.7). **α-Linolenate** is similarly a precursor on the way to **$\Delta^{5,8,11,14,17}$-eicosapentaenoate** (**EPA**), required for the synthesis of prostaglandins of the 'three' series, and it is also obtained from the diet. A similar chain extension process using further molecules of malonate is encountered in the sequence from α-linolenate in animal systems (Figure 3.7). Chain extension/dehydrogenations lead to formation of eicosapentaenoate (EPA) with further elaborations producing **docosapentaenoate** (**DPA**) and then **docosahexaenoate** (**DHA**). DHA is a component of lipids in sperm, the retina, and the brain. It is thought to be important for brain development, and deficiency is associated with abnormalities in brain function. Linoleate and α-linolenate are referred to as '**essential fatty acids**' (**EFAs**) since they and their metabolites are required in the diet for normal good health. Some food sources such as the oils present in fish are rich in the later metabolites derived from α-linolenic acid, e.g. EPA and DHA, and are also beneficial to health. Since these fatty acids all have a double bond three carbons from the methyl end of the chain, they are grouped together under the term ω-3 fatty acids (**omega-3 fatty acids**). Regular consumption of fish oils is claimed to reduce the risk of heart attacks and atherosclerosis.

Although most plant-derived oils contain high amounts of unsaturated fatty acid glycerides, including those of linoleic and α-linolenic acids, the conversion of linoleate into **γ-linolenate** can be blocked or inhibited in certain conditions in humans. This restricts synthesis of prostaglandins. In such cases, the use of food supplements, e.g. evening primrose oil* from *Oenothera biennis* (Onagraceae), which are rich in γ-linolenic esters, can be valuable and help in the disorder. Many plants in the Boraginaceae, e.g. borage (*Borago officinalis*), also accumulate significant amounts of γ-linolenic acid glycerides, as does evening primrose, indicating their unusual ability

Evening Primrose Oil

Evening primrose oil is extracted from the seeds of selected strains of the evening primrose (*Oenothera biennis*; Onagraceae), a biennial plant native to North America, which is now widely cultivated in temperate countries. The seeds contain about 24% fixed oil, which has a high content of glycerides of the unsaturated fatty acids linoleic acid (65–80%) and γ-linolenic acid (**gamolenic acid**) (7–14%). Because of this high γ-linolenic acid content, evening primrose oil is widely used as a dietary supplement, providing additional quantities of this essential fatty acid, which is a precursor in the biosynthesis of prostaglandins, which regulate many bodily functions (see page 54). Genetic and a number of other factors may inhibit the desaturation of linoleic acid into γ-linolenic acid. Ageing, diabetes, excessive alcohol intake, catecholamines, and zinc deficiency have all been linked to inhibition of the desaturase enzyme. The conversion may also be inhibited if there is a high proportion of fatty acids in the diet, which compete for the desaturase enzyme, including saturated and *trans*-unsaturated fatty acids. The latter group may be formed during the partial hydrogenation of polyunsaturated fatty acids which is commonly practised during food oil processing to produce semi-solid fats. Evening primrose oil appears to be valuable in the treatment of premenstrual tension, multiple sclerosis, breast pain (mastalgia), and perhaps also in eczema. There is potential for further applications, e.g. in diabetes, alcoholism, and cardiovascular disease. In evening primrose, γ-linolenic acid is usually present in the form of a dilinoleoylmono-γ-linolenylglycerol. This triglyceride is also being explored as a drug material for the treatment of diabetes-related neuropathy and retinopathy. γ-Linolenic acid is also found in the fixed oil of other plants, e.g. blackcurrant, comfrey, and borage, and in human milk. **Borage oil** (**starflower oil**) from the seeds of *Borago officinalis* (Boraginaceae) is used in the same way as evening primrose oil. It contains higher concentrations of γ-linolenic acid (23–26%), but rather less linoleic acid.

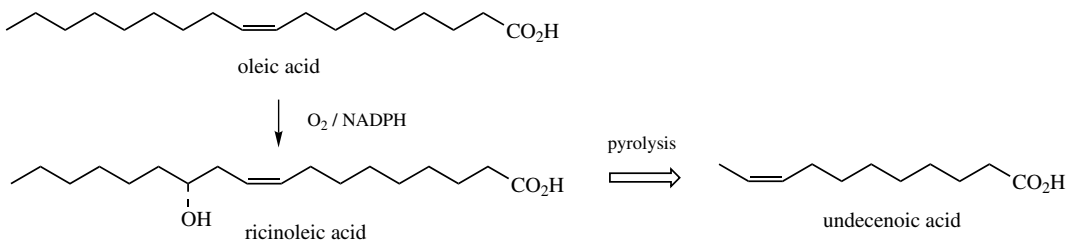


Figure 3.8

to desaturate linoleic esters towards the carboxyl terminus, rather than towards the methyl terminus as is more common in plants. Arachidonic acid itself has not been found in higher plants, but does occur in some algae, mosses, and ferns.

Ricinoleic acid (Figure 3.8) is the major fatty acid found in castor oil from seeds of the castor oil plant (*Ricinus communis*; Euphorbiaceae), and is the 12-hydroxy derivative of oleic acid. It is formed by direct hydroxylation of oleic acid (usually esterified as part of a phospholipid) by the action of an O_2- and NADPH-dependent mixed function oxidase, but this is not of the cytochrome P-450 type. Castor oil has a long history of use as a domestic purgative, but it is now mainly employed as a cream base. **Undecenoic acid** (Δ^9-undecenoic acid) can be obtained from ricinoleic acid by thermal degradation, and as the zinc salt or in ester form is used in fungistatic preparations.

Primary amides of unsaturated fatty acids have been characterized in humans and other mammals, and although their biological role is not fully understood, they may represent a group of important signalling molecules. **Oleamide**, the simple amide of oleic acid, has been shown to be a sleep-inducing lipid, and the amide of erucic acid, **erucamide**, stimulates the growth of blood vessels.

ACETYLENIC FATTY ACIDS

Many unsaturated compounds found in nature contain one or more acetylenic bonds, and these are predominantly produced by further desaturation of olefinic systems in fatty acid-derived molecules. They are surprisingly widespread in nature, and are found in many organisms, but are especially common in plants of the Compositae/Asteraceae, the Umbelliferae/Apiaceae, and fungi of the group Basidiomycetes. These compounds tend to be highly unstable and some are even explosive if sufficient amounts are accumulated. Since only very small amounts are present in plants, this does not present any widespread hazard. Whilst fatty acids containing several double bonds usually have these in a non-conjugated array, molecules containing triple bonds tend to possess conjugated unsaturation. This gives the compounds intense and highly characteristic UV spectra which aids their detection and isolation.

The processes of desaturation are exemplified in Figure 3.9, in which oleic acid (probably as a thiol ester) features as a precursor of **crepenynic acid** and **dehydrocrepenynic acid**. The acetylenic bond is now indicated by *a* in the semi-systematic shorthand nomenclature. Chain shortening by β-oxidation (see page 18) is often a feature of these pathways, and formation of the C_{10} acetylenic acid **dehydromatricaria acid** proceeds through C_{18} intermediates, losing eight carbons, presumably via four β-oxidations. In the latter part of the pathway, the *Z*-double bond from oleic acid moves into conjugation with the polyacetylene chain via an allylic isomerization, giving the more favoured *E*-configuration. Some noteworthy acetylenic structures (though they are no longer acids and components of fats) are given in Figure 3.10. **Cicutoxin** from the water hemlock (*Cicuta virosa*; Umbelliferae/Apiaceae) and **oenanthotoxin** from the hemlock water dropwort (*Oenanthe crocata*; Umbelliferae/Apiaceae) are extremely toxic to mammals, causing persistent vomiting and convulsions, leading to respiratory paralysis. Ingestion of the roots of these plants may frequently lead to fatal poisoning. **Falcarinol** is a constituent of *Falcaria vulgaris* (Umbelliferae/Apiaceae), *Oenanthe crocata*, *Hedera helix* (Araliaceae), and several other plants, and is known to cause contact dermatitis in certain

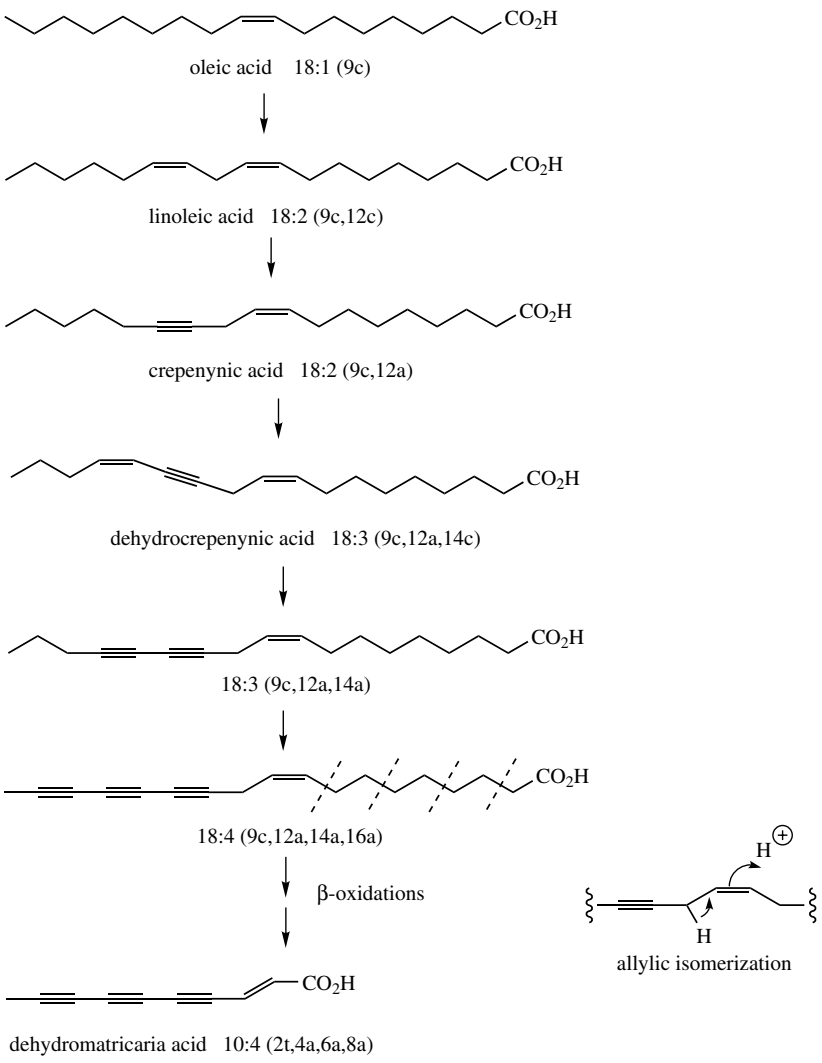


Figure 3.9

individuals when the plants are handled. Falcarinol (sometimes called panaxynol) and the structurally related **panaxytriol** are also characteristic polyacetylene components of ginseng (*Panax ginseng*; Araliaceae) (see page 222). **Wyerone** from the broad bean (*Vicia faba*; Leguminosae/Fabaceae) has antifungal properties, and its structure exemplifies how the original straight chain may be cross-linked to produce a ring system. The furan ring may originate from a conjugated diyne.

The herbal preparation echinacea* is derived from the roots of *Echinacea purpurea* (Compositae/Asteraceae) and is used for its immunostimulant properties, particularly as a prophylactic and treatment for the common cold. At least some of its activity arises from a series of alkylamides, amides of polyunsaturated acids with isobutylamine. These acids are predominantly C_{11} and C_{12} diene-diynes (Figure 3.11).

BRANCHED-CHAIN FATTY ACIDS

Whilst straight-chain fatty acids are the most common, branched-chain acids have been found to occur in mammalian systems, e.g. in wool fat and butter fat. They are also characteristic fatty acid constituents of the lipid part of cell walls in some

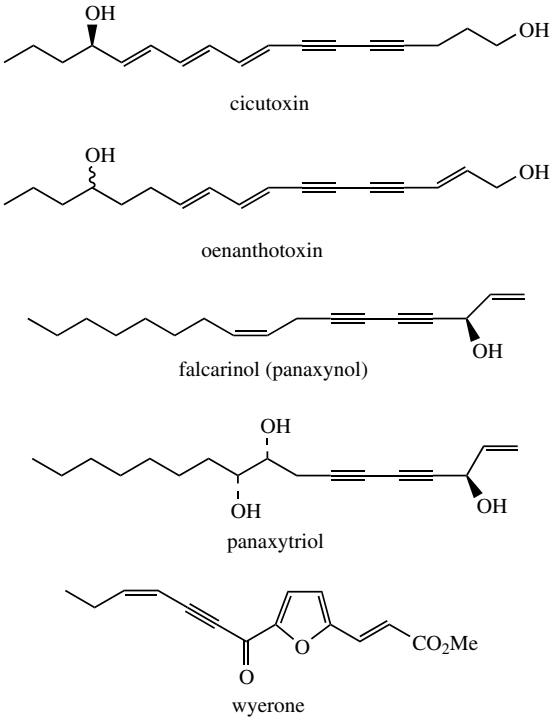


Figure 3.10

pathogenic bacteria. Several mechanisms appear to operate in their formation. Thus, the structure of **corynomycolic acid** from *Corynebacterium diphtheriae* can be rationalized from a combination of two palmitic acid units (Figure 3.12). Methyl side-chains can be introduced by using methylmalonyl-CoA instead of malonyl-CoA as the chain extending agent (Figure 3.13). Methylmalonyl-CoA arises by biotin-dependent carboxylation of propionyl-CoA in exactly the same way as malonyl-CoA was formed (see page 17). **2,4,6,8-Tetramethyldecanoic acid** found in the preen gland wax of the goose (*Anser anser*) is produced from an acetyl-CoA starter, and four methylmalonyl-CoA chain extender units. The incorporation of propionate as well as acetate is also a feature of many microbial antibiotic structures (see page 17). However, in other examples, methyl side-chains can be produced by a *C*-alkylation mechanism using *S*-adenosylmethionine (SAM). **Tuberculostearic acid** (Figure 3.14) found in *Mycobacterium tuberculosis*, the bacterium causing tuberculosis, is derived from oleic acid by alkylation on C-10,

Echinacea

Echinacea consists of the dried roots of *Echinacea purpurea*, *E. angustifolia*, or *E. pallida* (Compositae/Asteraceae), herbaceous perennial plants indigenous to North America, and widely cultivated for their large daisy-like flowers, which are usually purple or pink. Herbal preparations containing the dried root, or extracts derived from it, are promoted as immunostimulants, particularly as prophylactics and treatments for bacterial and viral infections, e.g. the common cold. Tests have validated stimulation of the immune response, though the origins of this activity cannot be ascribed to any specific substance. Activity has variously been assigned to lipophilic alkylamides, polar caffeic acid derivatives, high molecular weight polysaccharide material, or to a combination of these. Compounds in each group have been demonstrated to possess some pertinent activity, e.g. immunostimulatory, anti-inflammatory, antibacterial or antiviral effects. The alkylamides comprise a complex mixture of unsaturated fatty acids as amides with 2-methylpropanamine (isobutylamine) or 2-methylbutanamine, amines which are probably decarboxylation products from valine and isoleucine respectively. The acid portions are predominantly C_{11} and C_{12} diene-diynes or tetraenes (Figure 3.11). These compounds are found throughout the plant though relative proportions of individual components vary considerably. The root of *E. purpurea* contains at least 12 alkylamides (about 0.6%), of which C_{12} diene-diynes predominate; levels of these compounds fall significantly during drying and storage. Caffeic acid derivatives present include caffeic acid (see page 132), chlorogenic acid (5-*O*-caffeoylquinic acid, see page 132), 2-*O*-caffeoyltartaric acid, and cichoric acid (2,3-di-*O*-caffeoyltartaric acid) (Figure 3.11). Cichoric acid is a major component (0.6–2.1%) in *E. purpurea*, but only minor in the other species.

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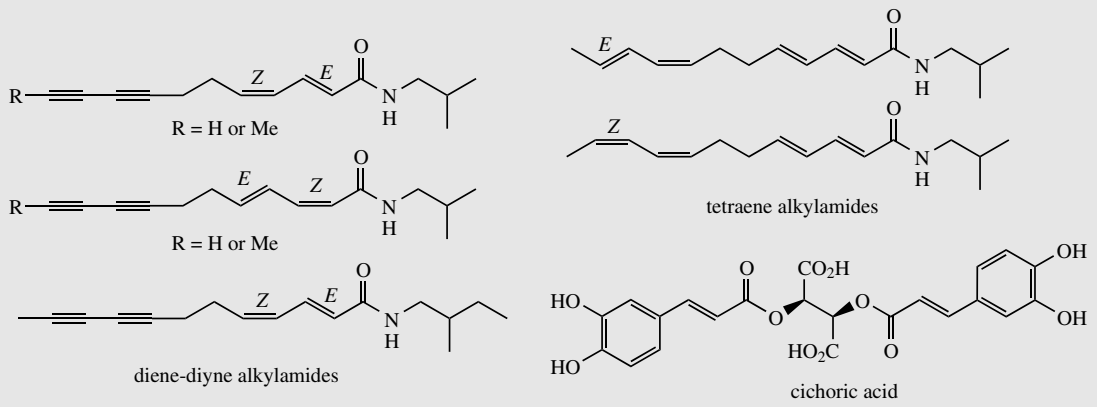

Figure 3.11

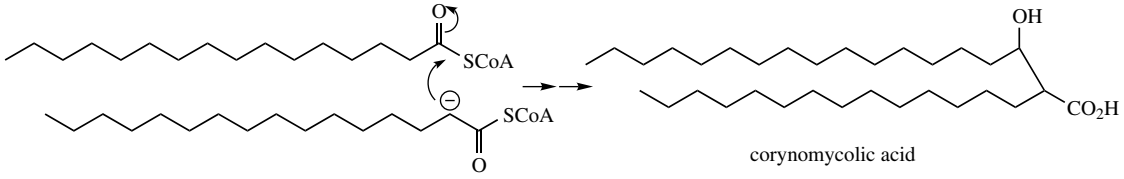

Figure 3.12

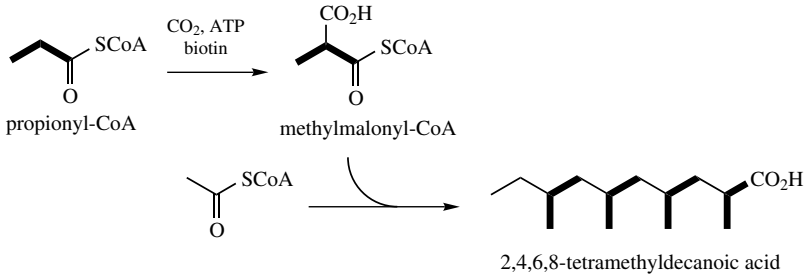

Figure 3.13

initiated by the double bond electrons. A postulated carbocation intermediate could then be discharged by accepting hydride from NADPH giving tuberculostearic acid. Alternatively, loss of a proton via cyclopropane ring formation could occur giving dihydrosterculic acid. This is known to be dehydrogenated to **sterculic acid**, an unusual fatty acid containing a highly strained cyclopropene ring. Sterculic acid is present in the seed oil from *Sterculia foetida* (Sterculiaceae) and with similar cyclopropene acids, e.g. malvalic acid, is present in edible cottonseed oil from *Gossypium* species (Malvaceae). **Malvalic acid** is produced from sterculic acid by chain shortening from the carboxyl end (Figure 3.14). Sterculic acid is an inhibitor of the Δ^9-desaturase which converts stearic acid into oleic acid and is potentially harmful to humans in that it can alter membrane permeability and inhibit reproduction.

Chaulmoogric and **hydnocarpic acids** (Figure 3.15) are cyclopentenyl fatty acids found in chaulmoogra oil expressed from seeds of *Hydnocarpus wightiana* (Flacourtiaceae). These acids are known to arise by malonate chain extension of the

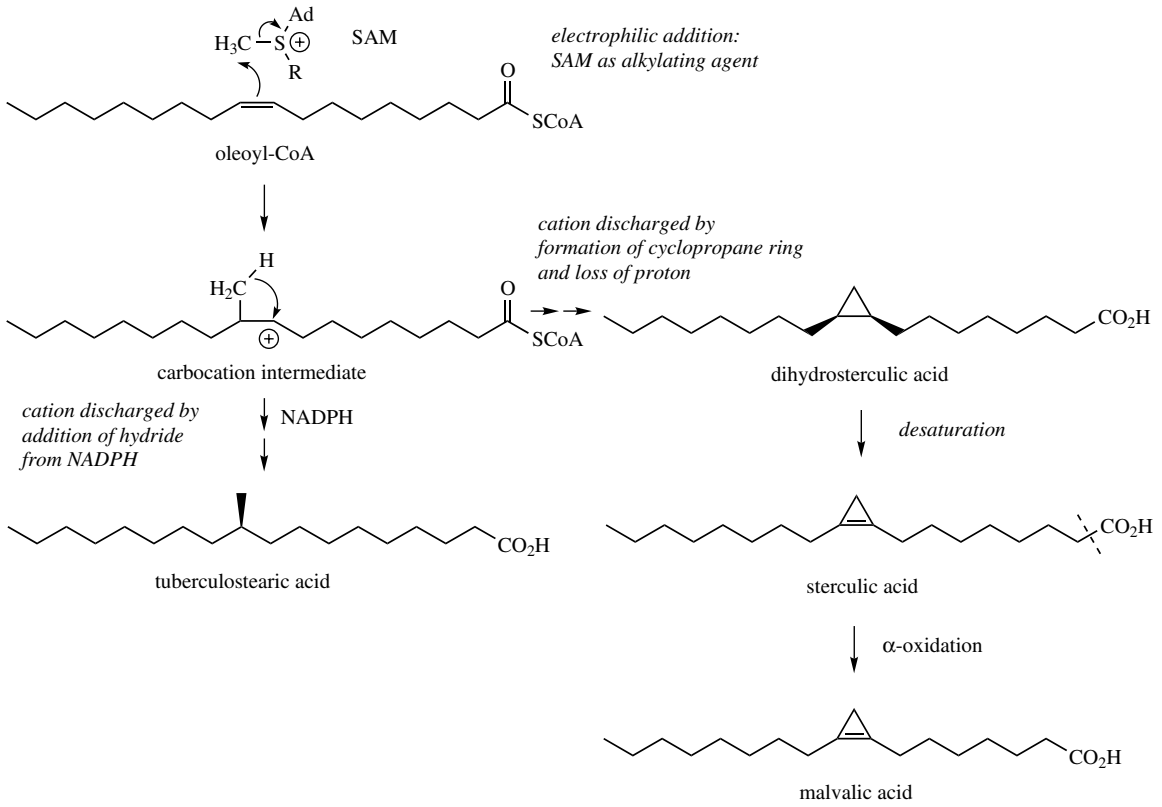

Figure 3.14

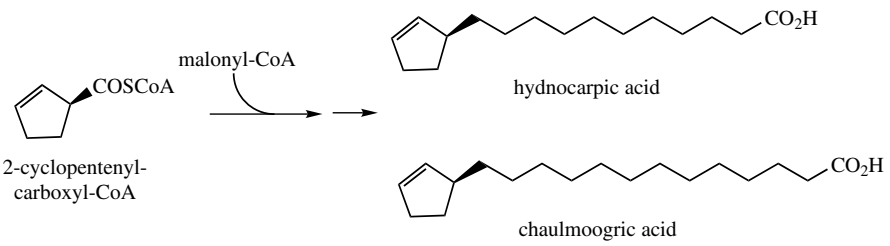

Figure 3.15

coenzyme A ester of 2-cyclopentenyl carboxylic acid as an alternative starter unit to acetate, demonstrating a further approach to unusual fatty acids. Chaulmoogra oil provided for many years the only treatment for the relief of leprosy, these two acids being strongly bactericidal towards the leprosy infective agent *Mycobacterium leprae*. Purified salts and esters of hydnocarpic and chaulmoogric acids were subsequently employed, until they were then themselves replaced by more effective synthetic agents.

PROSTAGLANDINS

The prostaglandins* are a group of modified C_{20} fatty acids first isolated from human semen and initially assumed to be secreted by the prostate gland. They are now known to occur widely in animal tissues, but only in tiny amounts, and they have been found to exert a wide variety of pharmacological effects on humans and animals. They are active at very low, hormone-like concentrations and can regulate blood pressure, contractions of smooth

muscle, gastric secretion, and platelet aggregation. Their potential for drug use is extremely high, but it has proved difficult to separate the various biological activities into individual agents.

The basic prostaglandin skeleton is that of a cyclized C_{20} fatty acid containing a cyclopentane ring, a C_7 side-chain with the carboxyl function, and a C_8 side-chain with the methyl terminus. Prostaglandins are biosynthesized from three essential fatty acids, **$\Delta^{8,11,14}$-eicosatrienoic acid** (**dihomo-γ-linolenic acid**), **$\Delta^{5,8,11,14}$-eicosatetraenoic acid** (**arachidonic acid**), and **$\Delta^{5,8,11,14,17}$-eicosapentaenoic acid**, which yield prostaglandins of the 1-, 2-, and 3-series, respectively (Figure 3.16) (see below for principles of nomenclature). The three precursors lead to products of similar structure, but with varying levels of unsaturation in the two side-chains. Some of the structures elaborated from arachidonic acid are shown in Figure 3.17. In the first reaction, arachidonic acid is converted into **prostaglandin G_2** (**PGG_2**) by an oxygenase (**cyclooxygenase**; **COX**) enzyme, which incorporates two molecules of oxygen, liberating a compound with both cyclic and acyclic peroxide functions. In arachidonic acid the methylene group flanked by two double bonds is susceptible to oxidation, probably via a free radical process. This may lead to incorporation of oxygen giving the proposed free radical intermediate. Formation of PGG_2 is then depicted as a concerted cyclization reaction, initiated by the peroxide radical, in which a second oxygen molecule is incorporated. The acyclic peroxide group in PGG_2 is then cleaved by a peroxidase to yield **prostaglandin H_2** (**PGH_2**), which occupies a central role and can be modified in several different ways. These modifications can be rationally accommodated by initial cleavage of the cyclic peroxide to the diradical; alternative ionic mechanisms may also be proposed. Quenching of the free radicals by abstraction of hydrogen atoms gives rise to **prostaglandin $F_{2\alpha}$** (**$PGF_{2\alpha}$**), whilst capture and loss of hydrogen atoms would provide either **prostaglandin E_2** (**PGE_2**) or **prostaglandin D_2** (**PGD_2**). The bicyclic system in **prostaglandin I_2** (**PGI_2**; **prostacyclin**) is envisaged as arising by involvement of a side-chain double bond, then loss of a hydrogen atom. Prostaglandin structures representative of the 1-series, e.g. **PGE_1**, or of the 3-series, e.g. **PGE_3**, can be formed in a similar way from the appropriate fatty acid precursor (Figure 3.16).

The basic skeleton of the prostaglandins is termed **prostanoic acid**, and derivatives of this system are collectively known as prostanoids. The term **eicosanoids** is also used to encompass prostaglandins, thromboxanes, and leukotrienes, which are all derived from C_{20} fatty acids (eicosanoic acids). Semi-systematic nomenclature of prostaglandins is based on the substitution pattern in the five-membered ring, denoted by a letter suffix (Figure 3.18), and the number of double bonds in the side-chains is given by a numerical subscript. Greek letters α and β are used to indicate the configuration at C-9, α indicating the substituent is below the plane (as

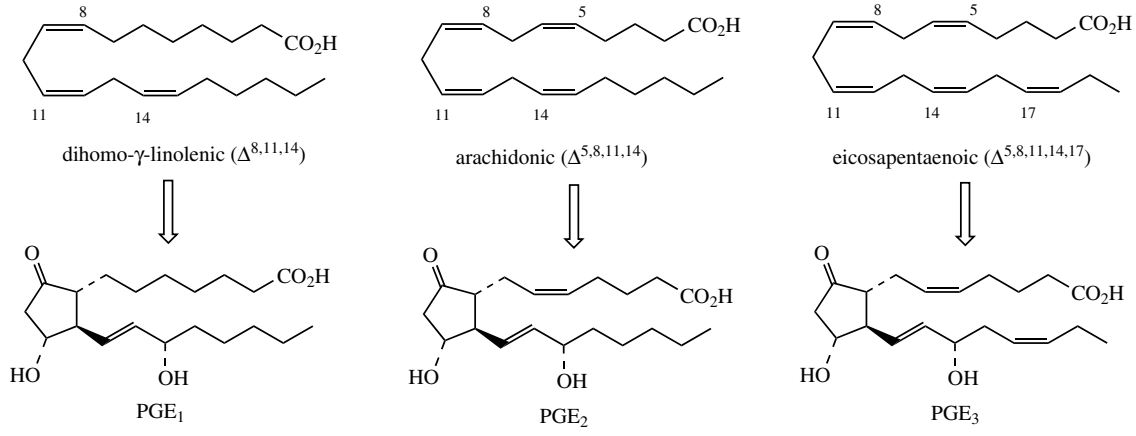

Figure 3.16

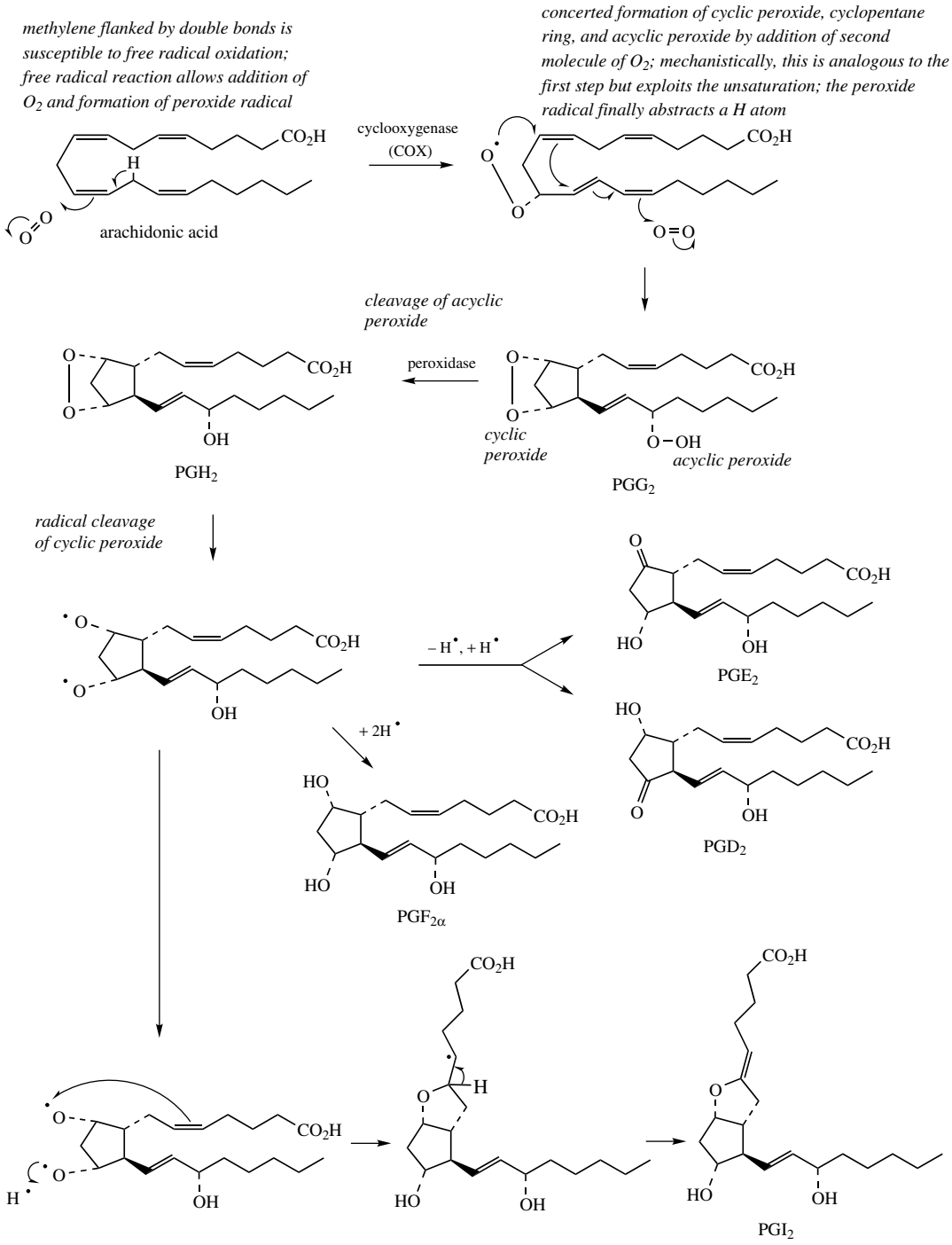

Figure 3.17

found in natural prostaglandins), and β indicating the substituent is above the plane (as in some synthetic analogues). 'Prostaglandin' is usually abbreviated to PG. Prostaglandins A, B, and C are inactive degradation products from the natural prostaglandins.

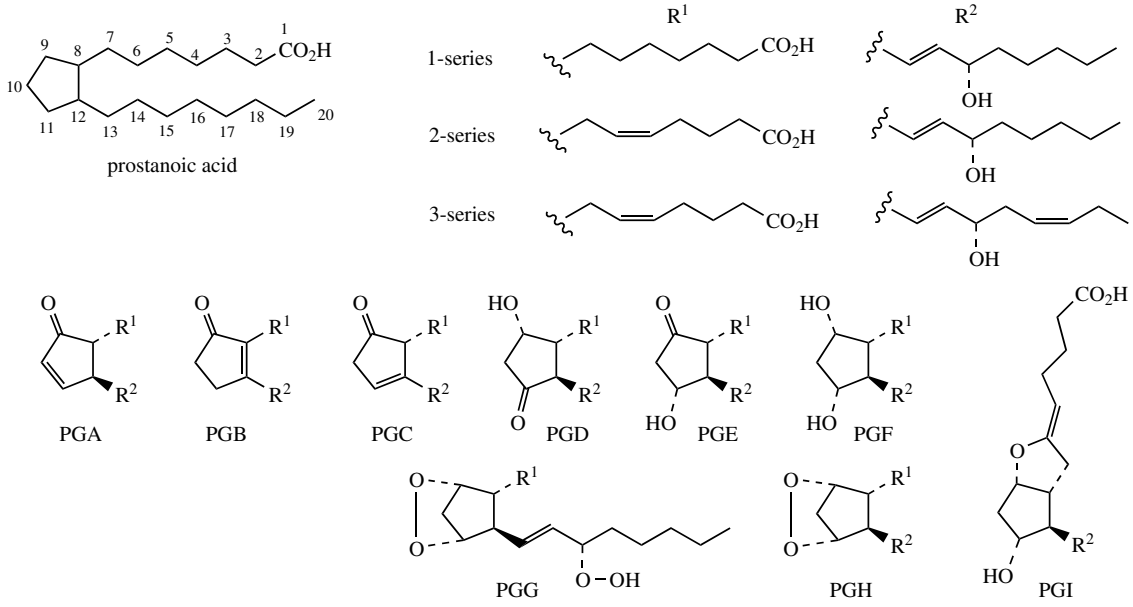

Figure 3.18

Prostaglandins

Prostaglandins occur in nearly all mammalian tissues, but only at very low concentrations. PGE_1 and $PGF_{1\alpha}$ were initially isolated from sheep seminal plasma, but these compounds and PGD_2, PGE_2, and $PGF_{2\alpha}$ are widely distributed. Animal sources cannot supply sufficient amounts for drug usage. The soft coral *Plexaura homomalla* (sea whip) from the Caribbean has been identified as having very high (2–3%) levels of prostaglandin esters, predominantly the C-15 epimer of PGA_2 (1–2%) with related structures. Prostaglandins of the A-, E-, and F-types are widely distributed in soft corals, especially *Plexaura*, but these are unlikely to provide a satisfactory and renewable natural source. Considerable effort has been exerted on the total synthesis of prostaglandins and their interconversions, and the high level of success achieved has opened up the availability of compounds for pharmacological testing and subsequent drug use. Synthetic analogues have also been developed to modify or optimize biological activity. The studies have demonstrated that biological activity is effectively confined to the natural enantiomers; the unnatural enantiomer of PGE_1 had only 0.1% of the activity of the natural isomer.

The prostaglandins display a wide range of pharmacological activities, including contraction and relaxation of smooth muscle of the uterus, the cardiovascular system, the intestinal tract, and of bronchial tissue. They may also inhibit gastric acid secretion, control blood pressure and suppress blood platelet aggregation. Some of these effects are consistent with the prostaglandins acting as second messengers, modulating transmission of hormone stimulation and thus metabolic response. Some prostaglandins in the A and J series have demonstrated potent antitumour properties. Since the prostaglandins control many important physiological processes in animal tissues, their drug potential is high, but the chances of precipitating unwanted side-effects are also high, and this has so far limited their therapeutic use.

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There is, however, much additional scope for controlling the production of natural prostaglandins in body tissues by means of specific inhibitors. Indeed it has been found that some established non-steroidal anti-inflammatory drugs (NSAIDs), e.g. aspirin, indometacin, and ibuprofen, inhibit early steps in the prostaglandin biosynthetic pathway that transform the unsaturated fatty acids into cyclic peroxides. Thus aspirin is known to irreversibly inactivate the cyclooxygenase activity (arachidonic acid $\rightarrow$ PGG_2), though not the peroxidase activity ($PGG_2 \rightarrow PGH_2$), by selective acetylation of a serine residue of the enzyme; ibuprofen and indometacin compete with arachidonic acid at the active site and are reversible inhibitors of the cyclooxygenase. A recent discovery is that two forms of the cyclooxygenase enzyme exist, designated COX-1 and COX-2. COX-1 is expressed constitutively in most tissues and cells and is thought to control synthesis of those prostaglandins important for normal cellular functions such as gastrointestinal integrity and vascular homeostasis. COX-2 is not normally present, but is inducible in certain cells in response to inflammatory stimuli, resulting in enhanced prostaglandin release in the CNS and inflammatory cells with the characteristic inflammatory response. Current NSAIDs do not discriminate between the two COX enzymes, and so this leads to both therapeutic effects via inhibition of COX-2, and adverse effects such as gastrointestinal problems, ulcers, and bleeding via inhibition of COX-1. Because of differences in the nature of the active sites of the two enzymes, it has now been possible to develop agents that can inhibit COX-2 rather than COX-1 as potential new anti-inflammatory drugs. The first of these, meloxicam and rofecoxib, have recently been introduced for relief of pain and inflammation in osteoarthritis. The anti-inflammatory activity of corticosteroids correlates with their preventing the release of arachidonic acid from storage phospholipids, but expression of COX-2 is also inhibited by glucocorticoids.

The role of essential fatty acids (see page 46) such as linoleic and γ-linolenic acids, obtained from plant ingredients in the diet, can now be readily appreciated. Without a source of arachidonic acid, or compounds which can be converted into arachidonic acid, synthesis of prostaglandins would be compromised, and this would seriously affect many normal metabolic processes. A steady supply of prostaglandin precursors is required since prostaglandins are continuously being synthesized and then deactivated. Prostaglandins are rapidly degraded by processes which include oxidation of the 15-hydroxyl to a ketone, reduction of the 13,14-double bond, and oxidative degradation of both side-chains.

A major area of application of prostaglandins as drugs is in obstetrics, where they are used to induce abortions during the early to middle stages of pregnancy, or to induce labour at term. **PGE_2** (**dinoprostone**) (Figure 3.19) is used in both capacities, whilst **$PGF_{2\alpha}$** (**dinoprost**) is now less commonly prescribed and restricted to abortions. $PGF_{2\alpha}$ is rapidly metabolized in body tissues (half-life less than 10 minutes), and the modified version **15-methyl $PGF_{2\alpha}$** (**carboprost**) has been developed to reduce deactivation by blocking oxidation at position 15. Carboprost is produced by oxidizing the 15-hydroxyl in a suitably-protected $PGF_{2\alpha}$, then alkylating the 15-carbonyl with a Grignard reagent. Carboprost is effective at much reduced dosage compared with dinoprost, and is of value in augmenting labour at term, especially in cases where ergometrine (see page 375) or oxytocin (see page 415) are ineffective. **Gemeprost** is another unnatural structure and is used to soften and dilate the cervix in early abortions. These agents are usually administered vaginally.

PGE_1 (**alprostadil**) differs from PGE_2 by having unsaturation in only one side-chain. Though having effects on uterine muscle, it also has vasodilator properties, and these are exploited for maintaining new-born infants with congenital heart defects, facilitating blood oxygenation prior to corrective surgery. The very rapid metabolism of PGE_1 means this drug must be

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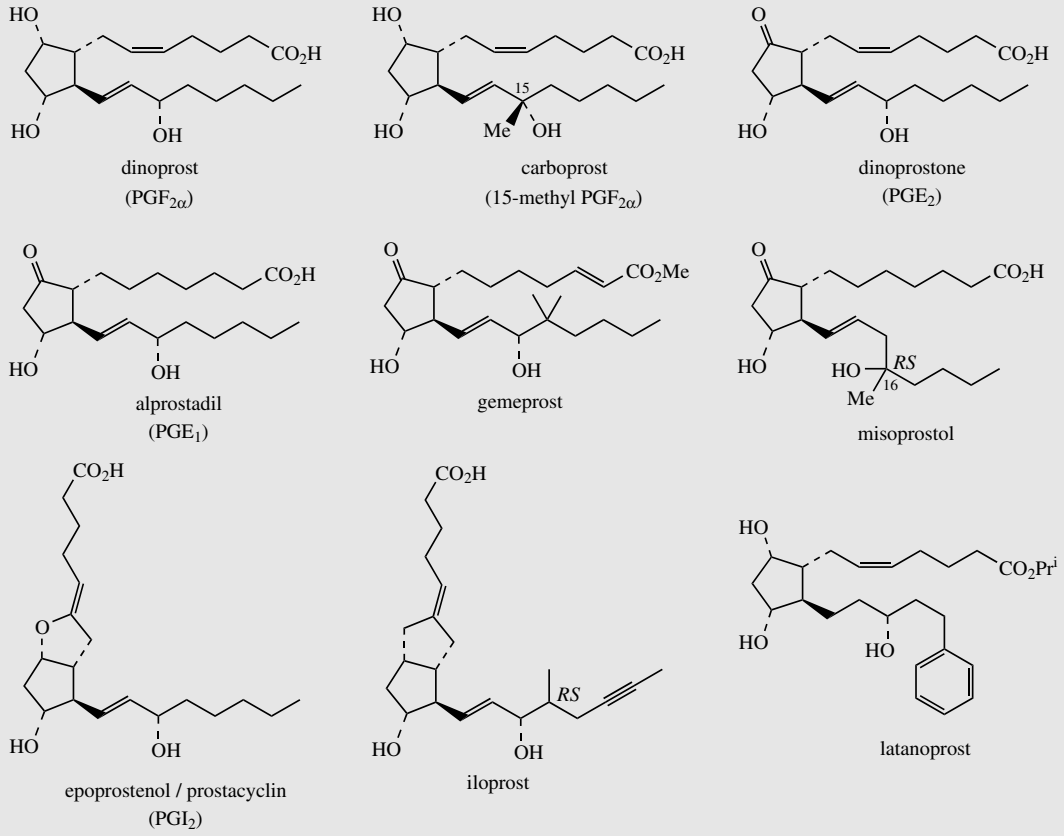

Figure 3.19

delivered by continuous intravenous infusion. Alprostadil is also of value in male impotence, self-injectable preparations being used to achieve erection of the penis. An interesting modification to the structure of PGE_1 is found in the analogue **misoprostol**. This compound has had the oxygenation removed from position 15, transferred to position 16, plus alkylation at position 16 to reduce metabolism (compare 15-methyl $PGF_{2\alpha}$ above). These modifications result in an orally active drug which inhibits gastric secretion effectively and can be used to promote healing of gastric and duodenal ulcers. In combination with non-specific NSAIDs, it can significantly lower the incidence of gastrointestinal side-effects such as ulceration and bleeding.

PGI_2 (**epoprostenol**, **prostacyclin**) reduces blood pressure and also inhibits platelet aggregation by reducing calcium concentrations. It is employed to inhibit blood clotting during renal dialysis, but its very low half-life (about 3 minutes) again necessitates continuous intravenous administration. The tetrahydrofuran ring is part of an enol ether and is readily opened by hydration, leading to 6-ketoprostaglandin $F_{1\alpha}$ (Figure 3.20). **Iloprost** (Figure 3.19) is a stable carbocyclic analogue of potential use in the treatment of thrombotic diseases.

Latanoprost (Figure 3.19) is a recently introduced prostaglandin analogue which increases the outflow of aqueous humour from the eye. It is thus used to reduce intraocular pressure in the treatment of the eye disease glaucoma.

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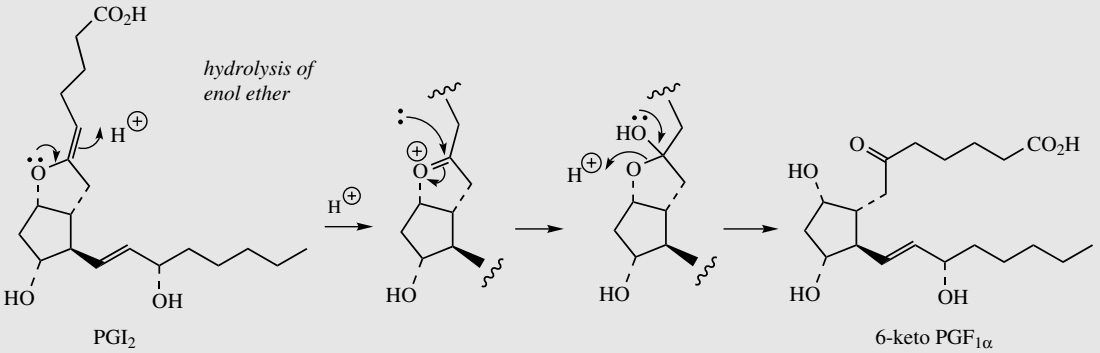

Figure 3.20

Isoprostanes

Isoprostanes represent a new class of prostaglandin-like compounds produced *in vivo* in humans and animals by non-enzymic free-radical-mediated oxidation of membrane-bound polyunsaturated fatty acids. An isomer of $PGF_{2\alpha}$ in which the two alkyl substituents on the five-membered ring were arranged *cis* rather than *trans* was detected in human urine and was the first of these compounds to be characterized. This compound was initially termed 8-*iso*-$PGF_{2\alpha}$, or 8-*epi*-$PGF_{2\alpha}$, though as many more variants in the isoprostane series were discovered it is now termed $iPF_{2\alpha}$-III (Figure 3.21). The last figure refers to the compound being of type III, with eight types being differentiated by the nature of the non-carboxylic chain. Compounds may be formed from linolenic acid and γ-linolenic acid, as well as from arachidonic, eicosapentaenoic, and dihomo-γ-linolenic acids. Structural characteristics of the four classes of isoprostanes derived from arachidonic acid are shown in Figure 3.22; the letter code as in prostaglandin nomenclature is used to define the ring substitution pattern. The four types of isoprostane shown in Figure 3.22 can be viewed as arising by a free radical mechanism which resembles the enzyme-mediated formation of prostaglandins shown in Figure 3.17. The varying side-chain substituents arise by utilizing different double bonds from the several available in the cyclization mechanism, and incorporating an oxygen atom from molecular oxygen at different positions. Many variants are produced because chemical processes rather than enzyme-controlled processes are employed. Free-radical-derived isomers of leukotrienes and thromboxanes have also been reported.

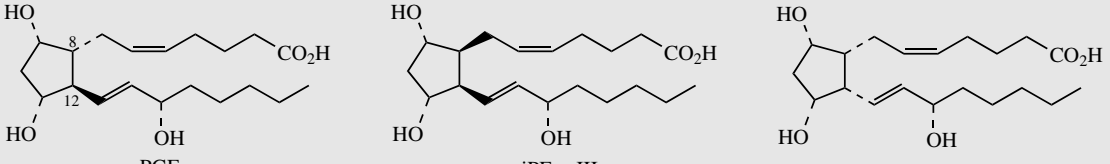

Figure 3.21

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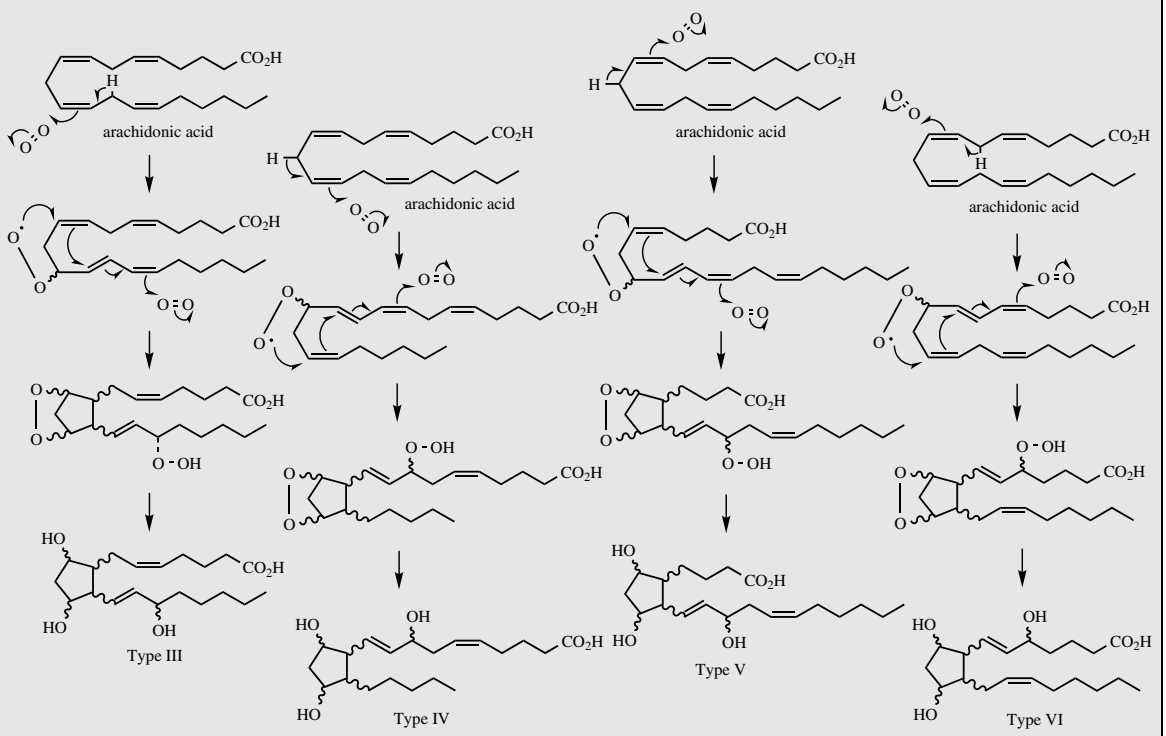

Figure 3.22

Interest in these isoprostanoid derivatives stems partly from the finding that certain compounds possess biological activity, probably via interaction with receptors for prostaglandins. For example, $iPF_{2\alpha}$-III is a potent vasoconstrictor and also aggregates platelets, whilst 8,12-*iso*-$iPF_{2\alpha}$-III (Figure 3.21) possesses activity similar to $PGF_{2\alpha}$. Another potential application relates to their origin via free radical peroxidation of unsaturated fatty acids. Free radicals are implicated in inflammatory and degenerative diseases such as atherosclerosis, cancer, and Alzheimer's disease. Isoprostane analysis of urine or serum may thus allow non-invasive monitoring of oxidative damage as a insight into these disease states.

THROMBOXANES

An intriguing side-branch from the prostaglandin pathway leads to thromboxanes* (Figure 3.23). The peroxide and cyclopentane ring functions of PGH_2 are cleaved and restructured to form **thromboxane A_2** (**TXA_2**), which contains a highly strained four-membered oxetane ring. TXA_2 is highly unstable, and reacts readily with nucleophiles. In an aqueous environment, it reacts to yield the hemiacetal **thromboxane B_2** (**TXB_2**).

LEUKOTRIENES

Yet another variant for the metabolism of arachidonic acid is the formation of leukotrienes*, a series of fatty acid derivatives with a conjugated triene functionality, and first isolated from leukocytes. In a representative pathway (others have

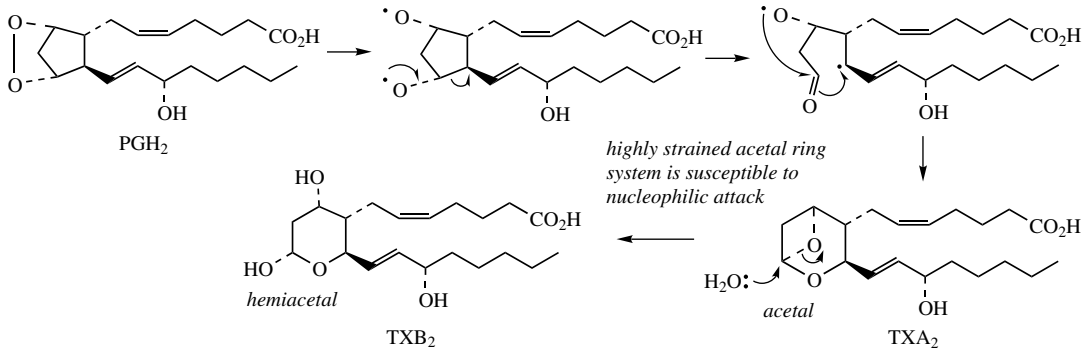

Figure 3.23

Thromboxanes

The thromboxanes were isolated from blood platelets, and whilst TXA_2 showed high biological activity TXB_2 was only weakly active. TXA_2 causes blood platelets to aggregate to form a clot or thrombus, by increasing cytoplasmic calcium concentrations and thus deforming the platelets which then fuse together. It has the opposite effect to PGI_2, and presumably the development of thrombosis reflects an imbalance in the two activities. Both compounds are produced from the same precursor, PGH_2, which is converted in the blood platelets to TXA_2, and in the blood vessel wall to PGI_2. Thromboxanes A_3 and B_3 have also been isolated from blood platelets, are structurally analogous to prostaglandins in the 3-series, and are derived from $\Delta^{5,8,11,14,17}$-eicosapentaenoic acid. TXA_3 is not strongly aggregatory towards blood platelets. The highly unstable nature of the biologically active thromboxanes has made their synthesis difficult, and drug use of natural structures will probably be impracticable. It is likely that most efforts will be directed towards thromboxane antagonists to help reduce blood platelet aggregation in thrombosis patients. The value of aspirin in preventing cardiovascular disease is now known to be related to inhibition of thromboxane A_2 biosynthesis in platelets.

Leukotrienes

The leukotrienes are involved in allergic responses and inflammatory processes. An antigen–antibody reaction can result in the release of compounds such as histamine (see page 379) or materials termed slow reacting substance of anaphylaxis (SRSA). These substances are then mediators of hypersensitive reactions such as hay fever and asthma. Structural studies have identified SRSA as a mixture of LTC_4, LTD_4 and LTE_4. These cysteine-containing leukotrienes are powerful bronchoconstrictors and vasoconstrictors, and induce mucus secretion, the typical symptoms of asthma. LTE_4 is some 10–100-fold less active than LTD_4, so that degradation of the peptide side-chain represents a means of eliminating leukotriene function. LTB_4 appears to facilitate migration of leukocytes in inflammation, and is implicated in the pathology of psoriasis, inflammatory bowel disease, and arthritis. The biological effects of leukotrienes are being actively researched to define the cellular processes involved. This may lead to the development of agents to control allergic and inflammatory reactions. Drugs inhibiting the formation of LTC_4 and LTB_4 are in clinical trials, whilst montelukast and zafirlukast have been introduced as orally active leukotriene (LTD_4) receptor antagonists for the prophylaxis of asthma.

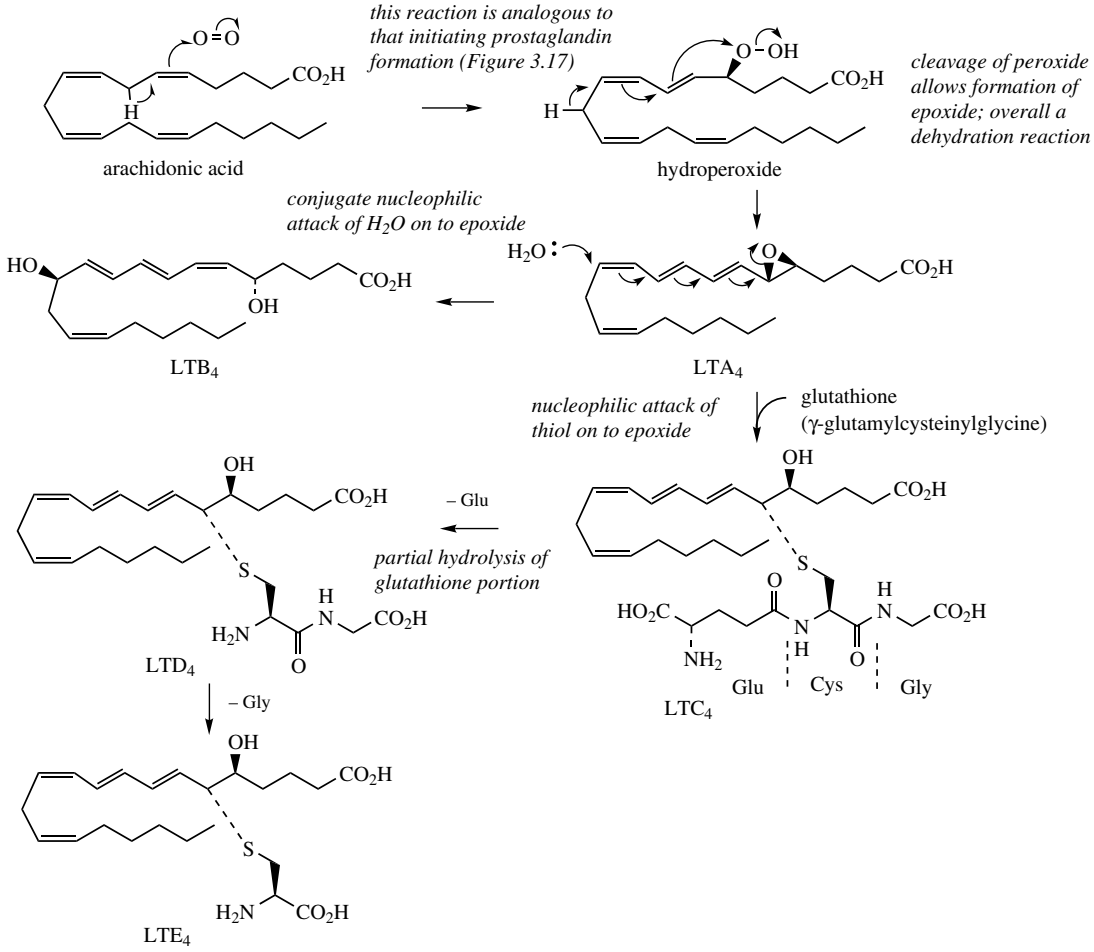

Figure 3.24

been characterized) (Figure 3.24), **arachidonic acid** is converted into a hydroperoxide, the point of oxygenation being C-5, rather than C-11 as in the prostaglandin pathway (Figure 3.17). This compound loses water via formation of an epoxide ring, giving **leukotriene A_4** (**LTA_4**). This unstable allylic epoxide may hydrolyse by conjugate addition giving **leukotriene B_4** (**LTB_4**), or alternatively the epoxide may be attacked directly by a nucleophile, in this case the sulphur atom of the tripeptide glutathione (γ-glutamylcysteinylglycine) (Figure 3.24). The adduct produced in the latter reaction is termed **leukotriene C_4** (**LTC_4**). Partial hydrolysis in the tripeptide fragment then leads to **leukotriene D_4** (**LTD_4**) and **leukotriene E_4** (**LTE_4**). Analogues, e.g. LTA_3 and LTA_5, are also known, and these are derived from **$\Delta^{5,8,11}$-eicosatrienoic acid** and **$\Delta^{5,8,11,14,17}$-eicosapentaenoic acid** respectively. The subscript numeral indicates the total number of double bonds in the leukotriene chain.

AROMATIC POLYKETIDES

For fatty acid biosynthesis, reduction after each condensation step affords a growing hydrocarbon chain. In the absence of this reduction process, the growing poly-β-keto chain needs to be stabilized on the enzyme surface until the chain length is complete, at which point cyclization or other reactions can occur. The poly-β-keto ester is very reactive, and there are various possibilities

for undergoing intramolecular Claisen or aldol reactions, dictated of course by the nature of the enzyme and how the substrate is folded. Methylenes flanked by two carbonyls are activated, allowing formation of carbanions/enolates and subsequent reaction with ketone or ester carbonyl groups, with a natural tendency to form strain-free six-membered rings.

Cyclization: Simple Phenols

The polyketo ester (Figure 3.25), formed from four acetate units (one acetate starter group and three malonate chain extension units) is capable of being folded in at least two ways, A and B (Figure 3.25). For A, ionization of the α-methylene allows aldol addition on to the carbonyl six carbons distant along the chain, giving the tertiary alcohol. Dehydration occurs as in most chemical aldol reactions, giving the alkene, and enolization follows to attain the stability conferred by the aromatic ring. The thioester bond (to coenzyme A or ACP) is then hydrolysed to produce **orsellinic acid**. Alternatively, folding of the polyketo ester as in B allows a Claisen reaction to occur, which, although mechanistically analogous to the aldol reaction, is

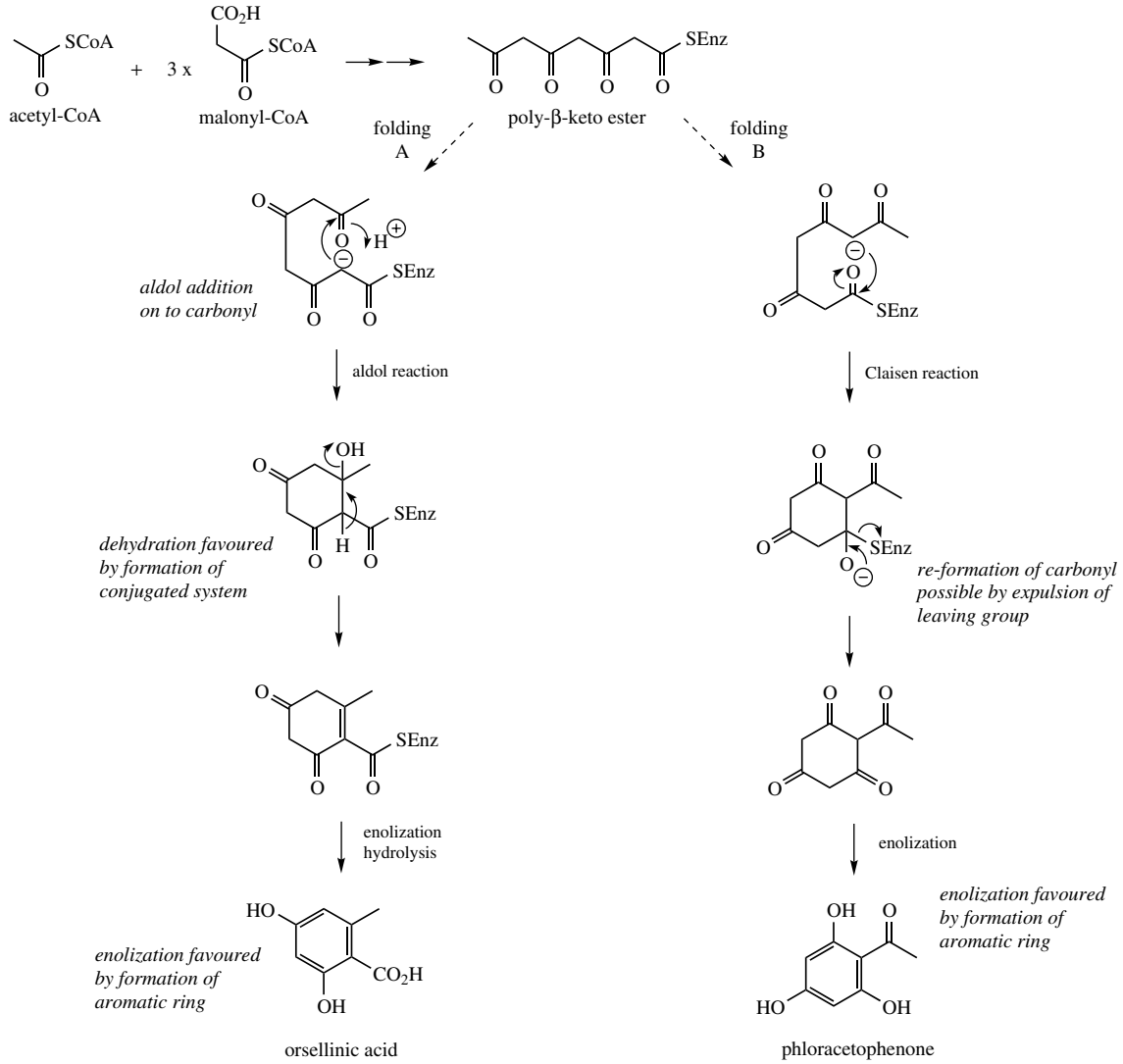

Figure 3.25

terminated by expulsion of the thiol leaving group, and direct release from the enzyme. Enolization of the cyclohexatrione produces **phloracetophenone**. As with fatty acid synthases, the whole sequence of reactions is carried out by an enzyme complex which converts acetyl-CoA and malonyl-CoA into the final product without giving any detectable free intermediates. These enzyme complexes combine **polyketide synthase** and **polyketide cyclase** activities and share many structural similarities with fatty acid synthases, including an acyl carrier protein with a phosphopantatheine group, a reactive cysteine residue, and an analogous β-ketoacyl synthase activity.

A distinctive feature of an aromatic ring system derived through the acetate pathway is that several of the carbonyl oxygens of the poly-β-keto system are retained in the final product. These end up on alternate carbons around the ring system. Of course, one or more might be used in forming a carbon–carbon bond, as in orsellinic acid. Nevertheless, this oxygenation on alternate carbon atoms, a *meta* oxygenation pattern, is usually easily recognizable, and points to the biosynthetic origin of the molecule. This *meta* oxygenation pattern contrasts to that seen on aromatic rings formed via the shikimate pathway (see Chapter 4).

6-methylsalicylic acid (Figure 3.26) is a metabolite of *Penicillium patulum*, and differs from orsellinic acid by the absence of a phenol group at position 4. It is also derived from acetyl-CoA and three molecules of malonyl-CoA, and the 'missing' oxygen function is removed during the biosynthesis. Orsellinic acid is not itself deoxygenated to 6-methylsalicylic acid. The enzyme 6-methylsalicylic acid synthase requires NADPH as cofactor, and removes the oxygen function by reduction of a ketone to an alcohol, followed by a dehydration step (Figure 3.26). Whilst on paper this could be carried out on an eight-carbon intermediate involved in orsellinic acid biosynthesis (Figure 3.25), there is evidence that the reduction/dehydration actually occurs on a six-carbon intermediate as the chain is growing (compare fatty acid biosynthesis, page 36), prior to the final chain extension (Figure 3.26). Aldol condensation, enolization, and release from the enzyme then generate 6-methylsalicylic acid. Important evidence for reduction occurring at the C_6 stage as shown in Figure 3.26 comes from the formation of triacetic acid lactone if NADPH is omitted from the enzymic incubation.

The folding of a polyketide chain can be established by labelling studies, feeding carbon-labelled sodium acetate to the appropriate organism and establishing the position of labelling in the final product by chemical degradation and counting (for the radioactive isotope ^{14}C), or by NMR spectrometry (for the stable isotope ^{13}C). ^{13}C NMR spectrometry is also valuable in establishing the location of intact C_2 units derived from feeding $^{13}C_2$-labelled acetate. This is exemplified in

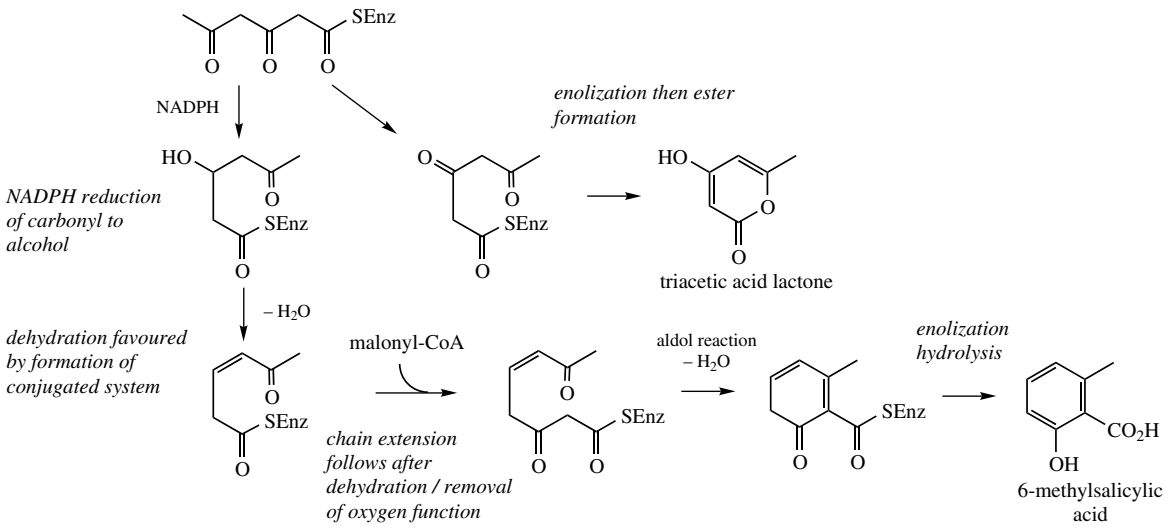

Figure 3.26

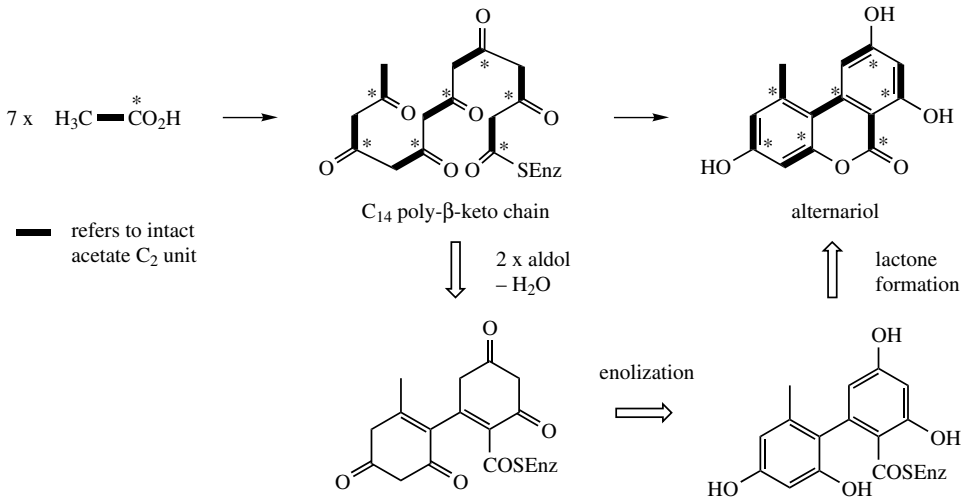

Figure 3.27

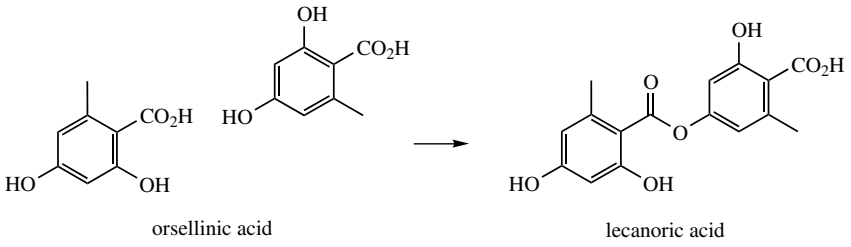

Figure 3.28

Figure 3.27, where **alternariol**, a metabolite from the mould *Alternaria tenuis*, can be established to be derived from a single C_{14} polyketide chain, folded as shown, and then cyclized. Whilst the precise sequence of reactions involved is not known, paper chemistry allows us to formulate the essential features. Two aldol condensations followed by enolization in both rings would give a biphenyl, and lactonization would then lead to alternariol. The oxygenation pattern in alternariol shows alternate oxygens on both aromatic rings, and an acetate origin is readily surmised, even though some oxygens have been used in ring formation processes. The lone methyl 'start-of-chain' is also usually very obvious in acetate-derived compounds, though the carboxyl 'end-of-chain' can often react with convenient hydroxyl functions, which may have arisen through enolization, and lactone or ester functions are thus reasonably common. For example, **lecanoric acid** is a **depside** (an ester formed from two phenolic acids) found in lichens and produced from two orsellinic acid molecules (Figure 3.28).

Structural Modifications: Anthraquinones

A number of natural anthraquinone derivatives are also excellent examples of acetate-derived structures. **Endocrocin** (Figure 3.29) found in species of *Penicillium* and *Aspergillus* fungi is formed by folding a polyketide containing eight C_2 units to form the periphery of the carbon skeleton. Three aldol-type condensations would give a hypothetical intermediate 1, and, except for a crucial carbonyl oxygen in the centre ring, endocrocin results by enolization reactions, one of which involves the vinylogous enolization $-CH_2-CH=CH-CO- \rightarrow -CH=CH-CH=C(OH)-$. The additional carbonyl oxygen must be introduced at some stage

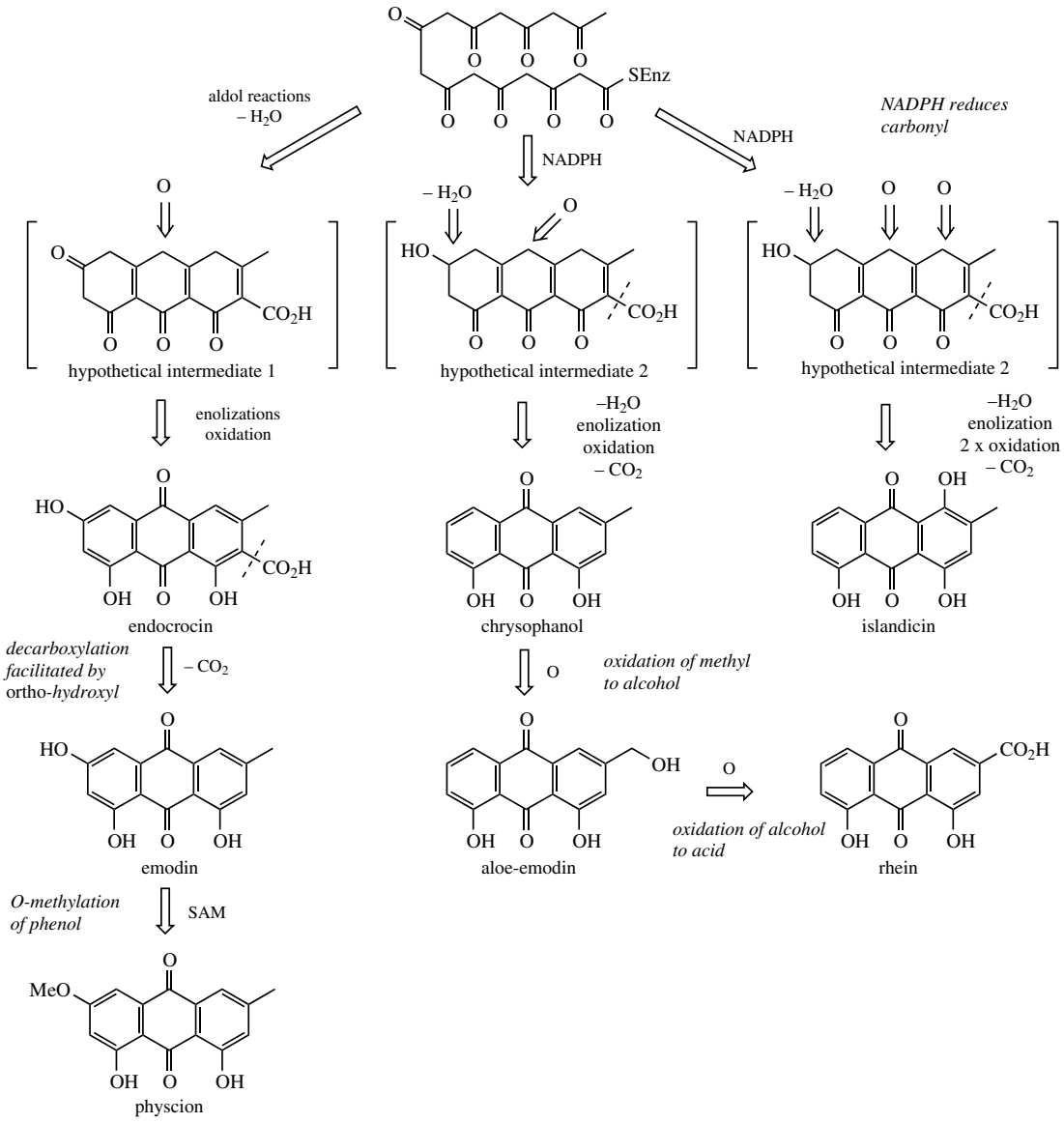

Figure 3.29

during the biosynthesis by an oxidative process, for which we have little information. **Emodin**, a metabolite of some *Penicillium* species, but also found in higher plants, e.g. *Rhamnus* and *Rumex* species, would appear to be formed from endocrocin by a simple decarboxylation reaction. This is facilitated by the adjacent phenol function (see page 20). *O*-Methylation of emodin would then lead to **physcion**. **Islandicin** is another anthraquinone pigment produced by *Penicillium islandicum*, and differs from emodin in two ways. One hydroxyl is missing, and a new hydroxyl has been incorporated adjacent to the methyl. Without any evidence for the sequence of such reactions, the structure of intermediate 2 shows the result of three aldol condensations and reduction of a carbonyl. A dehydration reaction, two oxidations, and a decarboxylation are necessary to attain the islandicin structure. In **chrysophanol**, **aloe-emodin**, and **rhein**, the same oxygen function is lost by reduction as in islandicin, and decarboxylation also occurs. The three compounds

are interrelated by a sequential oxidation of the methyl in chrysophanol to a hydroxymethyl in aloe-emodin, and a carboxyl in rhein.

These structural modifications undergone by the basic polyketide are conveniently considered under two main headings, according to the timing of the steps in the synthetic sequence. Thus, 'missing' oxygen functions appear to be reduced out well before the folded and cyclized polyketide is detached from the enzyme, and are mediated by a reductase component of the enzyme complex during chain elongation *before the cyclization reaction*. On the other hand, reactions like the decarboxylation, *O*-methylation, and sequential oxidation of a methyl to a carboxyl are representative of transformations occurring *after the cyclization reaction*. It is often possible to demonstrate these later conversions by the isolation of enzymes catalysing the individual steps. Most of the secondary transformations are easily rationalized by careful consideration of the reactivity conferred on the molecule by the alternating and usually phenolic oxygenation pattern. These oxygens activate adjacent sites creating nucleophilic centres. Introduction of additional hydroxyl groups *ortho* or *para* to an existing phenol will be facilitated (see page 26), allowing the extra hydroxyl of islandicin to be inserted, for example. *Ortho*- or *para*-diphenols are themselves susceptible to further oxidation in certain circumstances, and may give rise to *o*- and *p*-quinones (see page 25). The quinone system in anthraquinones is built up by an oxidation of the central cyclohexadienone ring, again at a nucleophilic centre activated by the enone system. Methyls on an aromatic ring are also activated towards oxidation, facilitating the chrysophanol → aloe-emodin oxidation, for example. Decarboxylation, e.g. endocrocin → emodin, is readily achieved in the presence of an *ortho* phenol function, though a *para* phenol can also facilitate this (see page 20).

It is now appreciated that the assembly of the anthraquinone skeleton (and related polycyclic structures) is achieved in a step-wise sequence. After the polyketide chain is folded, the ring at the centre of the fold is formed first, followed in turn by the next two rings. The pathway outlined for the biosynthesis of endocrocin and emodin is shown in Figure 3.30. Mechanistically, there is little difference between this and the speculative pathway of Figure 3.29, but the sequence of reactions is altered. Decarboxylation appears to take place before aromatization of the last-formed ring system, and tetrahydroanthracene intermediates such as atrochrysone carboxylic acid and atrochrysone are involved. These dehydrate to the anthrones **endocrocin anthrone** and **emodin anthrone**, respectively, prior to introduction of the extra carbonyl oxygen as a last transformation in the production of anthraquinones. This oxygen is derived from O_2.

Note that many other natural anthraquinone structures are not formed via the acetate pathway, but by a more elaborate sequence involving shikimate and an isoprene unit (see page 158). Such structures do not contain the characteristic *meta* oxygenation pattern, and often have oxygenation in only one aromatic ring (see page 164).

Emodin, physcion, chrysophanol, aloe-emodin, and rhein form the basis of a range of purgative anthraquinone derivatives found in long-established laxatives such as Senna*, Cascara*, Frangula*, Rhubarb*, and Aloes*. The free anthraquinones themselves have little therapeutic activity and need to be in the form of water-soluble glycosides to exert their action. Although simple anthraquinone *O*-glycosides are present in the drugs, the major purgative action arises from compounds such as **cascarosides**, e.g. cascaroside A (Figure 3.33), which are both *O*- and *C*-glycosides, and **sennosides**, e.g. sennoside A (Figure 3.33), which are dianthrone *O*-glycosides. These types of derivative are likely to be produced from intermediate anthrone structures. This could act as substrate for both *O*- and *C*-glucosylation, employing the glucose donor UDPglucose (see page 29), and would generate a cascaroside structure (Figure 3.31). Alternatively, a one-electron oxidation allows oxidative coupling (see page 28) of two anthrone systems to give a dianthrone (Figure 3.32). This can be formulated as direct oxidation at the benzylic $-CH_2-$, or via the anthranol, which is the phenolic tautomer of the anthrone (Figure 3.32). Glycosylation of the dianthrone system would then give a sennoside-like product. However, further oxidative steps can create a dehydrodianthrone, and then allow coupling of the aromatic rings through **protohypericin** to give a naphthodianthrone, e.g. **hypericin** (Figure 3.32). The reactions of Figure 3.32 can be achieved

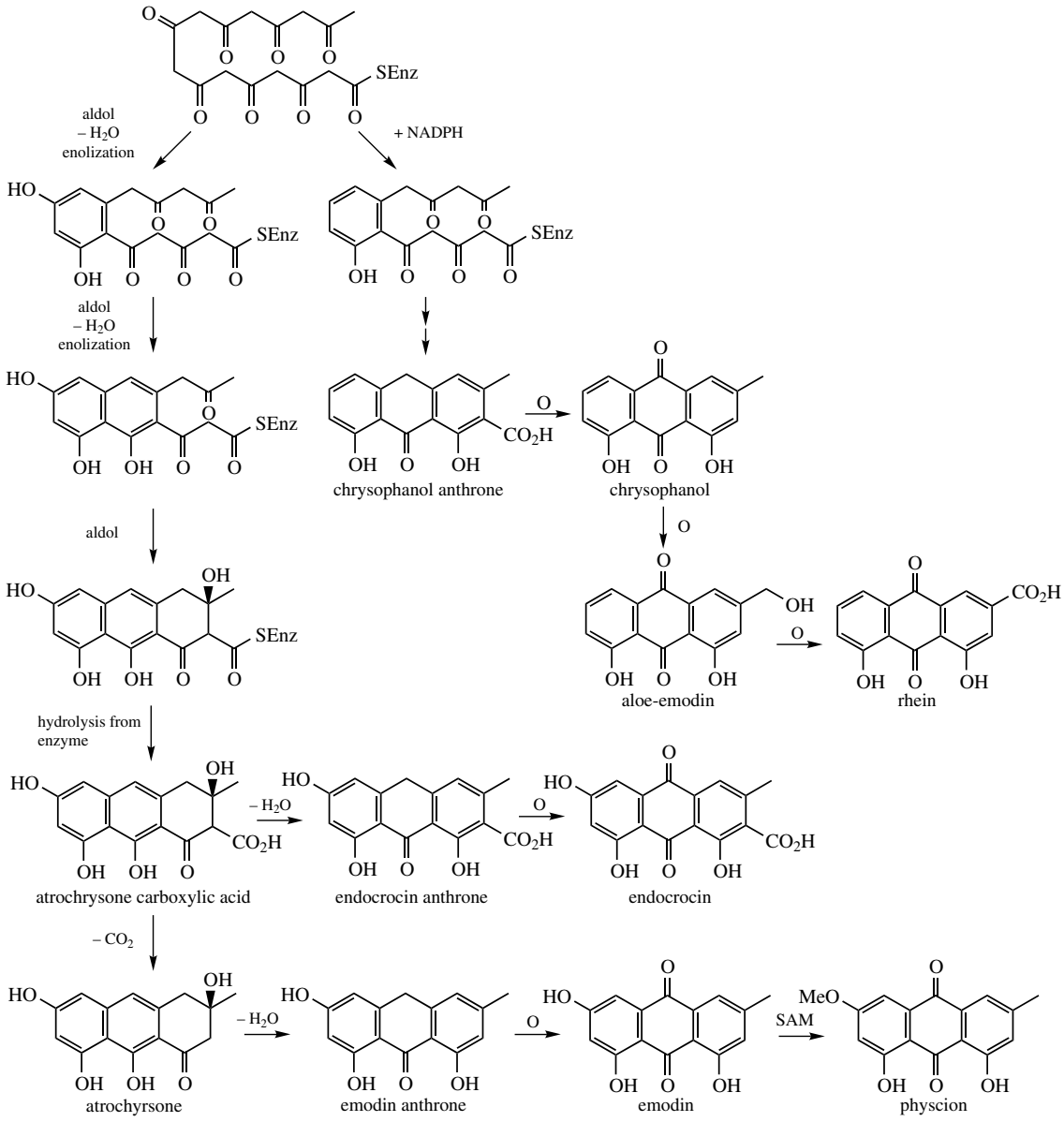

Figure 3.30

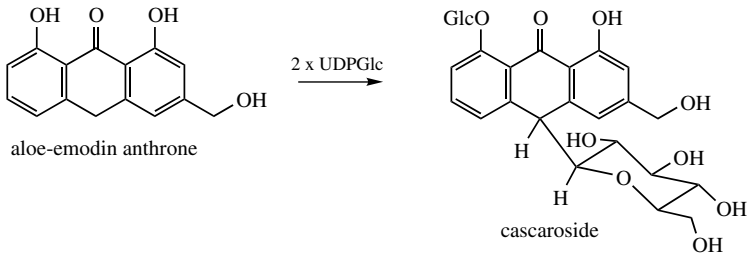

Figure 3.31

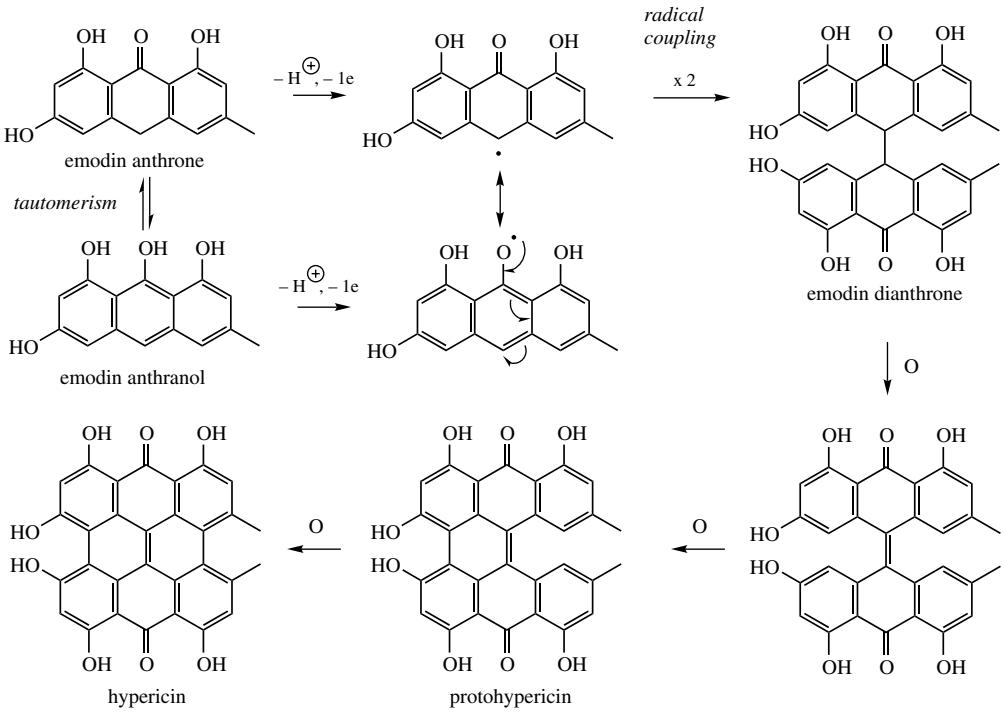

Figure 3.32

chemically by passing air into an alkaline solution of **emodin anthrone**. **Hypericin** is found in cultures of *Dermocybe* fungi, and is also a constituent of St John's Wort, *Hypericum perforatum* (Guttiferae/Hypericaceae), which is a popular herbal medicine in the treatment of depression. The naphthodianthrones have no purgative action, but hypericin can act as a photosensitizing agent in a similar manner to furocoumarins (see page 146). Thus ingestion of hypericin results in an increased absorption of UV light and can lead to dermatitis and burning. Hypericin is also being investigated for its antiviral activities, in particular for its potential activity against HIV.

Senna

Senna leaf and fruit are obtained from *Cassia angustifolia* (Leguminosae/Fabaceae), known as Tinnevelly senna, or less commonly from *Cassia senna* (*syn C. acutifolia*), which is described as Alexandrian senna. The plants are low, branching shrubs, *C. angustifolia* being cultivated in India and Pakistan, and *C. senna* being produced in the Sudan, much of it from wild plants. Tinnevelly senna is cultivated in wetter conditions than Alexandrian senna, which gives more luxuriant growth. Early harvests provide leaf material whilst later on, both leaf and fruit (senna pods) are obtained, a mixture which is separated by sieving (Alexandrian) or hand picking after drying (Tinnevelly). There are no significant differences in the chemical constituents of the two sennas, or between leaf and fruit drug. However, amounts of the active constituents do vary, and appear to be a consequence of cultivation conditions and the time of harvesting of the plant material.

The active constituents in both senna leaf and fruit are dianthrone glycosides, principally sennosides A and B (Figure 3.33). These compounds are both di-*O*-glucosides of rhein

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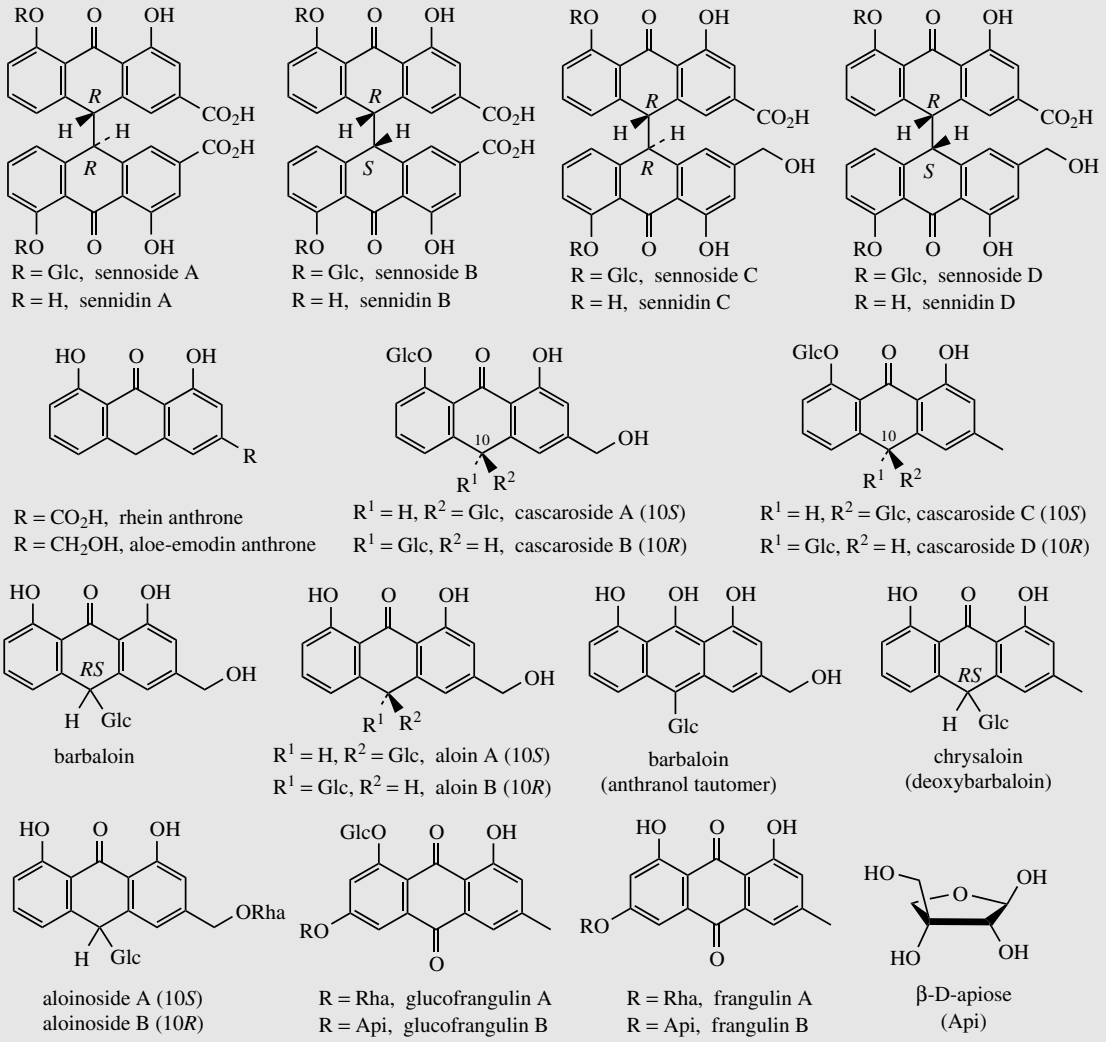

Figure 3.33

dianthrone (sennidins A and B), and liberate these aglycones on acid hydrolysis, or the anthraquinone rhein (Figure 3.30) on oxidative hydrolysis (e.g. aq HNO_3 or H_2O_2/HCl). Sennidins A and B are optical isomers: sennidin A is dextrorotatory (+) whilst sennidin B is the optically inactive *meso* form. Minor constituents include sennosides C and D (Figure 3.33), which are also a pair of optical isomers, di-*O*-glucosides of heterodianthrones sennidins C and D. Sennidin C is dextrorotatory, whilst sennidin D is optically inactive, approximating to a *meso* form in that the modest change in substituent does not noticeably affect the optical rotation. Oxidative hydrolysis of sennosides C and D would produce the anthraquinones rhein and aloe-emodin (Figure 3.30). Traces of other anthraquinone glycoside derivatives are also present in the plant material. Much of the sennoside content of the dried leaf appears to be formed by enzymic oxidation of anthrone glycosides during the drying process. Fresh leaves

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and fruits also seem to contain primary glycosides which are more potent than sennosides A and B, and which appear to be partially hydrolysed to sennosides A and B (the secondary glycosides) by enzymic activity during collection and drying. The primary glycosides contain additional glucose residues.

Senna leaf suitable for medicinal use should contain not less than 2.5% dianthrone glycosides calculated in terms of sennoside B. The sennoside content of Tinnevelly fruits is between 1.2 and 2.5%, that of Alexandrian fruits being 2.5–4.5%. Senna preparations, in the form of powdered leaf, powdered fruit, or extracts, are typically standardized to a given sennoside content. Non-standardized preparations have unpredictable action and should be avoided. Senna is a stimulant laxative and acts on the wall of the large intestine, increasing peristaltic movement. After oral administration, the sennosides are transformed by intestinal flora into rhein anthrone (Figure 3.33), which appears to be the ultimate purgative principle. The glycoside residues in the active constituents are necessary for water-solubility and subsequent transportation to the site of action. Although purgative action is provided by the aglycones, including anthraquinones, these materials are conjugated and excreted in the urine after oral administration rather than being transported to the colon. Senna is a purgative drug suitable for either habitual constipation, or for occasional use, and is widely prescribed.

Cascara

Cascara is the dried bark of the cascara buckthorn *Rhamnus purshianus* (Rhamnaceae), a small tree native to the forests of the Pacific coast of North America. Most of the drug material is gathered from wild trees in Oregon, Washington, and British Columbia. Trees are felled and the bark is stripped from the trunk and branches, then dried. The fresh bark is unsuitable for drug use, causing griping and nausea, and thus the bark is stored for at least a year before being processed. During this time, enzymic hydrolysis and oxidation modify the anthraquinone-based constituents and thus the cathartic activity. Cascara preparations are mainly formulated from extracts of the bark.

Cascara bark contains about 6–9% of anthracene derivatives, 80–90% of which are anthrone *C*-glycosides. The major constituents are cascarosides A and B (Figure 3.33), which contain both *O*- and *C*-glucoside linkages, and represent a pair of optical isomers differing only in the stereochemistry of the *C*-glucoside bond. These have a substitution pattern analogous to aloe-emodin (Figure 3.30) and oxidative hydrolysis (e.g. aq HNO_3 or H_2O_2/HCl) liberates aloe-emodin. Acid hydrolysis does not cleave the *C*-glucose linkage, and instead generates barbaloin (Figure 3.33), a mixture of two diastereoisomeric forms, which have been named aloin A and aloin B. It is likely that during any chemical manipulation, the two forms may interconvert via the anthranol tautomer (Figure 3.33). Similar components in the bark, though usually present in smaller amounts than cascarosides A and B, are cascarosides C and D (Figure 3.33). These are also a pair of diastereoisomers, and have a substitution pattern analogous to chrysophanol (Figure 3.30). Hydrolysis of the *O*-glucose linkage yields chrysaloin, sometimes referred to as deoxybarbaloin. Barbaloin and chrysaloin are also found in the bark, and are thought to be breakdown products formed by enzymic hydrolysis of the cascarosides. Other compounds identified in the bark include simple anthraquinones and their *O*-glycosides, and some dianthrone derivatives.

The principal purgative activity originates from the cascarosides, the *C*-glycosides barbaloin and chrysaloin being less active when taken orally. As with the sennosides, the actual purgative

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agent is produced by the action of intestinal flora, and the cascarosides are transformed into aloe-emodin anthrone (Figure 3.33). Cascara has a similar pharmacological action to senna, i.e. it stimulates peristalsis of the large intestine, and has found major use in the correction of habitual constipation. It has a stronger effect than senna, however, and its routine usage is not now recommended.

Frangula

The bark of the alder buckthorn, *Rhamnus frangula* (Rhamnaceae) is used in a similar way to cascara, and is preferred to cascara in many European countries, though not in the UK. *Rhamnus frangula* is a small tree of European origin, and supplies of the bark come from South-Eastern Europe. The bark is also stored for a year before use. **Frangula** bark contains about 6% anthracene derivatives, mainly anthraquinone *O*-glycosides. These are derivatives of emodin (Figure 3.30) and comprise glucofrangulins A and B, and frangulins A and B (Figure 3.33). Free aglycones emodin, physcion and chrysophanol are also present.

Allied Drugs

Aloes and rhubarb have found considerable use as purgatives in the past, but they both have a rather drastic action and their use for this purpose has largely been abandoned.

Aloes consists of the dried juice from the leaves of various *Aloe* species (Liliaceae/Aloeaceae), including *A. ferox* (Cape aloes), *A. barbadensis* (Curacao aloes), and *A. perryi* (Socotrine aloes). The dark brown–black solid extract is extremely bitter, and contains 10–30% anthracene derivatives, the main component of which is barbaloin (Figure 3.33). Aloinosides A and B (Figure 3.33) are present in some varieties. Large amounts of resinous material form the bulk of the extract. Aloes is still used as a pharmaceutical aid in Compound Benzoin Tincture. The fresh mucilaginous gel obtained from *Aloe* species, particularly *Aloe vera* (= *A. barbadensis*), is held to assist wound healing, and is also widely used in skin cosmetics for its moisturizing and emollient properties. This material, mainly carbohydrate in nature (pectins and glucomannans), does not contain anthraquinone derivatives.

Rhubarb consists of the dried rhizome and root of *Rheum officinale*, *R. palmatum*, and other *Rheum* species (Polygonaceae). This contains 3–7.5% anthracene derivatives, mainly in the form of mono- and di-*O*-glucosides of rhein, physcion, and chrysophanol. Aglycones, especially rhein, are also present, and dianthrone derivatives have also been characterized. A high proportion of tannin-like materials gives rhubarb astringent as well as purgative properties. The common rhubarb cultivated for culinary use is *Rheum rhaponticum*, a species containing similar anthraquinone derivatives to the drug material, but which was not officially acceptable. In common with other *Rheum* species, this plant is considered poisonous due to the high concentration of oxalic acid present in the leaf (though not in the stem, which is edible). Toxic effects result from hypocalcaemia caused by removal of calcium from the bloodstream by formation of the insoluble calcium oxalate.

Dantron (**danthron**; 1,8-dihydroxyanthraquinone) (Figure 3.34) is known as a natural product, but for drug use is produced synthetically. It is prescribed to relieve constipation in geriatric and terminally ill patients. **Dithranol** (1,8-dihydroxyanthrone) is used as topical agent to treat troublesome cases of psoriasis. **Diacetylrhein** is marketed in some countries for the treatment of osteoarthritis.

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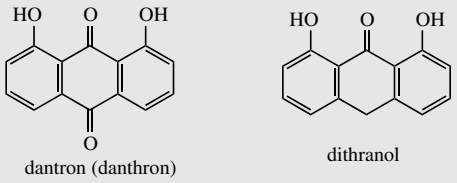

Figure 3.34

Hypericum/St John's Wort

The dried flowering tops of St John's Wort (*Hypericum perforatum*; Guttiferae/Hypericaceae) have been used as a herbal remedy for many years, an extract in vegetable oil being used for its antiseptic and wound healing properties. St John's Wort is now a major crop marketed as an antidepressant, that is claimed to be as effective in its action as the widely prescribed antidepressants of the selective serotonin re-uptake inhibitor (SSRI) class such as fluoxetine (Prozac ®), and with fewer side-effects. There is considerable clinical evidence that extracts of St John's Wort are effective in treating mild to moderate depression and improving mood. However, to avoid potentially dangerous side-effects, St John's Wort should not be used at the same time as prescription antidepressants. St John's Wort is a small to medium height herbaceous perennial plant with numerous yellow flowers characteristic of this genus. It is widespread throughout Europe, where it is generally considered a weed, and has also become naturalized in North America. The tops, including flowers at varying stages of development, which contain considerable amounts of the active principles, are harvested and dried in late summer.

The dried herb contains significant amounts of phenolic derivatives, including 4–5% of flavonoids, though the antidepressant activity is considered to derive principally from naphthodianthrone structures such as hypericin (about 0.1%) and pseudohypericin (about 0.2%), and a prenylated phloroglucinol derivative hyperforin (Figure 3.35). The fresh plant also contains significant levels of protohypericin and protopseudohypericin, which are converted into hypericin and pseudophypericin during drying and processing, as a result of irradiation with visible light. Hyperforin is a major lipophilic constituent in the leaves and flowers (2–3%),

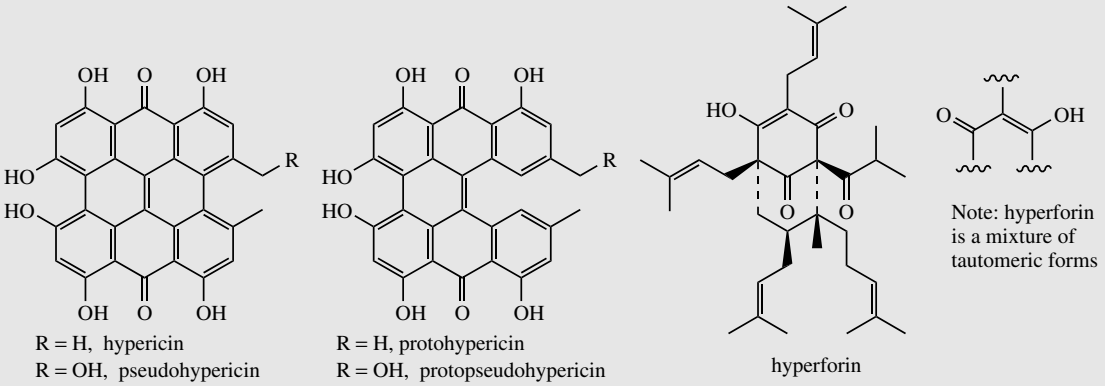

Figure 3.35

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and is now thought to be the major contributor to the antidepressant activity, as well as to the antibacterial properties of the oil extract. Studies show clinical effects of St John's Wort on depression correlate well with hyperforin content. Standardized aqueous ethanolic extracts containing 0.15% hypericin and 5% hyperforin are usually employed. The aqueous solubility of hypericin and pseudohypericin is markedly increased by the presence of flavonoid derivatives in the crude extract, particularly procyanidin B_2, a dimer of epicatechin (see page 151). *Hypericum* extracts have been demonstrated to increase levels of serotonin, noradrenaline, and dopamine, which may be responsible for the antidepressant activity.

Hypericin also possesses extremely high toxicity towards certain viruses, a property that requires light and may arise via photo-excitation of the polycyclic quinone system. It is currently under investigation as an antiviral agent against HIV and hepatitis C. Antiviral activity appears to arise from an inhibition of various protein kinases, including those of the protein kinase C family. Hypericin and pseudohypericin are potent photosensitizers initiating photochemical reactions, and are held responsible for hypericism, a photodermatosis seen in cattle after consuming *Hypericum* plants present in pasture. Patients using St John's Wort as an antidepressant should also be warned to avoid over-exposure to sunlight. There is also considerable evidence that St John's Wort interacts with a number of prescription drugs including the anticoagulant warfarin, the cardiac glycoside digoxin, the bronchodilator theophylline, the HIV protease inhibitor indinavir, the immunosuppressive drug cyclosporin, and oral contraceptives. In some cases, it is known to promote the cytochrome P-450-dependent metabolism of the co-administered drugs.

C-Alkylation Reactions

A common feature of many natural products containing phenolic rings is the introduction of alkyl groups at nucleophilic sites. Obviously, the phenol groups themselves are nucleophilic, and with a suitable alkylating agent, *O*-alkyl derivatives may be formed (see page 12), e.g. the *O*-methylation of emodin to physcion (Figure 3.30). However, a phenol group also activates the ring carbons at the *ortho* and *para* positions, so that these positions similarly become susceptible to alkylation, leading to *C*-alkyl derivatives. The *meta* oxygenation pattern, which is a characteristic feature of acetate-derived phenolics, has the effect of increasing this nucleophilicity considerably, and the process of *C*-alkylation is very much facilitated (see page 12). Suitable natural alkylating agents are *S*-adenosylmethionine (SAM), and dimethylallyl diphosphate (DMAPP). Other polyprenyl diphosphate esters may also be encountered in biological alkylation reactions (e.g. see vitamin K, page 159). A minor inconsistency has been discovered, in that, while *C*-alkylation with dimethylallyl and higher diphosphates is mediated *after* the initial polyketide cyclization product is liberated from the enzyme, there are several examples where *C*-methylation undoubtedly occurs *before* release of any aromatic compound from the enzyme. **5-methylorsellinic acid** (Figure 3.36) is a simple *C*-methylated analogue of orsellinic acid found in *Aspergillus flaviceps*, and the extra methyl is derived from SAM. However, orsellinic acid is not a precursor of 5-methylorsellinic acid and it is proposed that the poly-β-keto ester is therefore methylated as part of the series of reactions catalysed by the synthase complex (Figure 3.36). Similarly, 5-methylorsellinic acid, but not orsellinic acid is a precursor of **mycophenolic acid*** in *Penicillium brevicompactum* (Figure 3.36). However, *C*-alkylation by farnesyl diphosphate (see page 191) proceeds *after* the aromatization step, and a phthalide intermediate is the substrate involved. The phthalide is a lactone derived from 5-methylorsellinic acid by hydroxylation of its starter methyl group and reaction with the end-

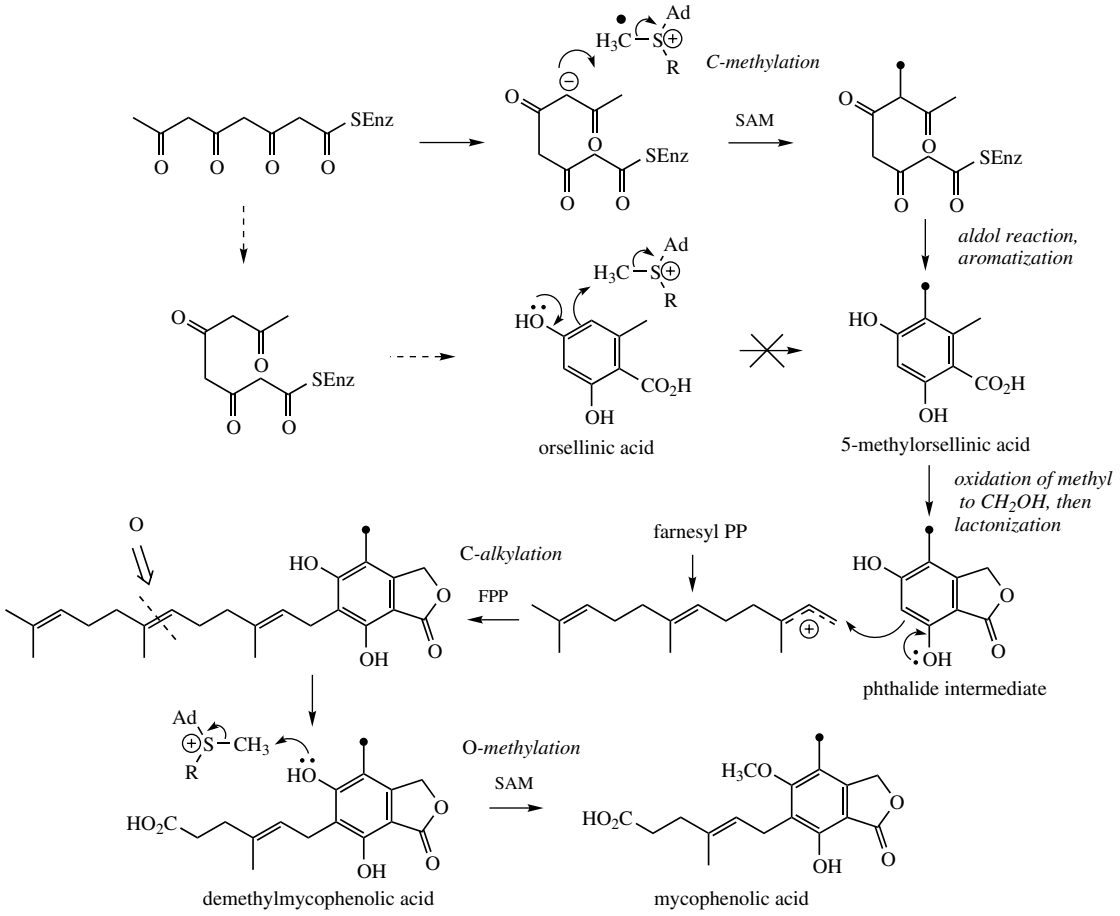

Figure 3.36

Mycophenolic Acid

Mycophenolic acid (Figure 3.36) is produced by fermentation cultures of the fungus *Penicillium brevicompactum*. It has been known for many years to have antibacterial, antifungal, antiviral, and antitumour properties. It has recently been introduced into medicine as an immunosuppressant drug, to reduce the incidence of rejection of transplanted organs, particularly kidney and heart transplants. It is formulated as the *N*-morpholinoethyl ester **mycophenolate mofetil** (Figure 3.37), which is metabolized after ingestion to mycophenolic

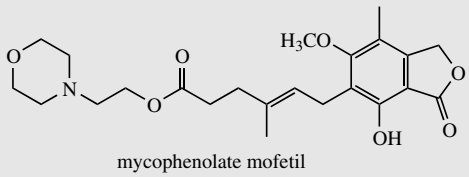

Figure 3.37

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acid, and is usually administered in combination with cyclosporin (see page 429). The drug is a specific inhibitor of mammalian inosine monophosphate dehydrogenase and has an antiproliferative activity on cells due to inhibition of guanosine nucleotide biosynthesis. This enzyme catalyses the NAD^+-dependent oxidation of inosine monophosphate (IMP) to xanthosine monophosphate (XMP), a key transformation in the synthesis of guanosine triphosphate (GTP) (see also caffeine biosynthesis, page 394). Rapidly growing cells have increased levels of the enzyme, so this forms an attractive target for anticancer, antiviral, and immunosuppressive therapy.

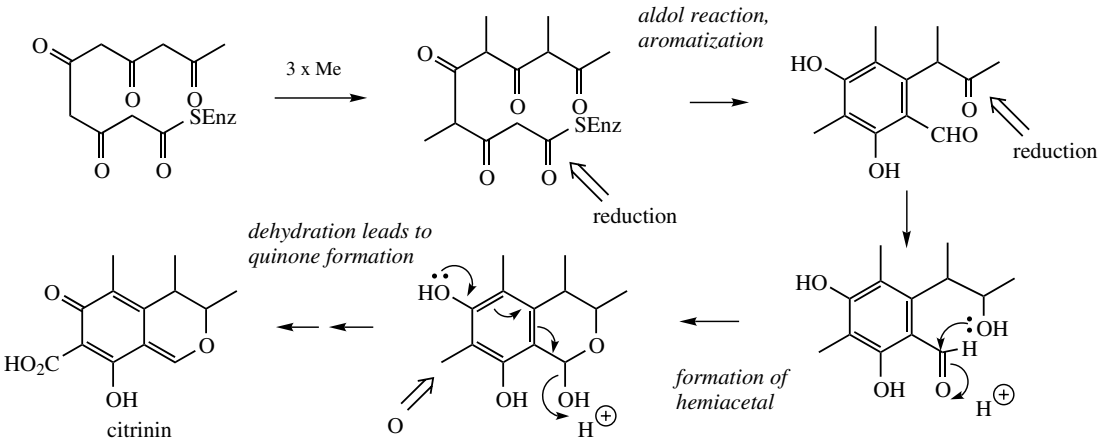

Figure 3.38

of-chain carboxyl. The chain length of the farnesyl alkyl group is then shortened by oxidation of a double bond giving demethylmycophenolic acid, which is then *O*-methylated, again involving SAM, to produce mycophenolic acid. Note that the *O*-methylation step only occurs after the *C*-alkylations, so that the full activating benefit of two *meta* positioned phenols can be utilized for the *C*-alkylation.

Three *C*-methyl substituents are inserted into the acetate-derived skeleton of **citrinin** (Figure 3.38), an antimicrobial metabolite from *Penicillium citrinum* and several *Aspergillus* species, which also displays potentially dangerous carcinogenic and nephrotoxic (kidney-damaging) activity. One of these introduced methyls has undergone oxidation to a carboxyl, adding to the difficulties in immediately recognizing the biosynthetic origins of this compound which contains a quinonemethide system rather than the simpler aromatic ring. The methyls are probably introduced into the polyketide prior to release of the first aromatic intermediate, which could well be an aldehyde rather than the corresponding acid if a reductase component also forms part of the synthase complex. The hemiacetal can be produced after reduction of the side-chain carbonyl, and then in the later stages, oxidation of one methyl to a carboxyl will follow. The quinonemethide system in citrinin is simply the result of a dehydration reaction on the hemiacetal (Figure 3.38).

Khellin* and **visnagin** (Figure 3.39) are furochromones found in the fruits of *Ammi visnaga* (Umbelliferae/Apiaceae), and the active principles of a crude plant drug which has a long history of use as an antiasthmatic agent. Figure 3.39 presents the sequence of steps utilized in the biosynthesis of these compounds, fully consistent with the biosynthetic rationale developed above. The two carbons C-2′ and C-3′ forming part of the furan

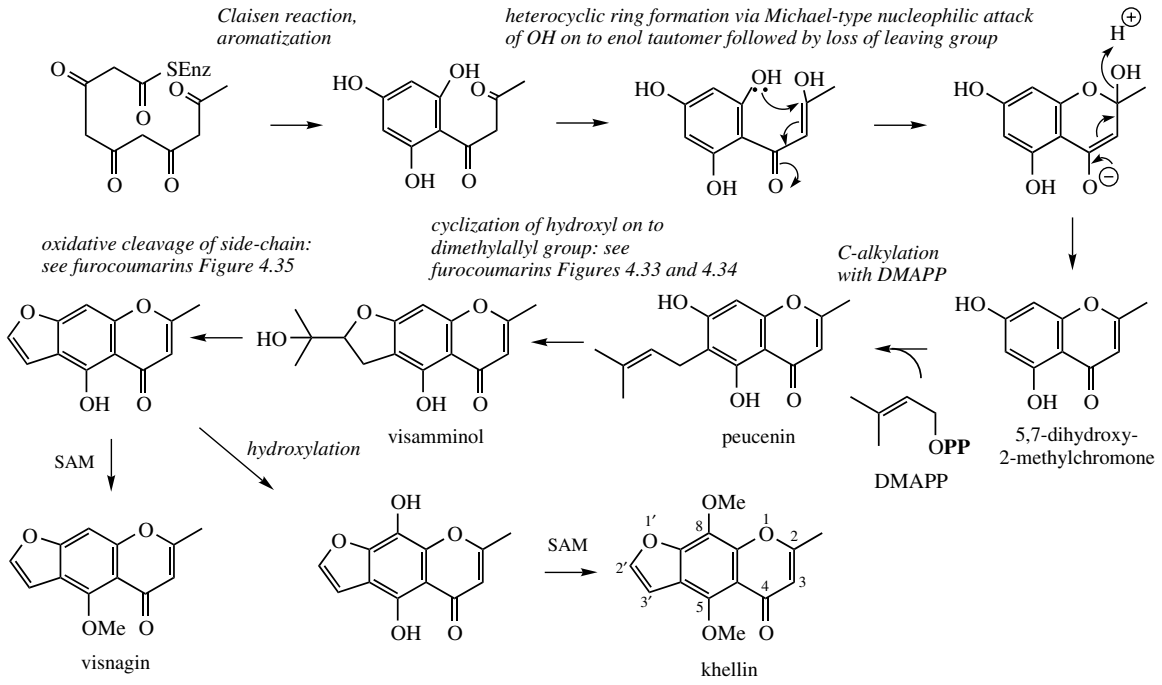

Figure 3.39

Khellin and Cromoglicate

The dried ripe fruits of *Ammi visnaga* (Umbelliferae/Apiaceae) have a long history of use in the Middle East as an antispasmodic and for the treatment of angina pectoris. The drug contains small amounts of coumarin derivatives, e.g. visnadin (Figure 3.40) (compare *Ammi majus*, a rich source of furocoumarins, page 146), but the major constituents (2–4%) are

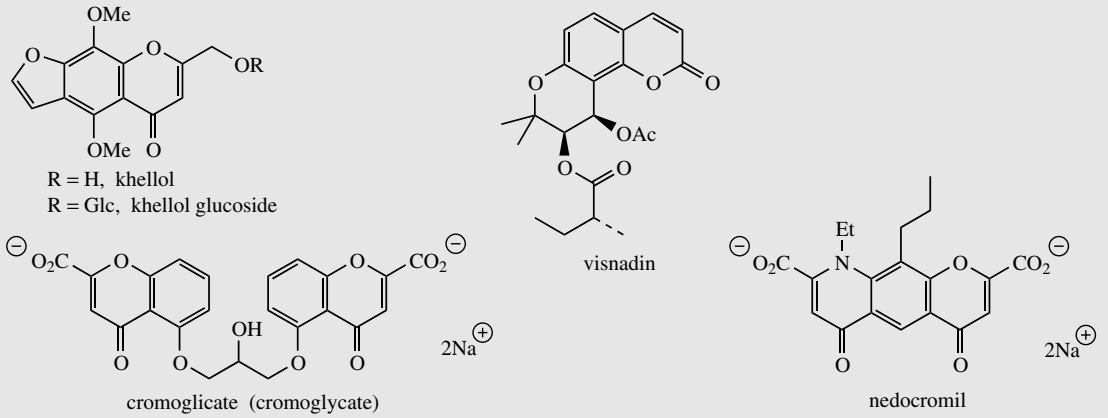

Figure 3.40

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furochromones, including khellin and visnagin (Figure 3.34), and khellol and khellol glucoside (Figure 3.40). Both khellin and visnadin are coronary vasodilators and spasmolytic agents, with visnadin actually being the more potent agent. Khellin has been used in the treatment of angina pectoris and bronchial asthma. The synthetic analogue **cromoglicate** (**cromoglycate**) (Figure 3.40) is a most effective and widely used agent for the treatment and prophylaxis of asthma, hay fever, and allergic rhinitis. Cromoglicate contains two chromone systems containing polar carboxylic acid functions, joined by a glycerol linker. The mode of action is not fully established. It was believed to prevent the release of bronchospasm mediators by stabilizing mast cell membranes, but an alternative suggestion is that it may act by inhibiting the effect of sensory nerve activation, thus interfering with bronchoconstriction. It is poorly absorbed orally and is thus administered as inhalation or nasal spray. Eyedrops for relief of allergic conjunctivitis are also available. The more potent **nedocromil** (Figure 3.40) has also been introduced.

ring originate by metabolism of a five-carbon dimethylallyl substituent attached to C-6 (for a full discussion, see furocoumarins, page 145). The 8-methoxy group in khellin is absent from visnagin, so must be introduced late in the sequence. The key intermediate is thus 5,7-dihydroxy-2-methylchromone. On inspection, this has the alternate acetate-derived oxygenation pattern and a methyl chain starter, so is formed from a poly-β-keto chain through Claisen condensation then heterocyclic ring formation by an overall dehydration reaction. After formation of the furan ring via the *C*-dimethylallyl derivative peucenin and then visamminol, **visnagin** can be obtained by *O*-methylation. Alternatively, further hydroxylation *para* to the free phenol, followed by two methylations, yields **khellin**. The antiasthmatic properties of khellin have been exploited by developing the more polar, water-soluble derivative **cromoglicate***.

Phenolic Oxidative Coupling

C-Methylation also features in the biosynthesis of **usnic acid** (Figure 3.41), an antibacterial metabolite found in many lichens, e.g. *Usnea* and *Cladonia* species, which are symbiotic combinations of alga and fungus. However, the principal structural modification encountered involves phenolic oxidative coupling (see page 28). Two molecules of **methylphloracetophenone** are incorporated, and these are known to derive from a pre-aromatization methylation reaction and not by methylation of phloracetophenone (Figure 3.41). The two molecules are joined together by an oxidative coupling mechanism which can be rationalized via the one-electron oxidation of a phenol group in methylphloracetophenone giving free radical A, for which resonance forms B and C can be written. Coupling of B and C occurs. Only the left-hand ring can subsequently be restored to aromaticity by keto–enol tautomerism, this state being denied to the right-hand ring because coupling occurred on to the methyl-containing position *para* to the original phenol. Instead, a heterocyclic ring is formed by attack of the phenol on to the enone system (see khellin, above). The outcome of this reaction is enzyme controlled, since two equivalent phenol groups are present as potential nucleophiles, and two equivalent enone systems are also available. Therefore, four different products could be formed, but only one is actually produced. Loss of water then leads to usnic acid.

Phenolic oxidative coupling is widely encountered in natural product biosynthesis, and many other examples are described in subsequent sections. A further acetate-derived metabolite formed as a result of oxidative coupling is the antifungal agent **griseofulvin*** (Figure 3.42) synthesized by cultures of *Penicillium griseofulvin*. The sequence of events leading to griseofulvin has now been established in detail, and the pathway also includes *O*-methylation steps and the introduction of a halogen (chlorine) atom at one of the nucleophilic sites, which is represented as involving the electrophile Cl^+ (Figure 3.42).

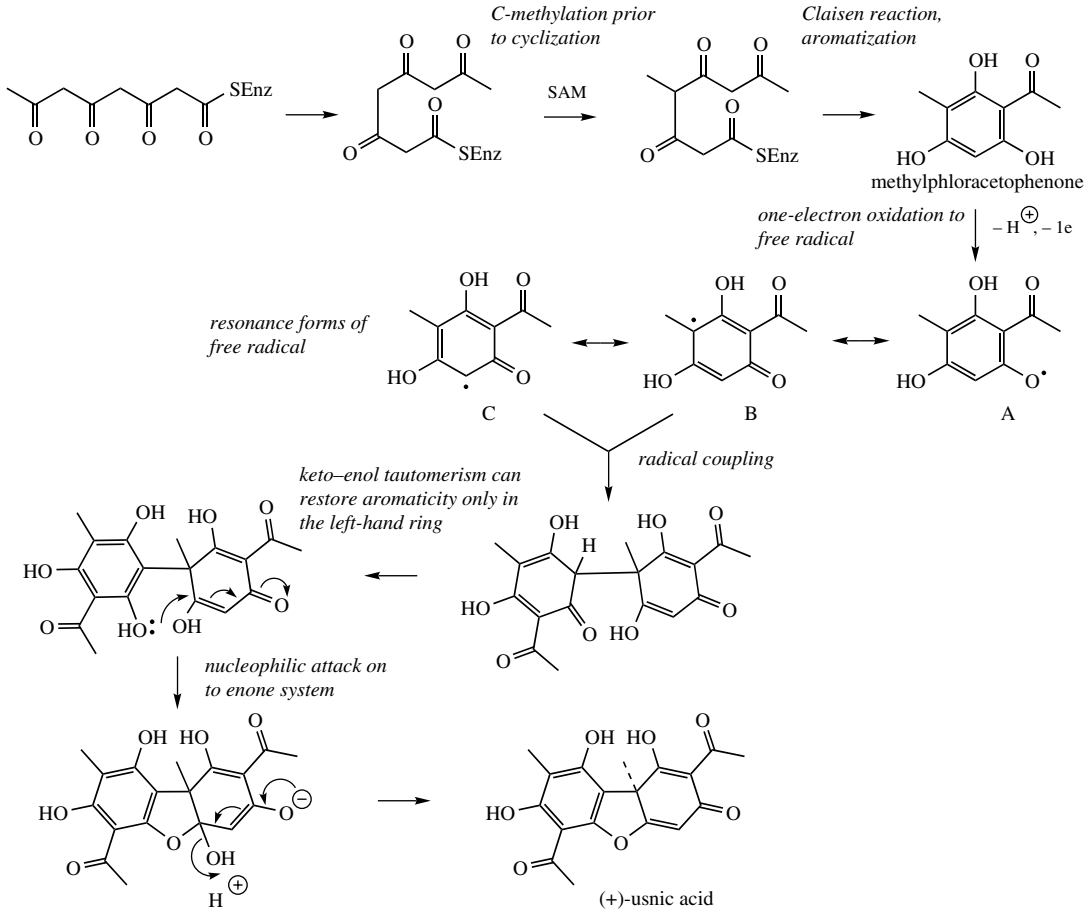

Figure 3.41

Griseofulvin

Griseofulvin is an antifungal agent produced by cultures of *Penicillium griseofulvum* and a number of other *Penicillium* species, including *P. janczewski*, *P. nigrum*, and *P. patulum*. Griseofulvin is the drug of choice for widespread or intractable dermatophyte infections, but is ineffective when applied topically. However, it is well absorbed from the gut and selectively concentrated into keratin, so may be used orally to control dermatophytes such as *Epidermophyton*, *Microsporium*, and *Trichophyton*. Treatment for some conditions, e.g. infections in fingernails, may have to be continued for several months, but the drug is generally free of side-effects. The antifungal action appears to be through disruption of the mitotic spindle, thus inhibiting fungal mitosis.

Initial inspection of the structure of griseofulvin shows the alternate oxygenation pattern, and also a methyl group which identifies the start of the polyketide chain. Cyclization of the C_{14} poly-β-keto chain folded as shown allows both Claisen (left-hand ring) and aldol (right-hand ring) reactions to occur giving a benzophenone intermediate. Two selective methylations lead to griseophenone C, which is the substrate for chlorination to griseophenone B; both these

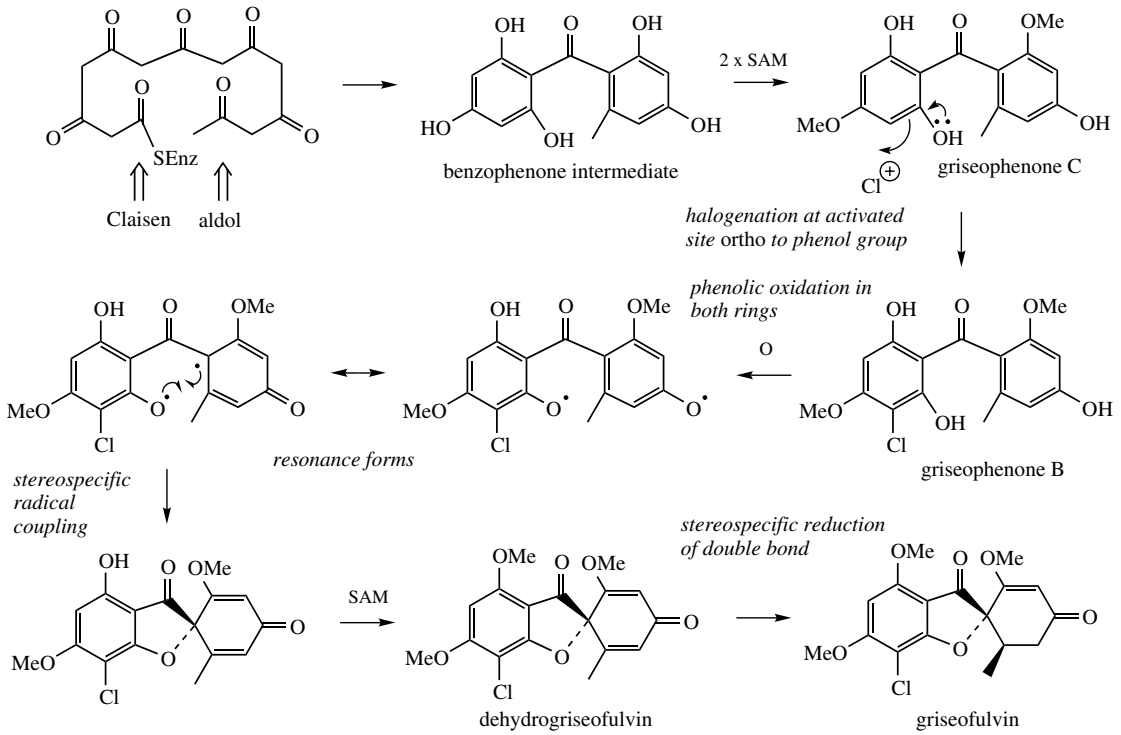

Figure 3.42

compounds appear as minor metabolites in *P. griseofulvin* cultures. One-electron oxidations on a phenolic group in each ring give a diradical and its mesomer, the latter allowing radical coupling to the basic grisan skeleton. **Griseofulvin** is then the result of methylation of the remaining phenol group and stereospecific reduction of the double bond in dehydrogriseofulvin.

Oxidative Cleavage of Aromatic Rings

Perhaps the most drastic modification which can happen to an aromatic ring is ring cleavage brought about by oxidative enzymes called dioxygenases (see page 27). These enzymes typically use catechol (1,2-dihydroxy) or quinol (1,4-dihydroxy) substrates, require molecular oxygen and Fe^{2+} cofactors, and incorporate both the oxygen atoms into the ring-cleaved product. In the case of catechols, cleavage may be between or adjacent to the two hydroxyls, giving products containing aldehyde and/or carboxylic acid functionalities (Figure 3.43). These groups are then able to react with other substituents in the molecule creating compounds in which the characteristic acetate-derived features are probably no longer apparent. Shikimate-derived aromatic rings can suffer similar oxidative cleavage reactions.

Patulin is an excellent example of an acetate-derived structure synthesized from an aromatic substrate via oxidative cleavage and subsequent modifications (Figure 3.44). Patulin is a potent carcinogen produced by *Penicillium patulum*, a common contaminant on apples. If mould-infected apples find their way into food products, e.g.

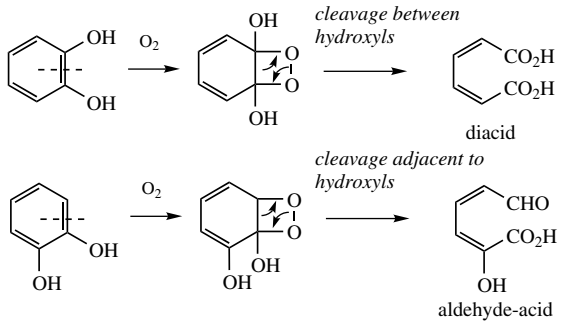

Figure 3.43

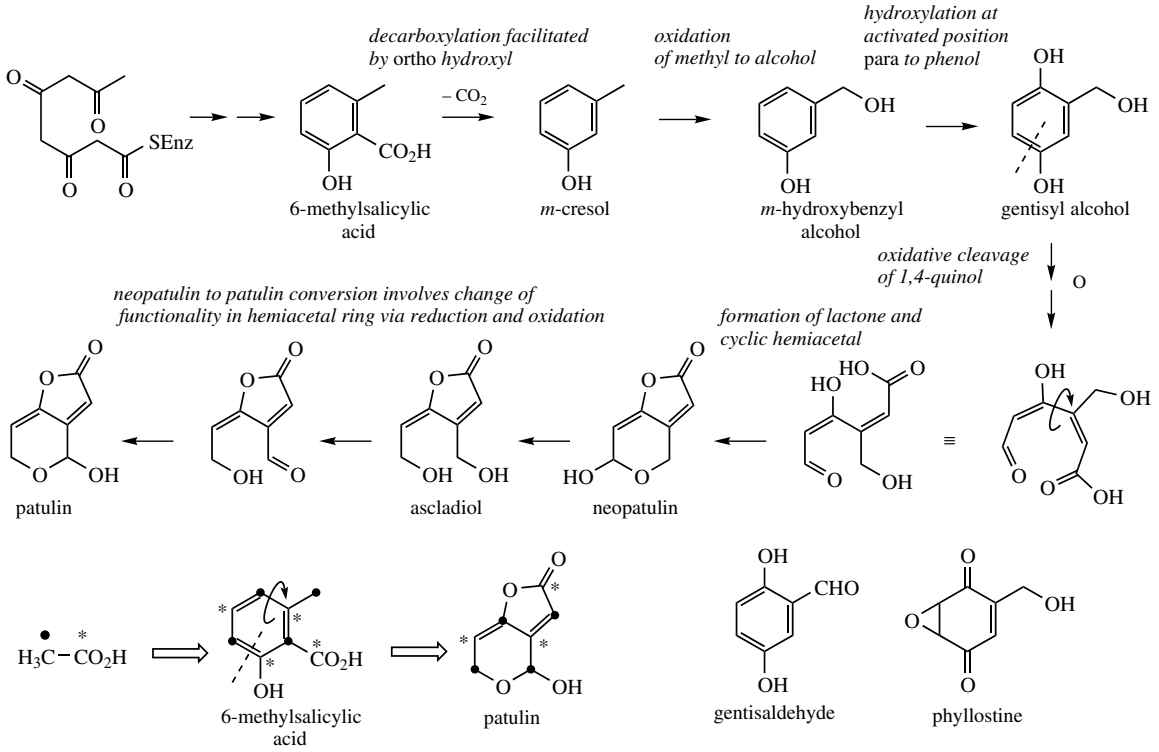

Figure 3.44

apple juice, fruit pies, etc, then these products may contain unacceptable and dangerous levels of patulin. Such food materials are routinely screened for patulin content, with a tolerance level set at 50 μg kg^{-1}. Patulin is derived from acetate via **6-methylsalicylic acid** (Figure 3.26). Decarboxylation and hydroxylation reactions then lead to **gentisyl alcohol** (Figure 3.44), which may suffer oxidative cleavage as shown. Cleavage of the aromatic ring would generate aldehyde and carboxylic acid functions. By rotating the molecule around the carbon–carbon single bond as shown, it is easy to see that **neopatulin** can result by formation of hemiacetal and lactone groups. The reversal of functionality in the hemiacetal ring to produce **patulin** is achieved by reduction and oxidation reactions involving aldehyde and alcohol components of the hemiacetal. The sequence shown in Figure 3.44 has been deliberately simplified to rationalize the oxidative cleavage. The true sequence involves gentisaldehyde and the epoxyquinone phyllostine as intermediates between gentisyl alcohol and neopatulin.

Penicillic acid (Figure 3.45), another microbially produced food contaminant with carcinogenic properties, is synthesized by cultures of *Penicillium cyclopium* and *P. baarnense*, and also features oxidative ring fission of an aromatic compound. This time **orsellinic acid** (Figure 3.25) is a precursor, and ring fission appears to proceed via a quinone, which is the result of decarboxylation, oxidation, and methylation reactions. Figure 3.45 also represents an over-simplistic rationalization of the ring fission process.

Starter Groups Other Than Acetate

In the examples so far discussed, the basic carbon skeleton has been derived from an acetate starter group, with malonate acting as the chain extender. The molecule has then, in some cases, been made more elaborate by the inclusion of other carbon atoms, principally via alkylation reactions. However, the range of natural product structures that are at least partly derived from acetate is increased enormously by altering the nature of the

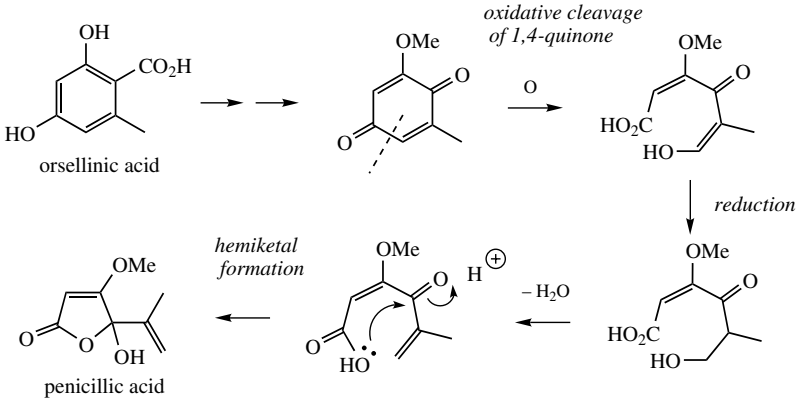

Figure 3.45

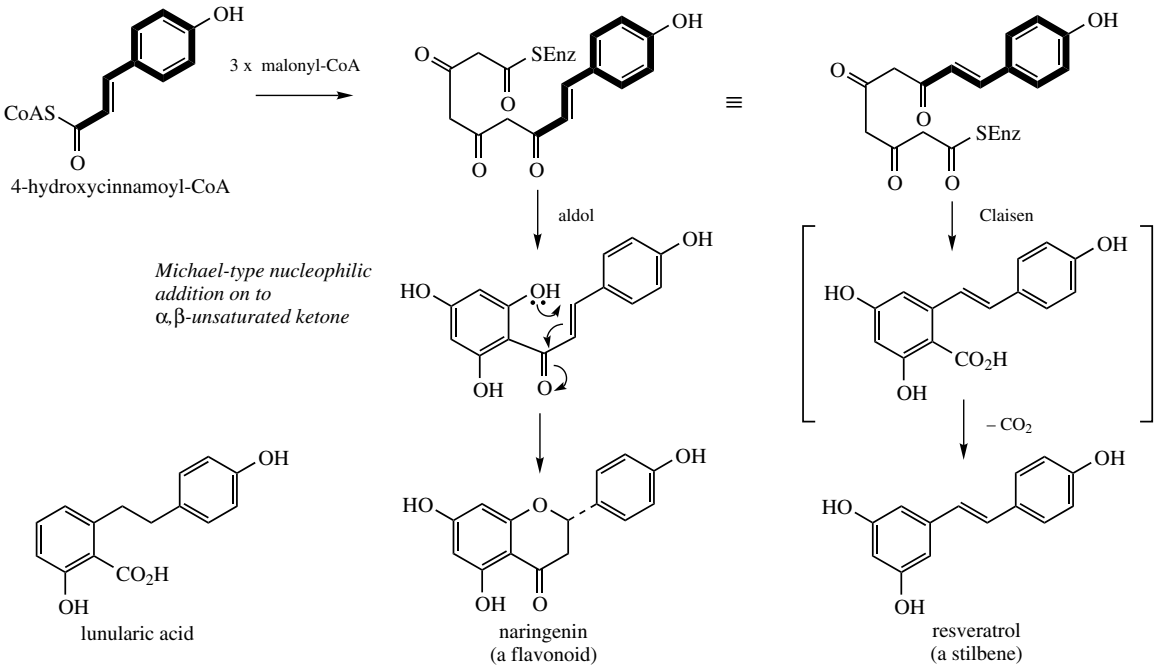

Figure 3.46

starter group from acetate to a different carboxylate system, as its coenzyme A ester, with malonyl-CoA again providing the chain extender. There is less detailed knowledge here about the precise nature of how substrates are bound to the enzyme, and whether coenzyme A esters are initially transformed into thio esters of the ACP type.

Flavonoids and **stilbenes** are simple examples of molecules in which a suitable cinnamoyl-CoA C_6C_3 precursor from the shikimate pathway (see page 130) has acted as a starter group. Thus, if **4-hydroxycinnamoyl-CoA** (Figure 3.46) is chain extended with three malonyl-CoA units, the poly-β-keto chain can then be folded in two ways, allowing aldol or Claisen-type cyclizations to occur, respectively. The six-membered heterocyclic ring characteristic of most flavonoids, e.g. **naringenin**, is formed by nucleophilic attack of a phenol group from the acetate-derived ring on to the α,β-unsaturated ketone. Stilbenes, such as

resveratrol, incorporate the carbonyl carbon of the cinnamoyl unit into the aromatic ring, and typically lose the end-of-chain carboxyl by a decarboxylation reaction. Although some related structures, e.g. **lunularic acid** from the liverwort *Lunularia cruciata*, still contain this carboxyl, in general it is lost in a pre-cyclization modification, and intermediates of the type shown in brackets are not produced. Flavonoids and stilbenes are discussed in more detail in Chapter 4 (see page 149).

Anthranilic acid (2-aminobenzoic acid) (see page 126) is another shikimate-derived compound which, as its CoA ester anthraniloyl-CoA, can act as a starter unit for malonate chain extension. Aromatization of the acetate-derived portion then leads to **quinoline** or **acridine** alkaloids, according to the number of acetate units incorporated (Figure 3.47). These products are similarly discussed elsewhere, under alkaloids (Chapter 6, page 376).

Fatty acyl-CoA esters are similarly capable of participating as starter groups. Fatty acid biosynthesis and aromatic polyketide biosynthesis are distinguished by the sequential reductions as the chain length increases in the former, and by the stabilization of a reactive poly-β-keto chain in the latter, with little or no reduction involved. It is thus interesting to see natural product structures containing both types of acetate–malonate-derived chains. In plants of the Anacardiaceae, e.g. poison ivy* (*Rhus radicans*) and poison oak* (*Rhus toxicodendron*), contact allergens called **urushiols** are encountered, which derive from just such a pathway. Thus, **palmitoleoyl-CoA** (Δ^9-hexadecenoyl-CoA) can act as starter group for extension by three malonyl-CoA units, with a reduction step during chain extension (Figure 3.48). Aldol cyclization then gives **anacardic acid**, which is likely to be the precursor of **urushiol** by decarboxylation/hydroxylation. It is likely that different fatty acyl-CoAs can participate in this sequence, since urushiols from poison ivy can contain up to three double bonds in the C_{15} side-chain, whilst those from poison oak also have variable unsaturation

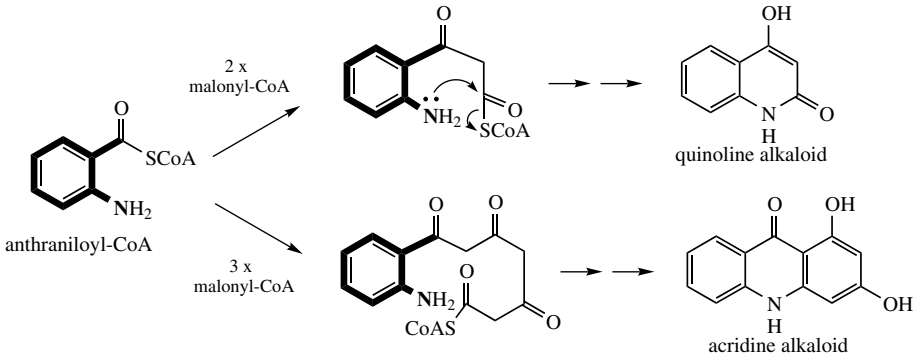

Figure 3.47

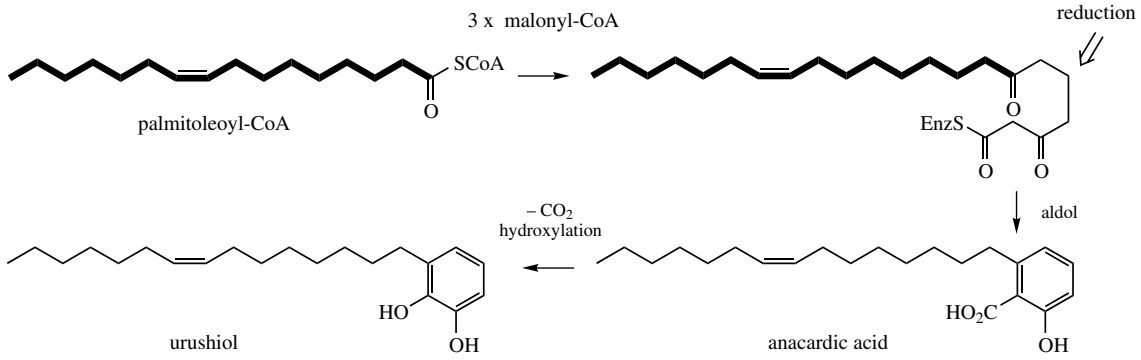

Figure 3.48

Poison Ivy and Poison Oak

Poison ivy (*Rhus radicans* or *Toxicodendron radicans*; Anacardiaceae) is a woody vine with three-lobed leaves that is common in the USA. The plant may be climbing, shrubby, or may trail over the ground. It presents a considerable hazard to humans should the sap, which exudes from damaged leaves or stems, come into contact with the skin. The sap sensitizes most individuals, producing delayed contact dermatitis after a subsequent encounter. This results in watery blisters that break open, the fluid quickly infecting other parts of the skin. The allergens may be transmitted from one person to another on the hands, on clothing, or by animals. The active principles are urushiols, a mixture of alkenyl polyphenols. In poison ivy, these are mainly pentadecylcatechols with varying degrees of unsaturation (Δ^8, $\Delta^{8,11}$, $\Delta^{8,11,14}$) in the side-chain. Small amounts of C_{17} side-chain analogues are present. These catechols become oxidized to an *ortho*-quinone, which is then attacked by nucleophilic groups in proteins to yield an antigenic complex.

Poison oak (*Rhus toxicodendron* or *Toxicodendron toxicaria*: Anacardiaceae) is nearly always found as a low-growing shrub, and has lobed leaflets similar to those of oak. It is also common throughout North America. There appears considerable confusion over nomenclature, and *Rhus radicans* may also be termed poison oak, and *R. toxicodendron* oakleaf poison ivy. Poison oak contains similar urushiol structures in its sap as poison ivy, though heptadecylcatechols (i.e. C_{17} side-chains) predominate over pentadecylcatechols (C_{15} side-chains).

Related species of *Rhus*, e.g. *R. diversiloba* (Pacific poison oak) and *R. vernix* (poison sumach, poison alder, poison dogwood) are also allergenic with similar active constituents. The allergen-containing species of *Rhus* have been reclassified under the genus *Toxicodendron*, though this nomenclature is not commonly employed. Dilute purified extracts containing urushiols may be employed to stimulate antibody production and thus build up immunity to the allergens.

in a C_{17} side-chain. Large quantities of anacardic acids containing C_{15} side-chains with one, two, and three double bonds are also found in the shells of cashew nuts (*Anacardium occidentale*; Anacardiaceae).

A saturated C_6 **hexanoate** starter unit is used in the formation of the **aflatoxins***, a group of highly toxic metabolites produced by *Aspergillus flavus*, and probably responsible for the high incidence of liver cancer in some parts of Africa. These compounds were first detected following the deaths of young turkeys fed on mould-contaminated peanuts (*Arachis hypogaea*; Leguminosae/Fabaceae). Peanuts still remain one of the crops most likely to represent a potential risk to human health because of contamination with fungal toxins. These and other food materials must be routinely screened to ensure levels of aflatoxins do not exceed certain set limits. The aflatoxin structures contain a bisfuran unit fused to an aromatic ring, e.g. **aflatoxin B_1** and **aflatoxin G_1**, and their remarkably complex biosynthetic origin begins with a poly-β-keto chain derived from a hexanoyl-CoA starter and seven malonyl-CoA extender units (Figure 3.49). This gives an anthraquinone **norsolorinic acid** by now-familiar condensation reactions, but the folding of the chain is rather different from that seen with simpler anthraquinones (see page 64). The six-carbon side-chain of norsolorinic acid is cyclized to give, in several steps, the ketal **averufin**. **Versiconal acetate** is another known intermediate, and its formation involves a Baeyer–Villiger oxidation (see page 28), resulting principally in transfer of a two-carbon fragment (the terminal ethyl of hexanoate) to become an ester function. These two carbons can then be lost in formation of **versicolorin B**, now containing the tetrahydrobisfuran moiety, oxidized in **versicolorin A** to a dihydrobisfuran system. **Sterigmatocystin** is derived from versicolorin A by

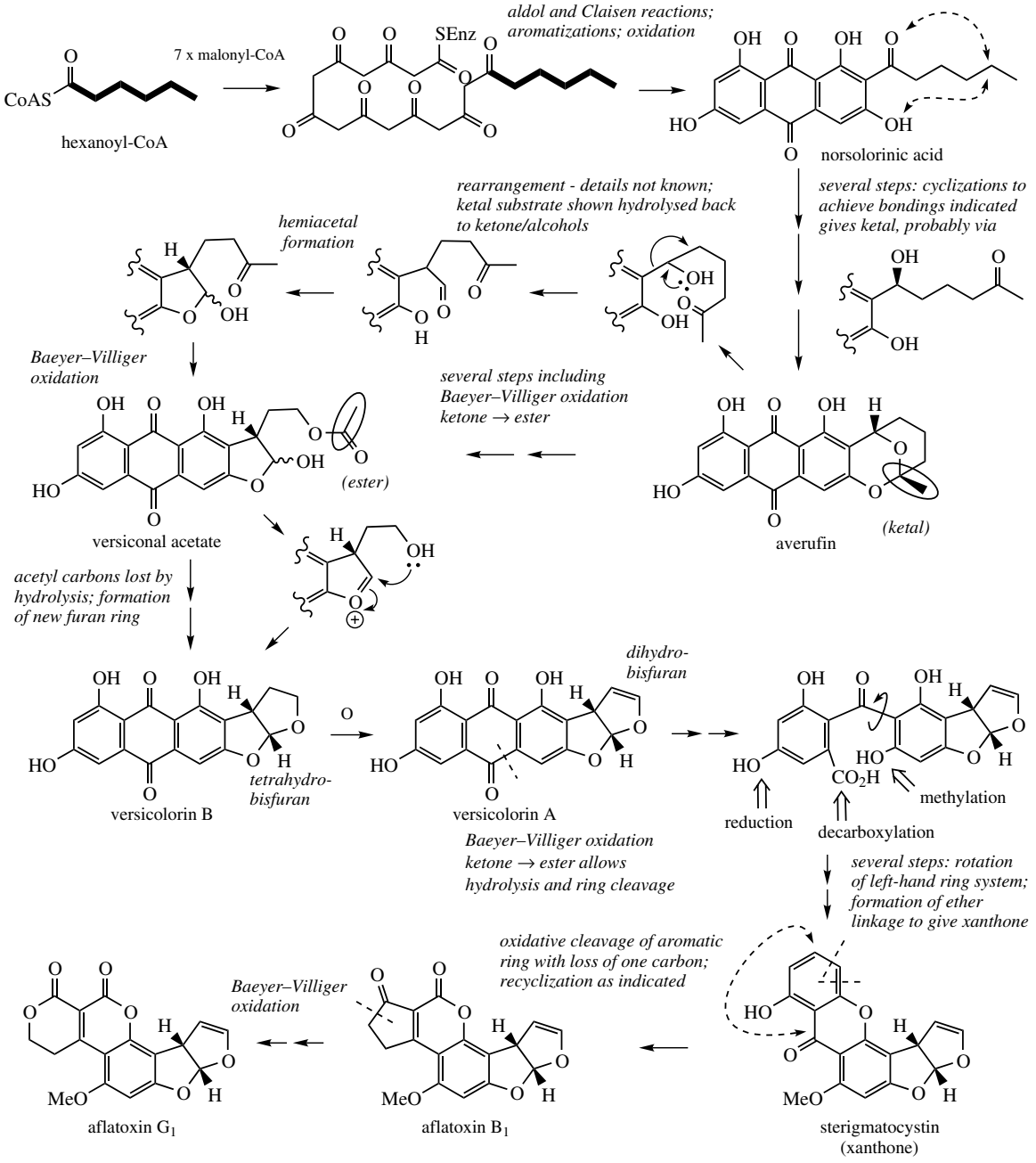

Figure 3.49

oxidative cleavage of the anthraquinone system involving a second Baeyer–Villiger oxidation, and recyclization through phenol groups to give a xanthone skeleton. Rotation of an intermediate leads to the angular product as opposed to a linear product. One phenol group is methylated, and, quite unusually, another phenol group is lost (contrast loss of oxygen functions via reduction/dehydration prior to cyclization, see page 62). **Aflatoxin B_1** formation requires oxidative cleavage of an aromatic ring in sterigmatocystin, loss of one carbon and recyclization exploiting the

Aflatoxins

Aflatoxins are potent mycotoxins produced by the fungi *Aspergillus flavus* and *A. parasiticus*. Four main naturally occurring aflatoxins, aflatoxins B_1, B_2, G_1, and G_2 (Figure 3.50), are recognized, but these can be metabolized by microorganisms and animals to other aflatoxin structures, which are also toxic. Aflatoxin B_1 is the most commonly encountered member of the group, and is also the most acutely toxic and carcinogenic example. Aflatoxin B_2 is a dihydro derivative of aflatoxin B_1, whilst aflatoxins G_1 and G_2 are an analogous pair with a six-membered lactone rather than a five-membered cyclopentenone ring. These toxins are most commonly associated with peanuts (groundnuts), maize, rice, pistachio nuts, and Brazil nuts, though other crops can be affected, and, although found world-wide, they are particularly prevalent in tropical and subtropical regions. Aflatoxin M_1 (Figure 3.50) is a hydroxy derivative of aflatoxin B_1 and equally toxic. It may occur in cow's milk as a result of mammalian metabolism of aflatoxin B_1 originally contaminating the animal's food. Because these compounds fluoresce strongly under UV light, they are relatively easily detected and monitored.

The aflatoxins primarily affect the liver, causing enlargement, fat deposition, and necrosis, at the same time causing cells of the bile duct to proliferate, with death resulting from irreversible loss of liver function. In the case of aflatoxin B_1, this appears to be initiated by cytochrome P-450-dependent metabolism in the body to the epoxide (Figure 3.50). The epoxide intercalates with DNA, and in so doing becomes orientated towards nucleophilic attack from guanine residues. This leads to inhibition of DNA replication and of RNA synthesis, and initiates mutagenic activity. Aflatoxins are also known to cause hepatic carcinomas, this varying with the species of animal. The above normal incidence of liver cancer in parts of Africa and Asia has been suggested to be linked to the increased amounts of aflatoxins found in foodstuffs, and a tolerance level of 30 ppb has been recommended. Acute hepatitis may result from food containing aflatoxin B_1 at levels of the order of 0.1 ppm, and levels of more than 1 ppm are frequently encountered.

The biosynthesis of aflatoxins proceeds through intermediates sterigmatocystin and versicolorin (see Figure 3.49). Toxins related to these structures but differing in aromatic substituents are also produced by various fungi. The sterigmatocystins are synthesized by species of *Aspergillus* and *Bipolaris*, and contain a reduced bifuran fused to a xanthone, whilst the versicolorins from *Aspergillus versicolor* contain the same type of reduced bisfuran system but fused to an anthraquinone. Like the aflatoxins, the sterigmatocystins are acutely toxic and carcinogenic. The versicolorins are less toxic though still carcinogenic.

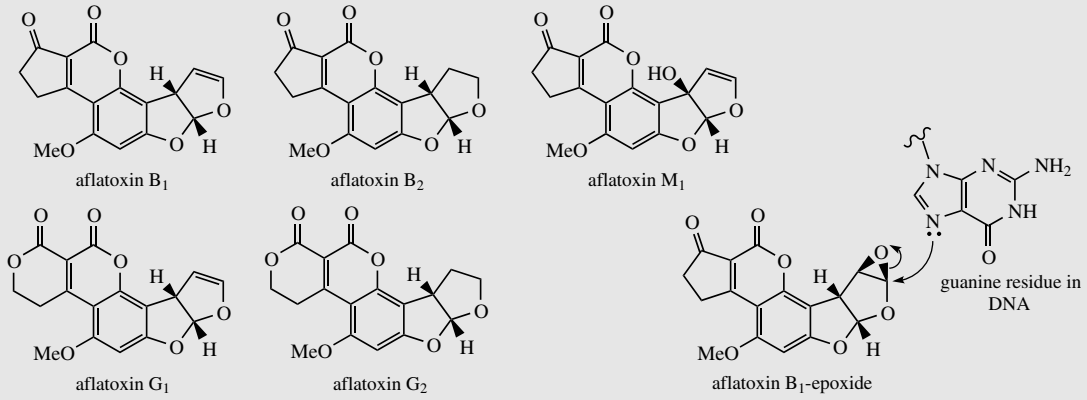

Figure 3.50

carbonyl functionality. **Aflatoxin G_1** is derived by further modification of aflatoxin B_1, cleaving the cyclopentenone ring and forming a lactone, perhaps via a further Baeyer–Villiger reaction.

Hexanoate is also likely to feature as a starter unit in the formation of the **cannabinoids**, a group of **terpenophenolics** found in Indian hemp (*Cannabis sativa*; Cannabaceae). This plant, and preparations from it, known under a variety of names including hashish, marihuana, pot, bhang, charas, and dagga, have been used for centuries for the pleasurable sensations and mild euphoria experienced after its consumption, usually by smoking. The principal psychoactive component is **tetrahydrocannabinol** (**THC**) (Figure 3.51), whilst structurally similar compounds such as **cannabinol** (**CBN**) and **cannabidiol** (**CBD**), present in similar or larger amounts, are effectively inactive. In recent years, the beneficial effects of cannabis*, and especially THC, in alleviating nausea and vomiting in cancer patients undergoing chemotherapy, and in the treatment of glaucoma and multiple sclerosis, has led to a study of cannabinoid analogues for potentially useful medicinal activity. All the cannabinoid structures contain a monoterpene C_{10} unit attached to a phenolic ring having a C_5 alkyl chain. The aromatic ring/C_5 chain

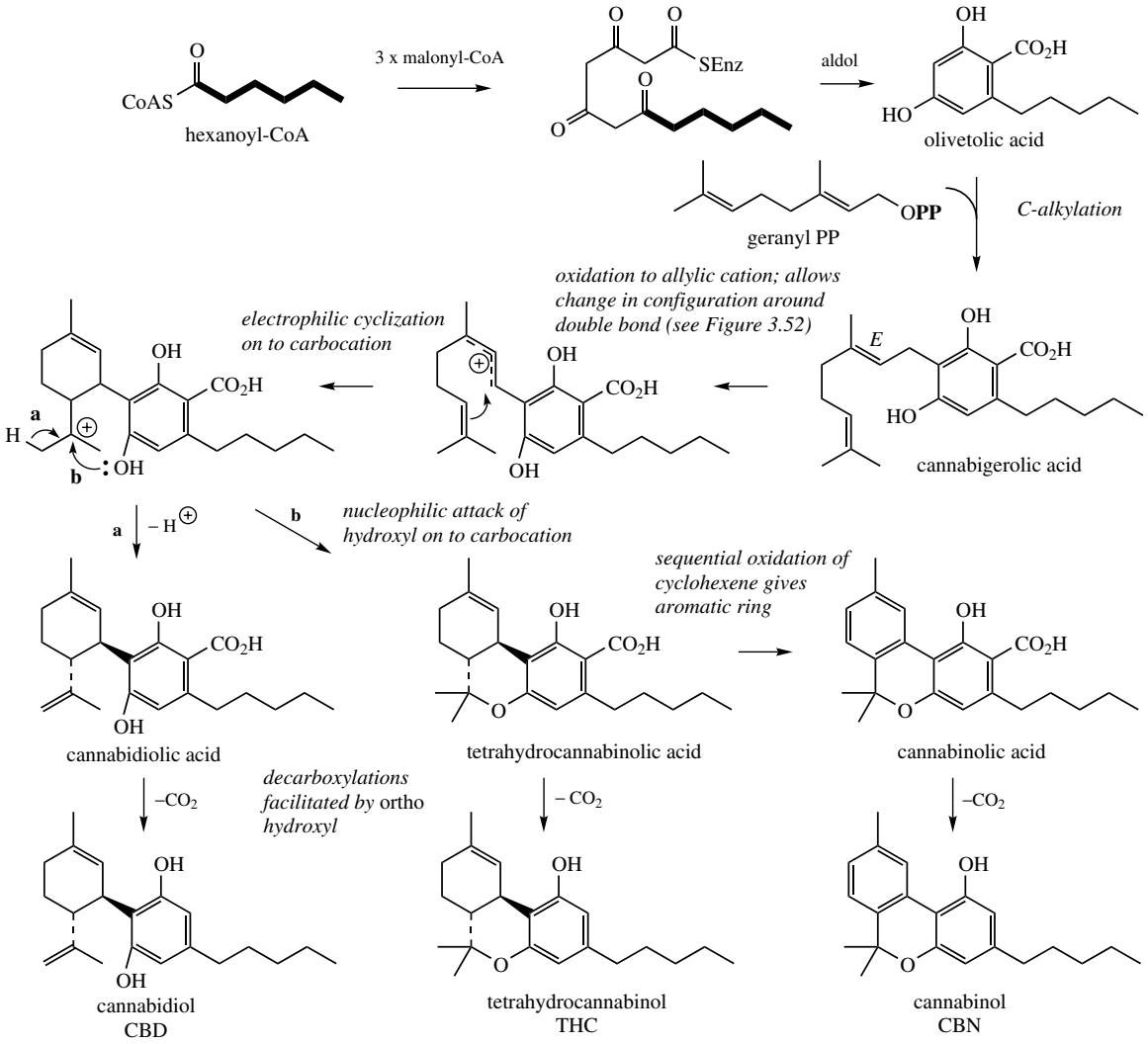

Figure 3.51

is likely to originate from hexanoate and malonate, cyclization to a polyketide giving **olivetolic acid**, from which **cannabigerolic acid** can be obtained by *C*-alkylation with the monoterpene unit geranyl diphosphate (Figure 3.51). Cyclization in the monoterpene unit necessitates a change in configuration of the double bond, and this may be rationalized as involving the allylic cation, which will then also allow electrophilic cyclization to proceed (for further detail see Figure 3.52, and compare terpenoid cyclization mechanisms, page 173). **Cannabidiolic acid** is the result of proton loss, whilst **tetrahydrocannabinolic acid** is the product from heterocyclic ring formation. **CBD** and **THC** are then the respective decarboxylation products from these two compounds. The aromatic terpenoid derived ring in **cannabinolic acid** and **cannabinol** can arise via a dehydrogenation process (compare thymol, page 186).

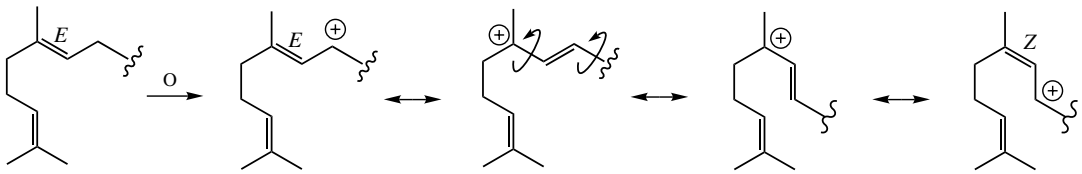

Figure 3.52

Cannabis

Indian hemp, *Cannabis sativa* (Cannabaceae) is an annual herb indigenous to Central and Western Asia, cultivated widely in India and many tropical and temperate regions for its fibre (hemp) and seed (for seed oil). The plant is also grown for its narcotic and mild intoxicant properties, and in most countries of the world its possession and consumption is illegal. Over many years, cannabis plants have been selected for either fibre production or drug use, the former resulting in tall plants with little pharmacological activity, whilst the latter tend to be short, bushy plants. Individual plants are almost always male or female, though the sex is not distinguishable until maturity and flowering. Seeds will produce plants of both sexes in roughly equal proportions. The active principles are secreted as a resin by glandular hairs, which are more numerous in the upper parts of female plants, and resin is produced from the time flowers first appear until the seeds reach maturity. However, all parts of the plant, both male and female, contain cannabinoids. In a typical plant, the concentration of cannabinoids increases in the following order: large leaves, small leaves, flowers, and bracts (which surround the ovaries), with stems containing very little. Material for drug use (ganja) is obtained by collecting the flowering tops (with little leaf) from female plants, though lower quality material (bhang) consisting of leaf from both female and male plants may be employed. By rubbing the flowering tops, the resin secreted by the glandular hairs can be released and subsequently scraped off to provide cannabis resin (charas) as an amorphous brown solid or semi-solid. A potent form of cannabis, called cannabis oil, is produced by alcoholic extraction of cannabis resin. A wide variety of names are used for cannabis products according to their nature and the geographical area. In addition to the Indian words above, the names hashish (Arabia), marihuana (Europe, USA), kief and dagga (Africa) are frequently used. The term ‘assassin’ is a corruption of ‘hashishin’, a group of 13th century murderous Persians who were said to have been rewarded for their activities with hashish. The names grass, dope, pot, hash, weed, and wacky backy are more likely to be in current usage.

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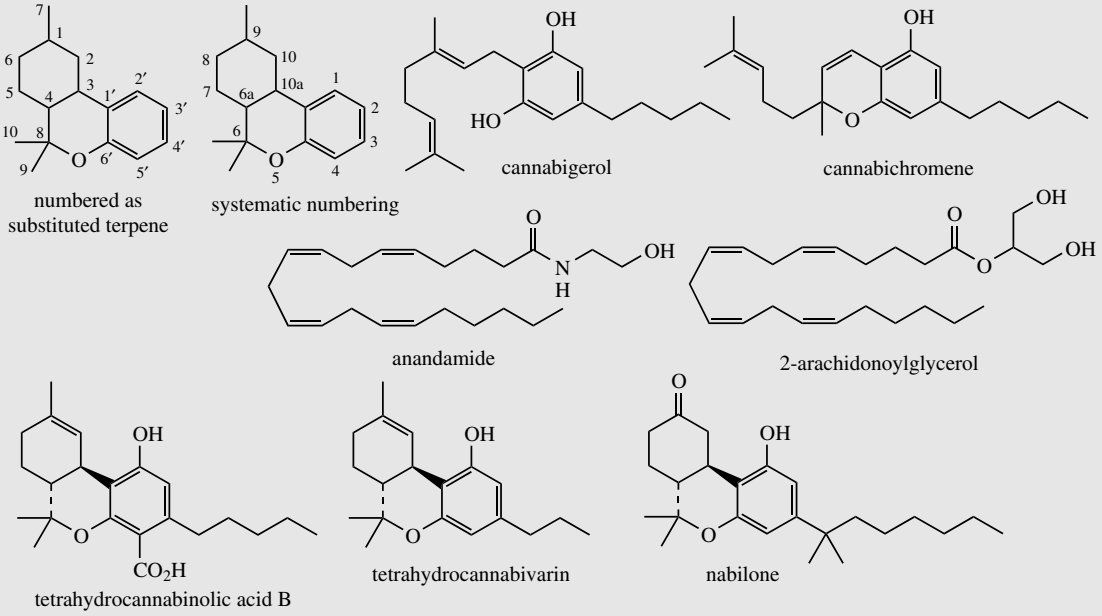

Figure 3.53

The quantity of resin produced by the flowering tops of high quality Indian cannabis is about 15–20%. The amount produced by various plants is dependent on several features, however, and this will markedly alter biological properties. Thus, in general, plants grown in a tropical climate produce more resin than those grown in a temperate climate. The tall fibre-producing plants are typically low resin producers, even in tropical zones. However, the most important factor is the genetic strain of the plant, and the resin produced may contain high levels of psychoactive compounds, or mainly inactive constituents. The quality of any cannabis drug is potentially highly variable.

The major constituents in cannabis are termed cannabinoids, a group of more than 60 structurally related terpenophenolics. The principal psychoactive agent is tetrahydrocannabinol (THC) (Figure 3.51). This is variously referred to as Δ^1-THC or Δ^9-THC according to whether the numbering is based on the terpene portion, or as a systematic dibenzopyran (Figure 3.53). Both systems are currently in use. Also found, often in rather similar amounts, are cannabinol (CBN) and cannabidiol (CBD) (Figure 3.51), which have negligible psychoactive properties. These compounds predominate in the inactive resins. Many other cannabinoid structures have been characterized, including cannabigerol and cannabichromene (Figure 3.53). A range of cannabinoid acids, e.g. cannabidiolic acid, tetrahydrocannabinolic acid, and tetrahydrocannabinolic acid B (Figure 3.53) are also present, as are some analogues of the other compounds mentioned, where a propyl side-chain replaces the pentyl group, e.g. tetrahydrocannabivarin (Figure 3.53). The latter compounds presumably arise from the use of butyrate rather than hexanoate as starter unit in the biosynthetic sequence.

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The THC content of high quality cannabis might be in the range 0.5–1% for large leaves, 1–3% for small leaves, 3–7% for flowering tops, 5–10% for bracts, 14–25% for resin, and up to 60% in cannabis oil. Higher amounts of THC are produced in selected strains known as skunk cannabis, so named because of their powerful smell; flowering tops from skunk varieties might contain 10–15% THC. The THC content in cannabis products tends to deteriorate on storage, an effect accelerated by heat and light. Cannabis leaf and resin stored under ordinary conditions rapidly lose their activity and can be essentially inactive after about 2 years. A major change which occurs is oxidation in the cyclohexene ring resulting in conversion of THC into CBN. THC is more potent when smoked than when taken orally, its volatility allowing rapid absorption and immediate effects, so smoking has become the normal means of using cannabis. Any cannabinoid acids will almost certainly be decarboxylated upon heating, and thus the smoking process will also effectively increase somewhat the levels of active cannabinoids available, e.g. THC acid $\rightarrow$ THC (Figure 3.51). The smoking of cannabis produces a mild euphoria similar to alcohol intoxication, inducing relaxation, contentment, and a sense of well-being, with some changes in perception of sound and colour. However, this is accompanied by a reduced ability to concentrate and do complicated tasks, and a loss of short-term memory. Users claim cannabis is much preferable to alcohol or tobacco, insisting it does not cause dependence, withdrawal symptoms, or lead to the use of other drugs, and they campaign vociferously for its legalization. However, psychological dependence does occur, and cannabis can lead to hallucinations, depression, anxiety, and panic, with the additional risk of bronchitis and lung cancer if the product is smoked.

Cannabis has been used medicinally, especially as a mild analgesic and tranquillzer, but more effective and reliable agents replaced it, and even controlled prescribing was discontinued. In recent times, cannabis has been shown to have valuable anti-emetic properties, which help to reduce the side-effects of nausea and vomiting caused by cancer chemotherapeutic agents. This activity stems from THC, and has resulted in some use of **THC** (**dronabinol**) and the prescribing of cannabis for a small number of patients. A synthetic THC analogue, **nabilone** (Figure 3.53), has been developed as an anti-emetic drug for reducing cytotoxic-induced vomiting. Some of the psychoactive properties of THC, e.g. euphoria, mild hallucinations, and visual disturbances, may be experienced as side-effects of nabilone treatment. Cannabis has also been shown to possess properties which may be of value in other medical conditions. There is now ample evidence that cannabis can give relief to patients suffering from chronic pain, multiple sclerosis, glaucoma, asthma, migraine, epilepsy, and other conditions. Many sufferers who cannot seem to benefit from any of the current range of drugs are obtaining relief from their symptoms by using cannabis, but are breaking the law to obtain this medication. Current thinking is that cannabis offers a number of beneficial pharmacological responses and that there should be legal prescribing of cannabinoids or derivatives. Clinical trials have already confirmed the value of cannabis and/or THC taken orally for the relief of chronic pain and the painful spasms characteristic of multiple sclerosis, and in reducing intraocular pressure in glaucoma sufferers. In general, cannabis is only able to alleviate the symptoms of these diseases, and does not provide a cure. The non-psychoactive CBD has been shown to have anti-inflammatory properties potentially useful in arthritis treatment.

Recently, the ethanolamide of arachidonic acid (anandamide; ananda is the Sanskrit word for bliss) (Figure 3.53) has been isolated from animal brain tissue, and has been shown

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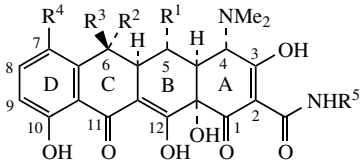
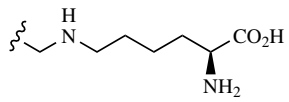
to mimic several of the pharmacological properties of THC. This appears to be a natural ligand which interacts with central receptors (CB1) to which cannabinoids also bind. Two other polyunsaturated fatty acid ethanolamides, namely dihomo-γ-linolenoyl- (20:3) and adrenoyl- (22:4) ethanolamides have also been isolated from mammalian brain, and shown to have THC-like properties. Another type of cannabinoid receptor (CB2), expressed mainly in the immune system, has been identified; its natural ligand is 2-arachidonoylglycerol (Figure 3.53). Since this compound also interacts with the anandamide receptor, and levels of 2-arachidonoylglycerol in the brain are some 800 times higher than those of anandamide, it is now thought to be the physiological ligand for both receptors, rather than anandamide. The identification of these endogenous materials may open up other ways of exploiting some of the desirable pharmacological features of cannabis.

Table 3.3 Tetracyclines



	R^1 5	R^2 6α	R^3 6β	R^4 7	R^5	
tetracycline	H	Me	OH	H	H	**natural**
chlortetracycline	H	Me	OH	Cl	H	
oxytetracycline	OH	Me	OH	H	H	
demeclocycline	H	H	OH	H	H	
methacycline	OH	$=CH_2$		H	H	**semi-synthetic**
doxycycline	OH	Me	H	H	H	
minocycline	H	H	H	NMe_2	H	
lymecycline	H	Me	OH	H		

The **tetracyclines*** (Table 3.3) are a group of broad spectrum, orally active antibiotics produced by species of *Streptomyces*, and several natural and semi-synthetic members are used clinically. They contain a linear tetracyclic skeleton of polyketide origin in which the starter group is **malonamyl-CoA** (Figure 3.54), i.e. the coenzyme A ester of malonate semi-amide. Thus, in contrast to most acetate-derived compounds, malonate supplies all carbon atoms of the tetracycline skeleton, the starter group as well as the chain extenders. The main features of the pathway (Figure 3.54) were deduced from extensive studies of mutant strains of *Streptomyces aureofaciens* with genetic blocks causing accumulation of mutant metabolites or production of abnormal tetracyclines. This organism typically produces **chlortetracycline**, whilst the parent compound **tetracycline** (Table 3.3) is in fact an aberrant product synthesized in mutants blocked in the chlorination step. The use of mutants with genetic blocks has also enabled the shikimate pathway (Chapter 4) to be delineated. In that case, since a primary metabolic pathway was affected, mutants tended to accumulate intermediates and could not grow unless later components of the pathway were supplied. With the tetracyclines, a secondary metabolic pathway is involved, and the relatively broad specificity of some of the

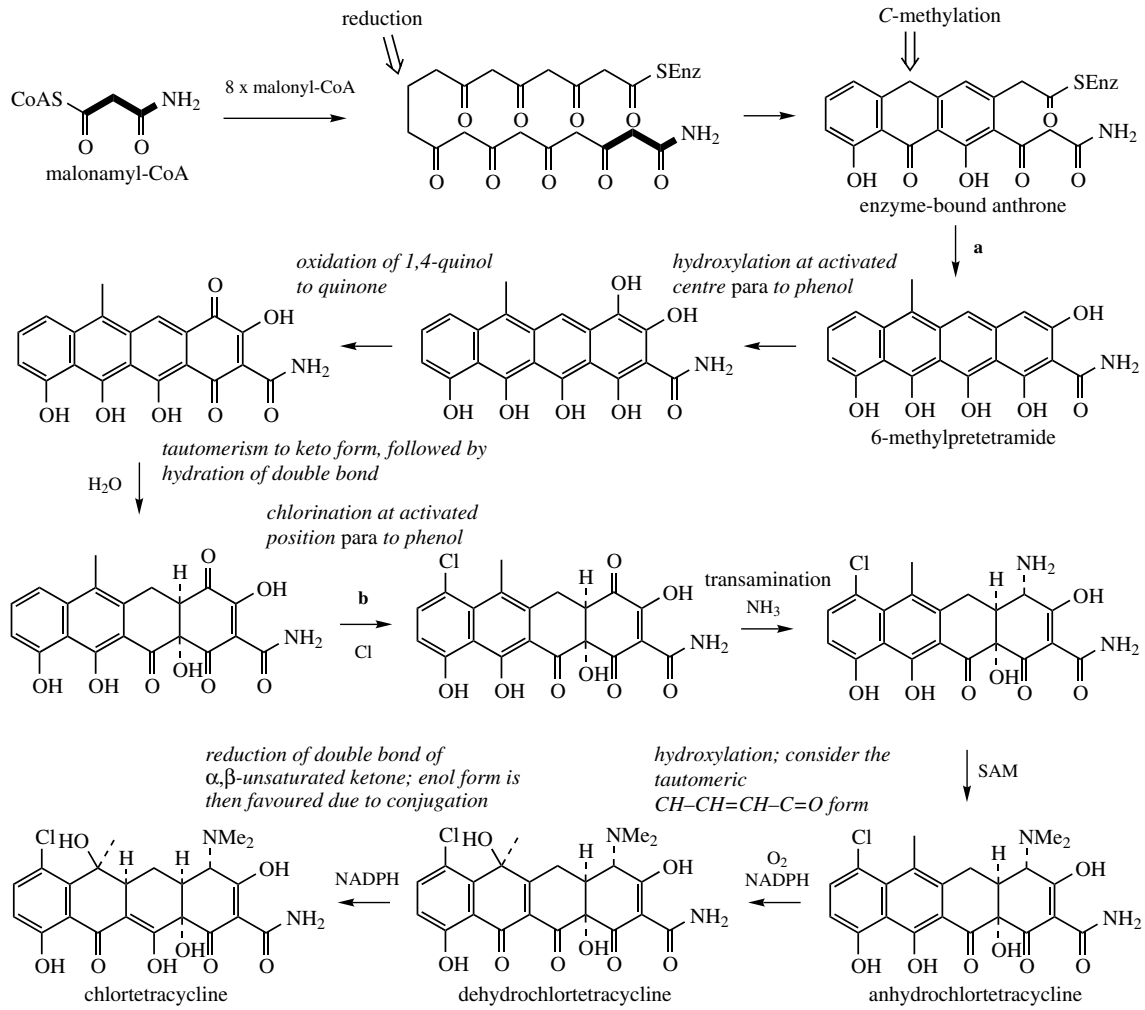


Figure 3.54

Tetracyclines

The tetracyclines (Table 3.3) are a group of broad spectrum, orally active antibiotics produced by cultures of *Streptomyces* species. **Chlortetracycline** isolated from *Streptomyces aureofaciens* was the first of the group to be discovered, closely followed by **oxytetracycline** from cultures of *S. rimosus*. **Tetracycline** was found as a minor antibiotic in *S. aureofaciens*, but may be produced in quantity by utilizing a mutant strain blocked in the chlorination step *b* (Figure 3.54). Similarly, the early C-6 methylation step (included in *a*) can also be blocked, and such mutants accumulate 6-demethyltetracyclines, e.g. **demeclocycline** (**deme**thyl**ch**l**o**rotetra**cycline**). These reactions can also be inhibited in the normal strain of *S. aureofaciens* by supplying cultures with either aminopterin (which inhibits C-6 methylation) or mercaptothiazole (which inhibits C-7 chlorination). Oxytetracycline from *S. rimosus* lacks

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the chlorine substituent, but has an additional 5α-hydroxyl group, probably introduced at a late stage. Only minor alterations can be made to the basic tetracycline structure to modify the antibiotic activity, and these are at positions 5, 6, and 7. Other functionalities in the molecule are all essential to retain activity. Semi-synthetic tetracyclines used clinically include **methacycline**, obtained by a dehydration reaction from oxytetracycline, and **doxycycline**, via reduction of the 6-methylene in methacycline. **Minocycline** contains a 7-dimethylamino group and is produced by a sequence involving aromatic nitration. **Lymecycline** is an example of an antibiotic developed by chemical modification of the primary amide function at C-2.

Having both amino and phenolic functions, tetracyclines are amphoteric compounds, and are more stable in acid than under alkaline conditions. They are thus suitable for oral administration, and are absorbed satisfactorily. However, because of the sequence of phenol and carbonyl substituents in the structures, they act as chelators and complex with metal ions, especially calcium, aluminium, iron, and magnesium. Accordingly, they should not be administered with foods such as milk and dairy products (which have a high calcium content), aluminium- and magnesium-based antacid preparations, iron supplements, etc, otherwise erratic and unsatisfactory absorption will occur. A useful feature of doxycycline and minocycline is that their absorptions are much less affected by metal ions. Chelation of tetracyclines with calcium also precludes their use in children developing their adult teeth, and in pregnant women, since the tetracyclines become deposited in the growing teeth and bone. In children, this would cause unsightly and permanent staining of teeth with the chelated yellow tetracycline.

Although the tetracycline antibiotics have a broad spectrum of activity spanning Gram-negative and Gram-positive bacteria, their value has decreased as bacterial resistance has developed in pathogens such as *Pneumococcus*, *Staphylococcus*, *Streptococcus*, and *E. coli*. These organisms appear to have evolved mechanisms of resistance involving decreased cell permeability; a membrane-embedded transport protein exports the tetracycline out of the cell before it can exert its effect. Nevertheless, tetracyclines are the antibiotics of choice for infections caused by *Chlamydia*, *Mycoplasma*, *Brucella*, and *Rickettsia*, and are valuable in chronic bronchitis due to activity against *Haemophilus influenzae*. They are also used systemically to treat severe cases of acne, helping to reduce the frequency of lesions by their effect on skin flora. There is little significant difference in the antimicrobial properties of the various agents, except for **minocycline**, which has a broader spectrum of activity, and being active against *Neisseria meningitidis* is useful for prophylaxis of meningitis. The individual tetracyclines do have varying bioavailabilities, however, which may influence the choice of agent. **Tetracycline** and **oxytetracycline** are probably the most commonly prescribed agents. Tetracyclines are formulated for oral application or injection, as ear and eye drops, and for topical use on the skin. **Doxycycline** also finds use as a prophylactic against malaria in areas where there is widespread resistance to chloroquine and mefloquine (see page 363).

Their antimicrobial activity arises by inhibition of protein synthesis. This is achieved by interfering with the binding of aminoacyl-tRNA to acceptor sites on the ribosome by disrupting the codon–anticodon interaction (see page 407). Evidence points to a single strong binding site on the smaller 30S subunit of the ribosome. Although tetracyclines can also bind to mammalian ribosomes, there appears to be preferential penetration into bacterial cells, and there are few major side-effects from using these antibiotics.

A series of tetracycline derivatives has recently been isolated from species of *Dactylosporangium*. These compounds, the dactylocyclines (Figure 3.55), are glycosides and have the opposite configuration at C-6 to the natural tetracyclines. Importantly, these compounds are active towards tetracycline-resistant bacteria.

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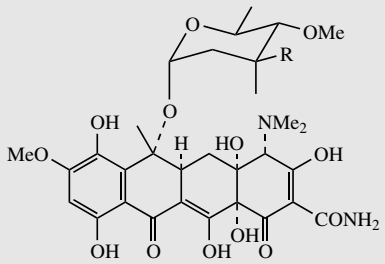

R = NHOH, dactylocycline-A
R = NO_2, dactylocycline-B
R = NHOAc, dactylocycline-C
R = OH, dactylocycline-E

Figure 3.55

enzymes concerned allows many of the later steps to proceed even if one step, e.g. the chlorination, is not achievable. This has also proved valuable for production of some of the clinical tetracycline antibiotics.

One of the early intermediates in the pathway to chlortetracycline is 6-methylpretetramide (Figure 3.54). This arises from the poly-β-keto ester via an enzyme-bound anthrone (compare Figure 3.30). Reduction of one carbonyl will occur during chain extension, whilst the methylation must be a later modification. Hydroxylation in ring A followed by oxidation gives a quinone, the substrate for hydration at the A/B ring fusion. The product now features the keto tautomer in ring B, since its aromaticity has been destroyed. Chlorination of ring D at the nucleophilic site *para* to the phenol follows, and an amine group is then introduced stereospecifically into ring A by a transamination reaction. This amino function is then di-*N*-methylated using SAM as the methylating agent yielding **anhydrochlortetracycline**. In the last two steps, C-6 is hydroxylated via an O_2-, NADPH-, and flavin-dependent oxygenase giving the enone **dehydrochlortetracycline**, and NADPH reduction of the C-5a/11a double bond generates **chlortetracycline**.

A number of **anthracycline antibiotics***, e.g. **doxorubicin** (Figure 3.56) from *Streptomyces peuceticus* and **daunorubicin** from *S. coeruleorubicus*, have structurally similar tetracyclic skeletons and would appear to be related to the tetracyclines. There are similarities in that the molecules are essentially acetate derived, but for the anthracyclines the starter group is **propionate** rather than malonamide, and labelling studies have demonstrated a rather different folding of the poly-β-keto chain (Figure 3.56). As a result, the end-of-chain carboxyl is ultimately lost through decarboxylation. This carboxyl is actually retained for a considerable portion of the pathway, and is even protected against decarboxylation by methylation to the ester, until no longer required. Most of the modifications which occur during the biosynthetic pathway are easily predictable. Thus, the anthraquinone portion is likely to be formed first, then the fourth ring can be elaborated by a aldol reaction (Figure 3.56). A feature of note in molecules such as doxorubicin and daunorubicin is the amino sugar L-daunosamine which originates from TDPglucose (thymidine diphosphoglucose; compare UDPglucose, page 29) and is introduced in the latter stages of the sequence. Hydroxylation of daunorubicin to doxorubicin is the very last step. Doxorubicin and daunorubicin are used as antitumour drugs rather than antimicrobial agents. They act primarily at the DNA level and so also have cytotoxic properties. Doxorubicin in particular is a highly successful and widely used antitumour agent, employed in the treatment of leukaemias, lymphomas, and a variety of solid tumours.

MACROLIDES AND POLYETHERS

Extender Groups other than Malonate

The use of propionate as a starter group as in the formation of the anthracyclines is perhaps

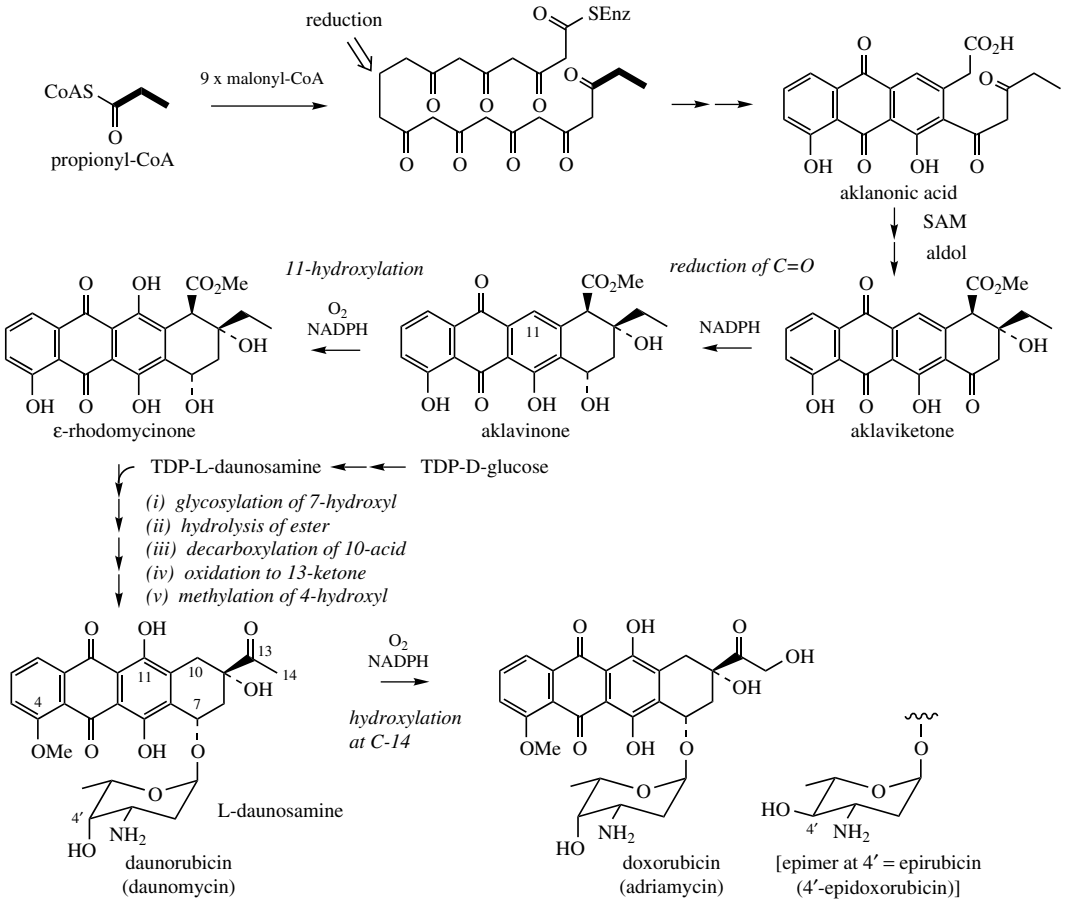

Figure 3.56

Anthracycline Antibiotics

Doxorubicin (adriamycin) (Figure 3.56) is produced by cultures of *Streptomyces peucetius* var *caesius* and is one of the most successful and widely used antitumour drugs. The organism is a variant of *S. peucetius*, a producer of daunorubicin (see below), in which mutagen treatment resulted in expression of a latent hydroxylase enzyme and thus synthesis of doxorubicin by 14-hydroxylation of daunorubicin. Doxorubicin has one of the largest spectra of antitumour activity shown by antitumour drugs and is used to treat acute leukaemias, lymphomas, and a variety of solid tumours. It is administered by intravenous injection and largely excreted in the bile. It inhibits the synthesis of RNA copies of DNA by intercalation of the planar molecule between base pairs on the DNA helix. The sugar unit provides further binding strength and also plays a major role in sequence-recognition for the binding. Doxorubicin also exerts some of its cytotoxic effects by inhibition of the enzyme topoisomerase II, which is responsible for cleaving and resealing of double-stranded DNA during replication (see page 137). Common toxic effects include nausea and vomiting, bone marrow suppression, hair loss, and local tissue necrosis, with cardiotoxicity at higher dosage.
Daunorubicin (Figure 3.56) is produced by *Streptomyces coeruleorubidus* and *S. peucetius*,

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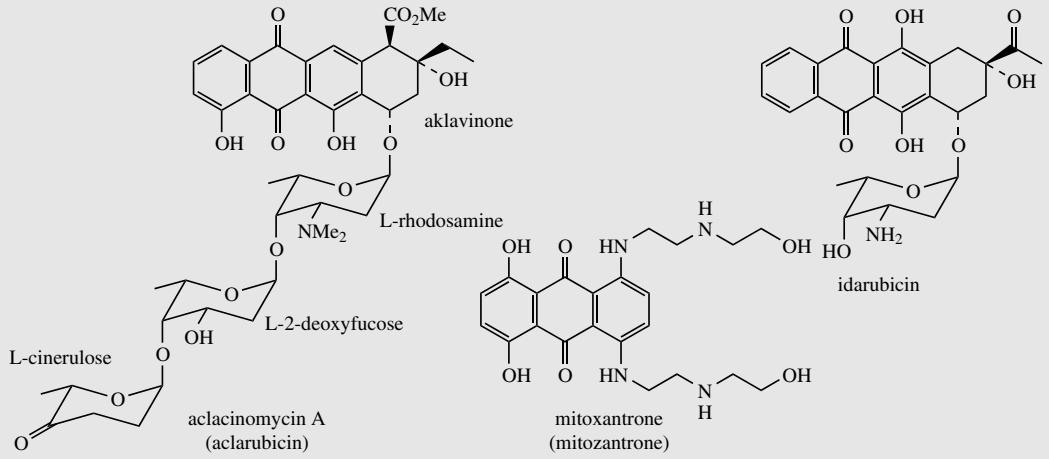

Figure 3.57

and, though similar to doxorubicin in its biological and chemical properties, it is no longer used therapeutically to any extent. It has a much less favourable therapeutic index than doxorubicin, and the markedly different effectiveness as an antitumour drug is not fully understood, though differences in metabolic degradation may be responsible. **Epirubicin** (Figure 3.56), the 4′-epimer of doxorubicin, is particularly effective in the treatment of breast cancer, producing lower side-effects than doxorubicin. The antileukaemics **aclarubicin** from *Streptomyces galilaeus*, a complex glycoside of aklavinone (Figure 3.56), and the semi-synthetic **idarubicin** are shown in Figure 3.57. These compounds are structurally related to doxorubicin but can show increased activity with less cardiotoxicity. The principal disadvantage of all of these agents is their severe cardiotoxicity which arises through inhibition of cardiac Na^+,K^+-ATPase.

Mitoxantrone (**mitozantrone**) (Figure 3.57) is a synthetic analogue of the anthracyclinones in which the non-aromatic ring and the aminosugar have both been replaced with aminoalkyl side-chains. This agent has reduced toxicity compared with doxorubicin, and is effective in the treatment of solid tumours and leukaemias.

less common than incorporating it as a chain extender via methylmalonyl-CoA. We have already encountered this process in the formation of some branched-chain fatty acids with methyl substituents on the basic chain (see page 49). Of course, methyl groups can also be added to a fatty acid chain via SAM (see page 49), and there are also many examples for the methylation of poly-β-keto chains, several of which have already been discussed. Accordingly, methylation using SAM, and incorporation of propionate via methylmalonyl-CoA, provide two different ways of synthesizing a methylated polyketide (Figure 3.58). The former process is the more common in fungi, whilst Actinomycetes (e.g. *Streptomyces*) tend to employ propionate by the latter route. The incorporation of propionate by methylmalonate extender units can frequently be interrupted and normal malonate extenders are added, thus giving an irregular sequence of methyl side-chains.

The **macrolide antibiotics*** provide us with excellent examples of natural products conforming to the acetate pathway, but composed principally of propionate units, or mixtures of propionate and acetate units. The macrolides are a large family of compounds, many with antibiotic activity, characterized by a macrocyclic lactone ring, typically 12, 14, or 16 membered, reflecting the number of units utilized. **Zearalenone** (Figure 3.59), a toxin produced by the

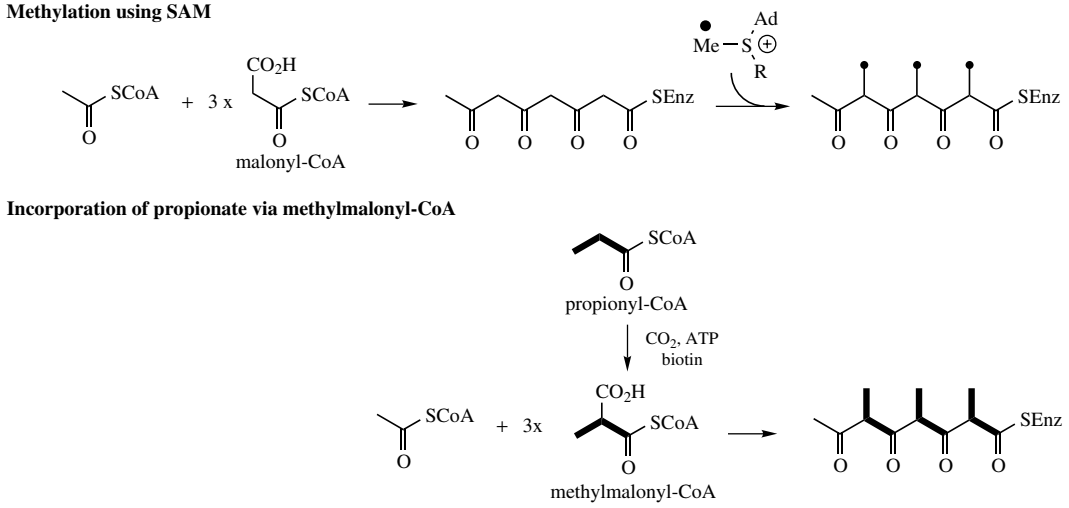

Figure 3.58

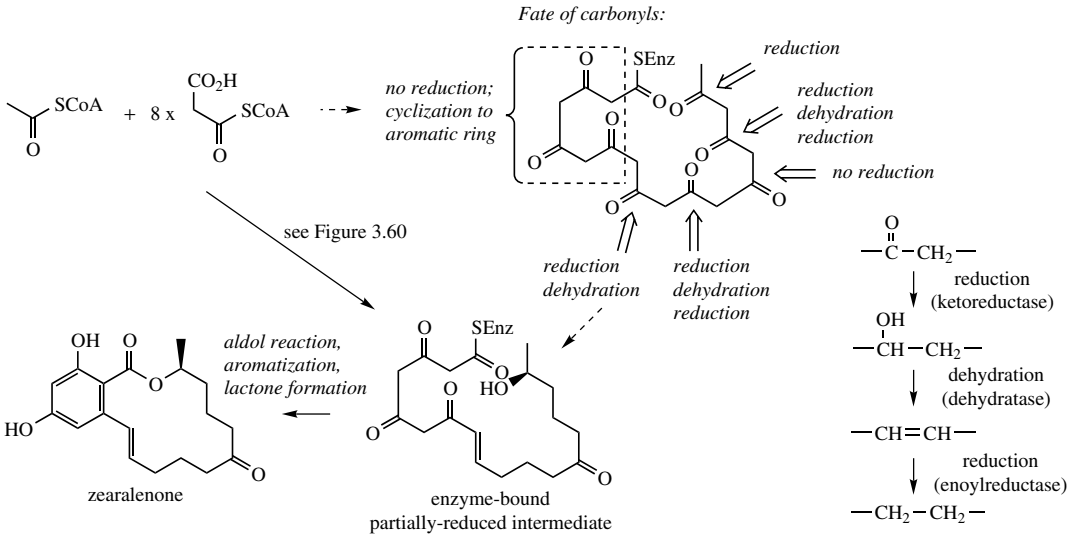

Figure 3.59

fungus *Gibberella zeae* and several *Fusarium* species, has a relatively simple structure which is derived entirely from acetate–malonate units. It could be envisaged as a cyclization product from a poly-β-keto ester, requiring a variety of reduction processes and formation of an aromatic ring by aldol condensation near the carboxyl terminus (Figure 3.59). However, the poly-β-keto ester shown in Figure 3.59 would not be produced, since its reactivity might tend to favour formation of a polycyclic aromatic system (compare anthraquinones, page 63, and tetracyclines, page 89). Instead, appropriate reductions, dehydrations, etc, involving the β-carbonyl group are achieved during the chain extension process as in the fatty acid pathway (see page 36), and *before* further malonyl-CoA extender units are added (Figure 3.60). In contrast to fatty acid biosynthesis, where there is total reduction of each carbonyl group before further chain extension, macrolide biosynthesis frequently involves partial reduction, with the enzymic machinery being accurately controlled to leave the units at the right oxidation level before further chain extension

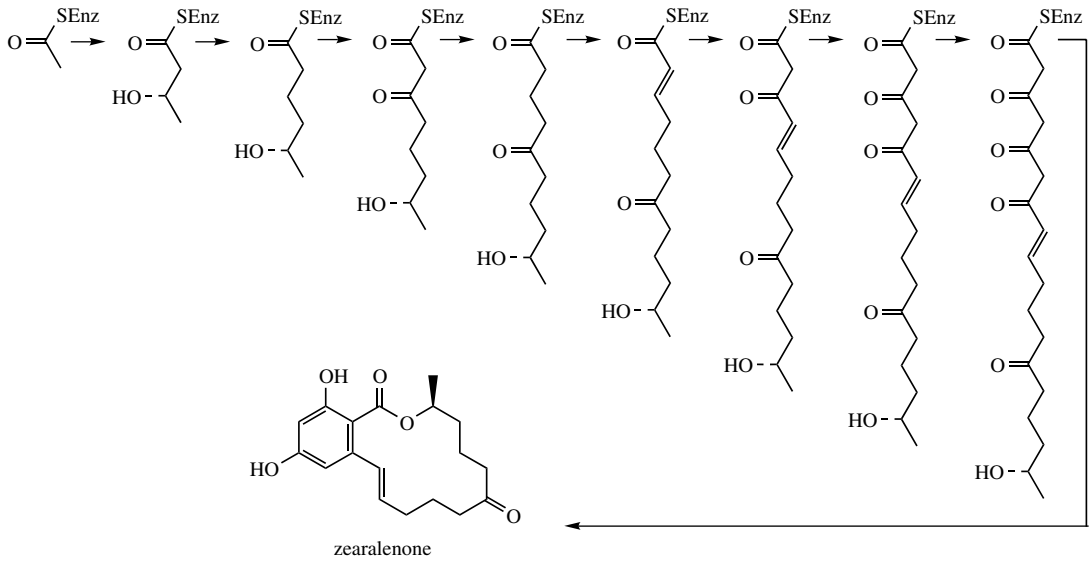

Figure 3.60

occurs. This then provides an enzyme-bound intermediate, which leads on to the final product (Figure 3.59). As a result, zearalenone is a remarkable example of an acetate-derived metabolite containing all types of oxidation level seen during the fatty acid extension cycle, i.e. carbonyl, secondary alcohol (eventually forming part of the lactone), alkene, and methylene, as well as having a portion which has cyclized to an aromatic ring because no reduction processes occurred in that fragment of the chain. There is now extensive genetic evidence from a variety of **polyketide synthase** systems to show that macrolide assembly is accomplished on a biological production line of multifunctional proteins organised as discrete modules, in which the developing polyketide chain attached to an acyl carrier protein is modified according to the appropriate enzyme activities encoded genetically, and is then passed on to another ACP prior to the next condensation and modification (see page 115 for more details).

Erythromycin A (Figure 3.61) from *Saccharopolyspora erythraea* is a valuable antibacterial drug and contains a 14-membered macrocycle composed entirely of propionate units, both as starter and extension units, the latter via methylmalonyl-CoA. In common with many antibacterial macrolides, sugar units, including amino sugars, are attached through glycoside linkages. These unusual 6-deoxy sugars are frequently restricted to this group of natural products. In erythromycin A, the sugars are L-cladinose and D-desosamine. Chain extension and appropriate reduction processes lead to an enzyme-bound polyketide in which one carbonyl group has suffered total reduction, four have been reduced to alcohols, whilst one carbonyl is not reduced, and remains throughout the sequence. These processes ultimately lead to release of the modified polyketide as the macrolide ester **deoxyerythronolide**, a demonstrated intermediate in the pathway to erythromycins (Figure 3.61; see also page 115). The stereochemistry in the chain is controlled by the condensation and reduction steps during chain extension, but a reassuring feature is that there appears to be a considerable degree of stereochemical uniformity throughout the known macrolide antibiotics. In the later stages of the biosynthesis of erythromycin, hydroxylations at carbons 6 and 12, and addition of sugar units, are achieved.

A combination of propionate and acetate units is used to produce the 14-membered macrocyclic ring of **oleandomycin** (Figure 3.62) from *Streptomyces antibioticus*, but otherwise many of the structural features and the stereochemistry of oleandomycin resemble those of erythromycin A. One acetate provides the starter unit, whilst seven propionates, via methylmalonyl-CoA, supply the extension units (Figure 3.62). One methyl group derived

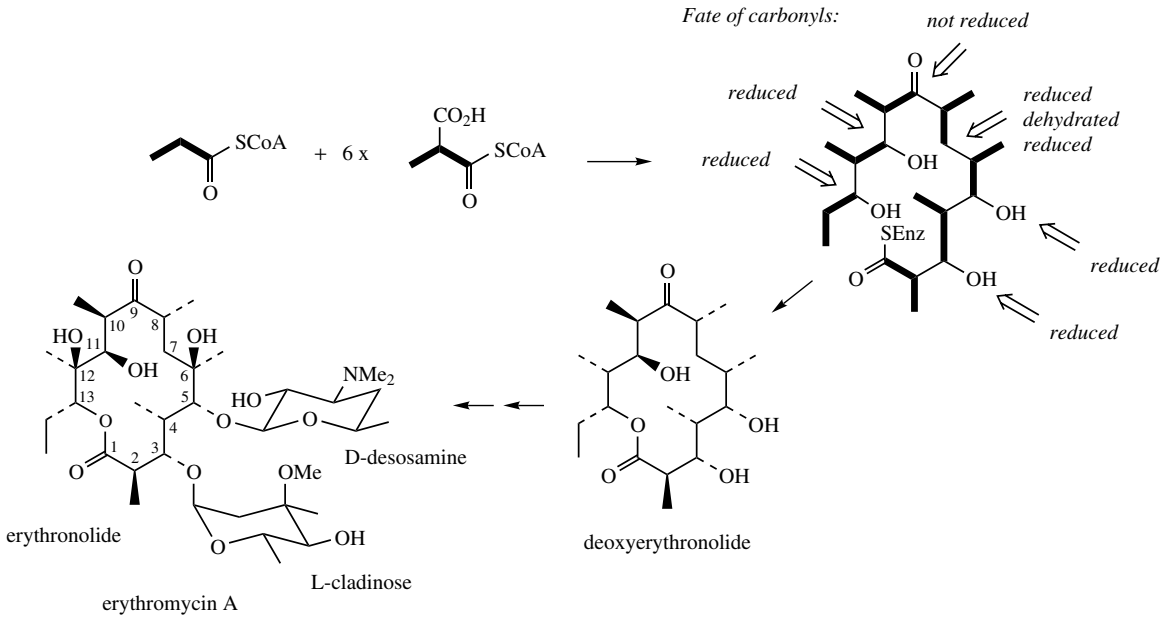

Figure 3.61

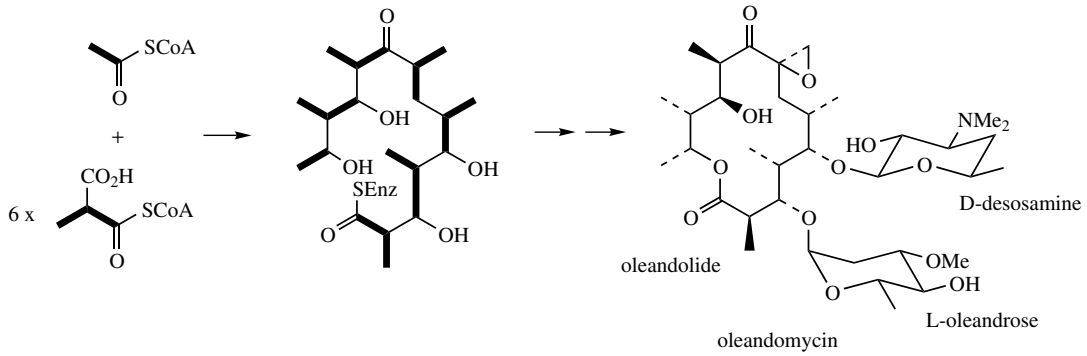

Figure 3.62

from propionate has been modified to give an epoxide function. The sugar units in oleandomycin are L-oleandrose and D-desosamine. **Spiramycin I** (Figure 3.63) from *Streptomyces ambofaciens* has a 16-membered lactone ring, and is built up from a combination of six acetate units (one as starter), one propionate extender, together with a further variant, butyrate as chain extender. Butyrate will be incorporated via ethylmalonyl-CoA and yield an extension unit having an ethyl side-chain. This is outlined in Figure 3.63. In due course, this ethyl group is oxidized generating an aldehyde. Spiramycin I also contains a conjugated diene, the result of carbonyl reductions being followed by dehydration during chain assembly. **Tylosin** (Figure 3.64) from *Streptomyces fradiae* has many structural resemblances to the spiramycins, but can be analysed as a propionate starter with chain extension from two malonyl-CoA, four methylmalonyl-CoA, and one ethylmalonyl-CoA.

The **avermectins*** (Figure 3.67) have no antibacterial activity, but possess anthelmintic, insecticidal, and acaricidal properties, and these are exploited in human and veterinary medicine. The avermectins are also 16-membered macrolides, but their structures are made up from a much longer

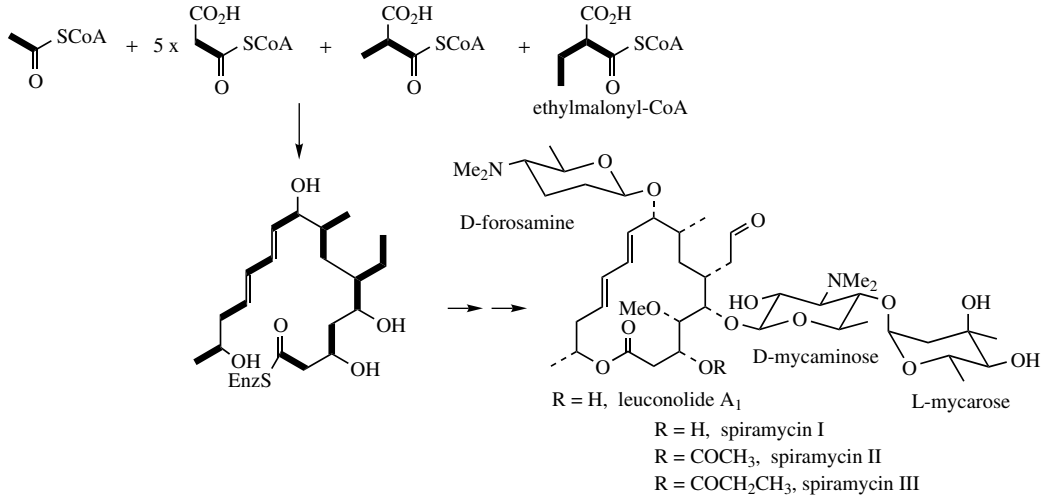

Figure 3.63

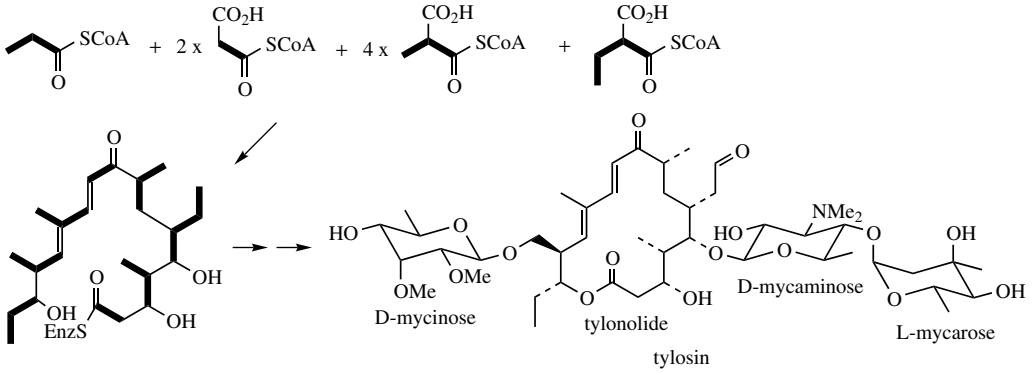

Figure 3.64

Macrolide Antibiotics

The macrolide antibiotics are macrocyclic lactones with a ring size typically 12–16 atoms, and with extensive branching through methyl substituents. Two or more sugar units are attached through glycoside linkages, and these sugars tend to be unusual 6-deoxy structures often restricted to this class of compounds. Examples include L-cladinose, L-mycarose, D-mycinose, and L-oleandrose. At least one sugar is an amino sugar, e.g. D-desosamine, D-forosamine, and D-mycaminose. These antibiotics have a narrow spectrum of antibacterial activity, principally against Gram-positive microorganisms. Their antibacterial spectrum resembles, but is not identical to, that of the penicillins, so they provide a valuable alternative for patients allergic to the penicillins. Erythromycin is the principal macrolide antibacterial currently used in medicine.

The erythromycins (Figure 3.65) are macrolide antibiotics produced by cultures of *Saccharopolyspora erythraea* (formerly *Streptomyces erythreus*). The commercial product

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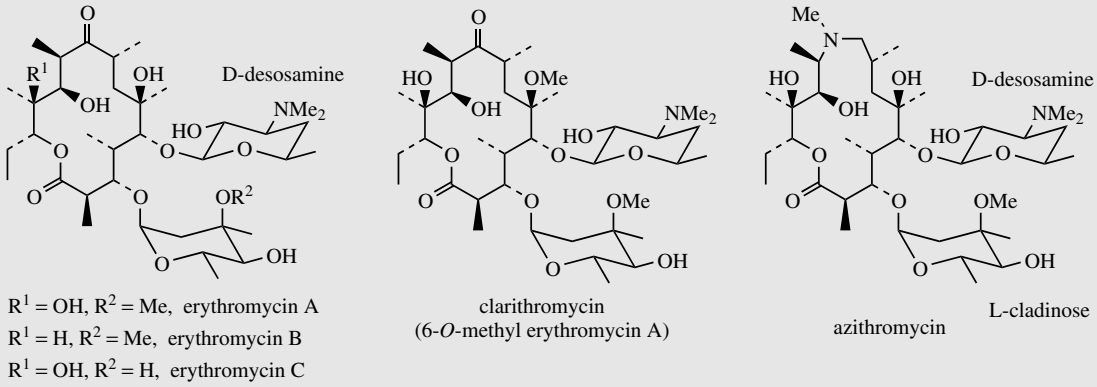

Figure 3.65

erythromycin is a mixture containing principally erythromycin A, plus small amounts of erythromycins B and C (Figure 3.65). Erythromycin activity is predominantly against Gram-positive bacteria, and the antibiotic is prescribed for penicillin-allergic patients. It is also used against penicillin-resistant *Staphylococcus* strains, in the treatment of respiratory tract infections, and systemically for skin conditions such as acne. It is the antibiotic of choice for infections of *Legionella pneumophila*, the cause of legionnaire's disease. Erythromycin exerts its antibacterial action by inhibiting protein biosynthesis in sensitive organisms. It binds reversibly to the larger 50S subunit of bacterial ribosomes and blocks the translocation step in which the growing peptidyl-tRNA moves from the aminoacyl acceptor site to the peptidyl donor site on the ribosome (see page 408). The antibiotic is a relatively safe drug with few serious side-effects. Nausea and vomiting may occur, and if high doses are prescribed, a temporary loss of hearing might be experienced. Hepatotoxicity may also occur at high dosage.

Erythromycin is unstable under acidic conditions, undergoing degradation to inactive compounds by a process initiated by the 6-hydroxyl attacking the 9-carbonyl to form a hemiketal. Dehydration then follows (Figure 3.66). The 14-membered ring in erythromycin A adopts a modified version of the diamond lattice chairlike conformation shown in Figure 3.66. Studies have indicated that carbon 6 is displaced from this conformation to reduce the 1,3-diaxial interactions at C-4 and C-6, and the two relatively large sugar units attached to the hydroxyls at C-3 and C-5 also distort the ring system further. The distortion of the chairlike conformation brings the 6-hydroxyl sufficiently close to react with the 9-carbonyl. A similar reaction may occur between the C-12 hydroxyl and the 9-carbonyl. Thus, to protect oral preparations of erythromycin against gastric acid, they are formulated as enteric-coated tablets, or as insoluble esters (e.g. ethyl succinate esters), which are then hydrolysed in the intestine. Esterification typically involves the hydroxyl of the amino sugar desosamine. To reduce this acid instability, semi-synthetic analogues of erythromycin have also been developed. **Clarithromycin** (Figure 3.65) is a 6-*O*-methyl derivative of erythromycin A; this modification blocks hemiketal formation as in Figure 3.66. **Azithromycin** (Figure 3.65) is a ring-expanded aza-macrolide in which the carbonyl function has been reduced. In both analogues, the changes enhance activity compared with that of erythromycin.

Bacterial resistance to erythromycin has become significant and has limited its therapeutic use against many strains of *Staphylococcus*. Several mechanisms of resistance have been

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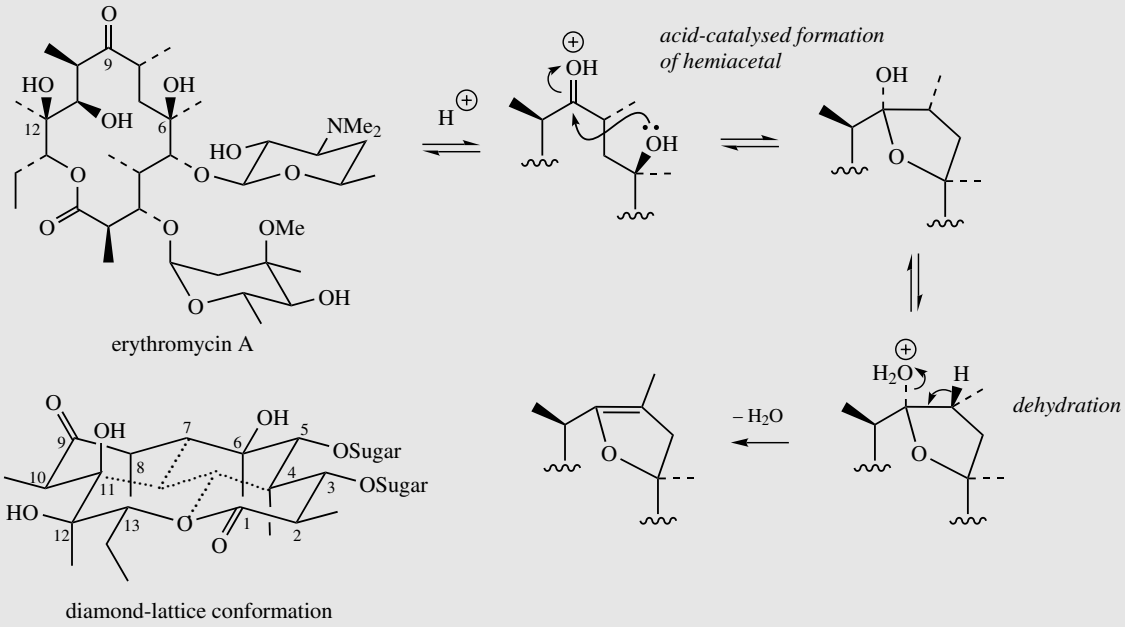

Figure 3.66

implicated, one of which is a change in permeability of the bacterial cell wall. Differences in permeability also appear to explain the relative insensitivity of Gram-negative bacteria to erythromycin when compared to Gram-positive bacteria. Resistant bacteria may also modify the chemical nature of the binding site on the ribosome, thus preventing antibiotic binding, and some organisms are now known to metabolize the macrolide ring to yield inactive products.

Oleandomycin (Figure 3.62) is produced by fermentation cultures of *Streptomyces antibioticus* and has been used medicinally as its triacetyl ester **troleandomycin** against Gram-positive bacterial infections. The **spiramycins** (Figure 3.63) are macrolides produced by cultures of *Streptomyces ambofaciens*. The commercial antibiotic is a mixture containing principally spiramycin I, together with smaller amounts (10–15% each) of the acetyl ester spiramycin II and the propionyl ester spiramycin III. This antibiotic has recently been introduced into medicine for the treatment of toxoplasmosis, infections caused by the protozoan *Toxoplasma gondii*.

Tylosin (Figure 3.64) is an important veterinary antibiotic. It is produced by *Streptomyces fradiae*, and is used to control chronic respiratory diseases caused by *Mycoplasma galliseptum* in poultry, and to treat Gram-positive infections in pigs.

polyketide chain, which is also used to form oxygen heterocycles fused to the macrolide. **Avermectin B_{1a}** exemplifies a typical structure and the basic carbon skeleton required to produce this can be postulated as in Figure 3.67. The starter unit in this case would be 2-methylbutyryl-CoA, which is derived from the amino acid L-isoleucine (compare necic acids, page 305, and tiglic acid, page 197). Both malonyl-CoA and methylmalonyl-CoA are then utilized as extender units. The heterocyclic rings are easily accounted for: the spiro system is merely a ketal, though the tetrahydrofuran ring requires further hydroxylations of the basic skeleton for its construction. Avermectins are usually isolated as a mixture in which the main *a* component has a 2-methylpropyl group

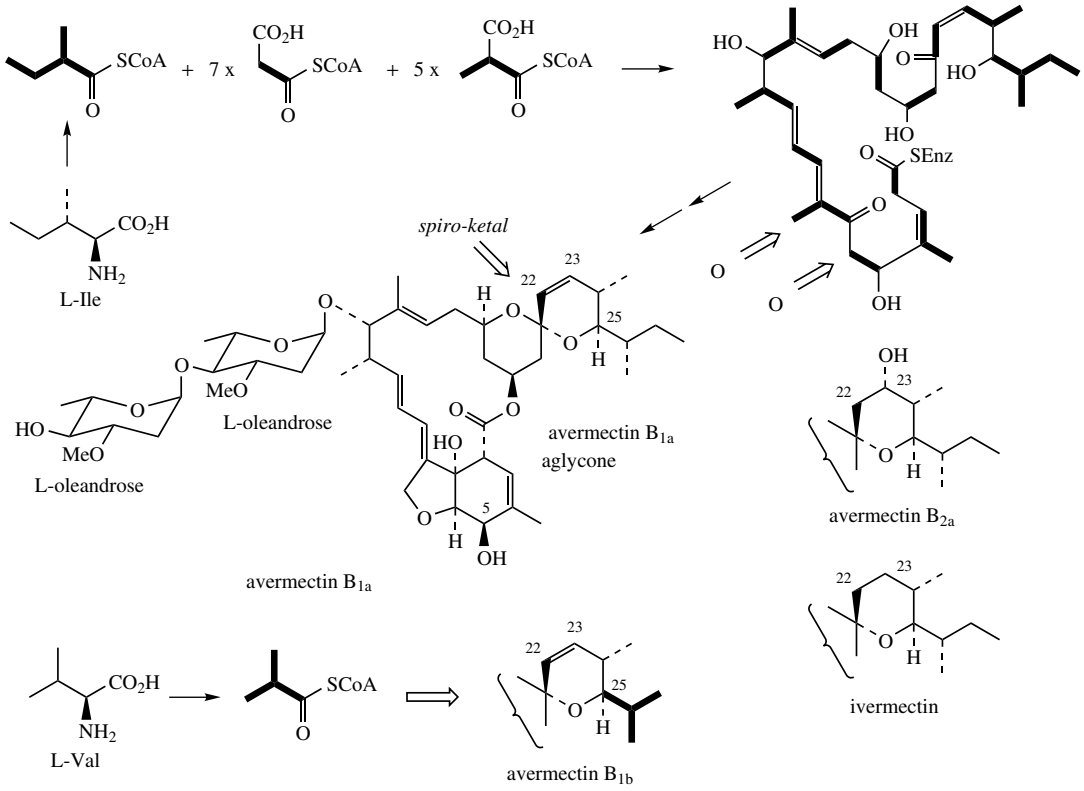

Figure 3.67

Avermectins

The avermectins (Figure 3.67) are a group of macrolides with strong anthelmintic, insecticidal, and acaricidal properties, but with low toxicity to animals and humans. They are produced by cultures of *Streptomyces avermectilis*. Some eight closely related structures have been identified, with avermectins B_{1a} and B_{2a} being the most active antiparasitic compounds. **Abamectin** (a mixture of about 85% avermectin B_{1a} and about 15% avermectin B_{1b}) is used on agricultural crops to control mites and insects. **Ivermectin** (Figure 3.67) is a semi-synthetic 22,23-dihydro derivative of avermectin B_{1a} and was first used in veterinary practice against insects, ticks, mites, and roundworms. Although it is a broad spectrum nematocide against roundworms, it is inactive against tapeworms and flatworms, or against bacteria and fungi. It is an extremely potent agent, and is effective at very low dosages. It has now been introduced for use against filarial and several other worm parasites in humans. Avermectins act by blocking neuromuscular transmission in sensitive organisms by acting on GABA (γ-aminobutyric acid) receptors.

(derived from isoleucine) at C-25, whilst the minor *b* component has an isopropyl group instead, e.g. **avermectin B_{1b}**. In this case, the starter group is 2-methylpropionyl-CoA, derived from the amino acid L-valine. The A-series of avermectins are the 5-methoxy analogues of the B-series.

Even larger macrolides are encountered in the **polyene macrolides***, most of which have antifungal properties, but not antibacterial activity. The macrolide ring size ranges from 26 to 38 atoms, and this also accommodates a conjugated polyene of up to seven *E* double bonds. Relatively

few methyl groups are attached to the ring, and thus malonyl-CoA is utilized more frequently than methylmalonyl-CoA as chain extender. Typical examples are **amphotericin B** (Figure 3.68) from *Streptomyces nodosus* and **nystatin A_1** from *Streptomyces noursei*. These have very similar structures and are derived from the same basic precursors (Figure 3.68). The ring size is contracted due to cross-linking by formation of a hemiketal. They have slightly different hydroxylation patterns, part of which is introduced by hydroxylation, and the two areas of conjugation in nystatin A_1 are extended into a heptaene system in amphotericin B. Both compounds are glycosylated with the amino sugar D-mycosamine, and both are carboxylic acids, a result of oxidation of a propionate-derived methyl group.

An unusual and clinically significant macrolide isolated from *Streptomyces tsukubaensis* is **FK-506** (**tacrolimus**)* (Figure 3.69), which contains a

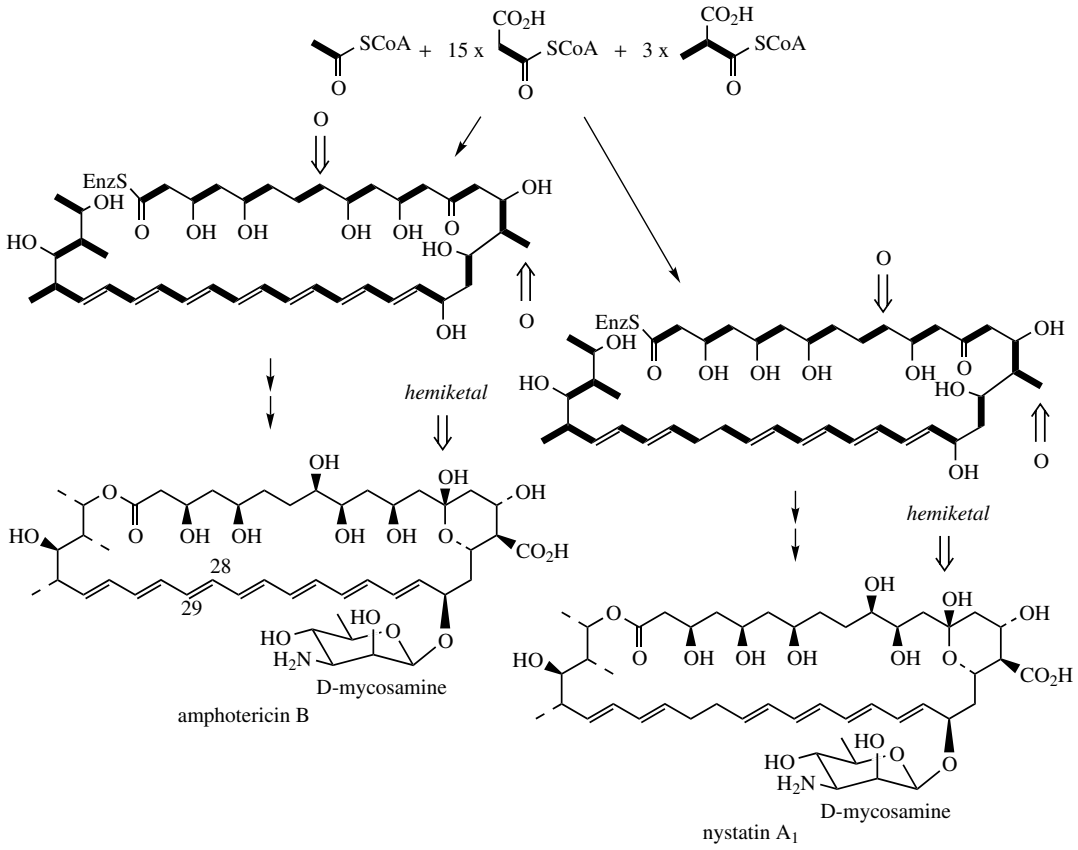

Figure 3.68

Polyene Antifungals

The polyene antifungals are a group of macrocyclic lactones with a very large 26–38-membered ring. They are characterized by the presence of a series of conjugated *E* double bonds and are classified according to the longest conjugated chain present. Medicinally important ones include the heptaene amphotericin B, and the tetraene nystatin. There are relatively few methyl branches in the macrocyclic chain. The polyenes have no antibacterial

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activity but are useful antifungal agents. Their activity is a result of binding to sterols in the eukaryotic cell membrane, which action explains the lack of antibacterial activity because bacterial cells do not contain sterol components. Fungal cells are also attacked rather than mammalian cells, since the antibiotics bind much more strongly to ergosterol, the major fungal sterol (see page 253), than to cholesterol, the main animal sterol component (see page 236). This binding modifies the cell wall permeability and leads to formation of transmembrane pores allowing K^+ ions, sugars, and proteins to be lost from the microorganism. Though binding to cholesterol is less than to ergosterol, it is responsible for the observed toxic side-effects of these agents on humans. The polyenes are relatively unstable, undergoing light-catalysed decomposition, and are effectively insoluble in water. This insolubility actually protects the antibiotic from gastric decomposition, allowing oral treatment of infections in the intestinal tract.

Amphotericin is an antifungal polyene produced by cultures of *Streptomyces nodosus* and contains principally the heptaene amphotericin B (Figure 3.68) together with structurally related compounds, e.g. the tetraene amphotericin A (about 10%), which is the 28,29-dihydro analogue of amphotericin B. Amphotericin A is much less active than amphotericin B. Amphotericin is active against most fungi and yeasts, but it is not absorbed from the gut, so oral administration is restricted to the treatment of intestinal candidiasis. It is administered intravenously for treating potentially life-threatening systemic fungal infections. However, it then becomes highly protein bound resulting in poor penetration and slow elimination from the body. After parenteral administration, toxic side-effects, including nephrotoxicity, are relatively common. Close supervision and monitoring of the patient is thus necessary, especially since the treatment may need to be prolonged. A liposome-encapsulated formulation of amphotericin has been shown to be much less toxic and may prove a significant advance. *Candida* infections in the mouth or on the skin may be treated with appropriate formulations.

Nystatin is a mixture of tetraene antifungals produced by cultures of *Streptomyces noursei*. The principal component is nystatin A_1 (Figure 3.68), but the commercial material also contains nystatin A_2 and A_3, which have additional glycoside residues. Nystatin is too toxic for intravenous use, but has value for oral treatment of intestinal candidiasis, as lozenges for oral infections, and as creams for topical control of *Candida* species.

23-membered macrolactone that also incorporates an *N*-heterocyclic ring. This compound is known to be derived from acetate and propionate, the fragments of which can readily be identified in the main chain. The starter unit is cyclohexanecarboxylic acid, a reduction product from shikimate, and the piperidine ring and adjacent carbonyl are incorporated as pipecolic acid (see page 310) via an amide linkage on to the end of the growing chain. An unusual pentanoic acid unit is also incorporated to provide the propenyl side-chain. FK-506 is a particularly effective immunosuppressant, and is proving valuable in organ transplant surgery. Although **rapamycin** (**sirolimus**)* contains a very large 31-membered macrocycle, several portions of the structure are identical to those of FK-506. Cyclohexanecarboxylic acid and pipecolic acid are again utilized in its formation, whilst the rest of the skeleton is supplied by simple acetate and propionate residues (Figure 3.69).

Attracting considerable interest at the present time are the **epothilones** (Figure 3.70), a group of macrolides produced by cultures of the bacterium *Sorangium cellulosum*. These compounds employ an unusual starter unit containing a thiazole ring, which is almost certainly constructed from the amino acid cysteine and an acetate unit (see also thiazole rings in bleomycin, page 429). The macrolide ring also contains an extra methyl group at C-4, the result of methylation after or during polyketide chain assembly. The other interesting feature is that this bacterium produces epothilone A and epothilone B in the ratio of about 2:1. These compounds differ in the nature

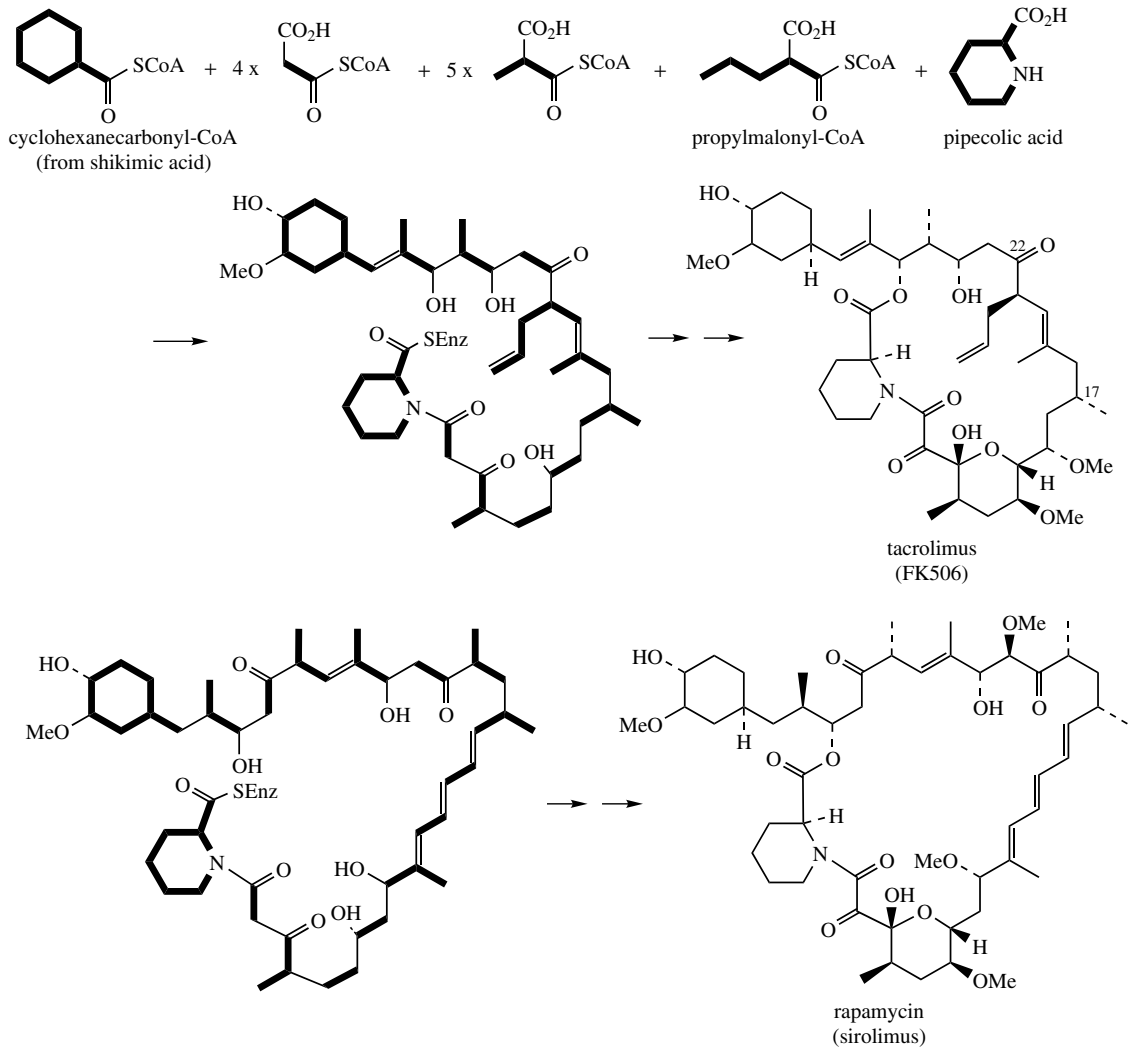

Figure 3.69

Tacrolimus and Sirolimus

Tacrolimus (**FK-506**) (Figure 3.69) is a macrolide immunosuppressant isolated from cultures of *Streptomyces tsukubaensis*. It is used in liver and kidney transplant surgery. Despite the significant structural differences between tacrolimus and the cyclic peptide cyclosporin A (ciclosporin; see page 429), these two agents have a similar mode of action. They both inhibit T-cell activation in the immunosuppressive mechanism by binding first to a receptor protein giving a complex, which then inhibits a phosphatase enzyme called calcineurin. The resultant aberrant phosphorylation reactions prevent appropriate gene transcription and subsequent T-cell activation. Structural similarities between the region C-17 to C-22 and fragments of the cyclosporin A peptide chain have been postulated to account for this binding. Tacrolimus is

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up to 100 times more potent than cyclosporin A, but produces similar side-effects including neurotoxicity and nephrotoxicity.

Rapamycin (**sirolimus**) (Figure 3.69) is produced by cultures of *Streptomyces hygroscopicus* and is also being investigated as an immunosuppressant drug. Although tacrolimus and rapamycin possess a common structural unit, and both inhibit T-cell activation, they appear to achieve this by somewhat different mechanisms. The first-formed rapamycin–receptor protein binds not to calcineurin, but to a different protein. Rapamycin suppresses lymphocyte production. Rapamycin also possesses pronounced antifungal activity, but is not active against bacteria.

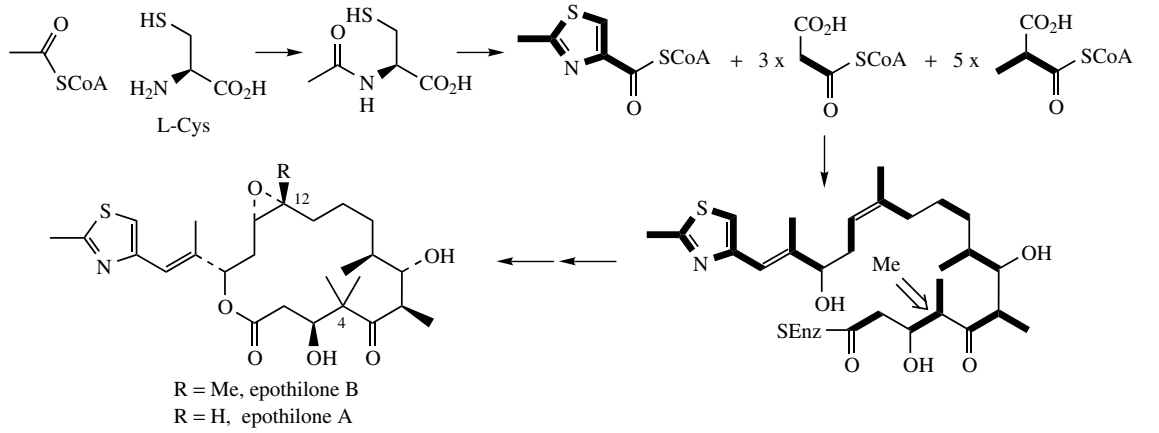

Figure 3.70

of the substituent at C-12, which is hydrogen in epothilone A but a methyl group in epothilone B. Genetic evidence shows that the polyketide synthase enzyme can accept either malonyl-CoA or methylmalonyl-CoA extender units for this position. Thus, epothilone B is constructed from three malonate and five methylmalonate extender units as shown in Figure 3.70, whilst epothiolone A requires four units of each type. The epothilones display marked antitumour properties with a mode of action paralleling that of the highly successful anticancer drug taxol (see page 205). However, the epothilones have a much higher potency (2000–5000 times) and are active against cell lines which are resistant to taxol and other drugs. There appears to be considerable potential for developing the epothilones or analogues into valuable anticancer drugs.

A further group of macrolides in which non-adjacent positions on an aromatic ring are bridged by the long aliphatic chain is termed **ansa macrolides***. These are actually lactams rather than lactones, and the nitrogen atom originates from **3-amino-5-hydroxybenzoic acid**, which acts as the starter unit for chain extension with malonyl-CoA or methylmalonyl-CoA. 3-Amino-5-hydroxybenzoic acid (Figure 3.71) is a simple phenolic acid derivative produced by an unusual variant of the shikimate pathway (see Chapter 4), in which aminoDAHP is formed in the initial step, and then the pathway continues with amino analogues. This proceeds through to aminodehydroshikimic acid which yields 3-amino-5-hydroxybenzoic acid on dehydration. In the biosynthesis of **rifamycin B** (Figure 3.71) in *Amycolatopsis mediterranei*, this starter unit, plus two malonyl-CoA and eight methylmalonyl-CoA extenders, are employed to fabricate **proansamycin X** as the first product released from the enzyme. The enzyme-bound intermediate shown in Figure 3.71 is not strictly correct, in that the naphthoquinone ring system is now

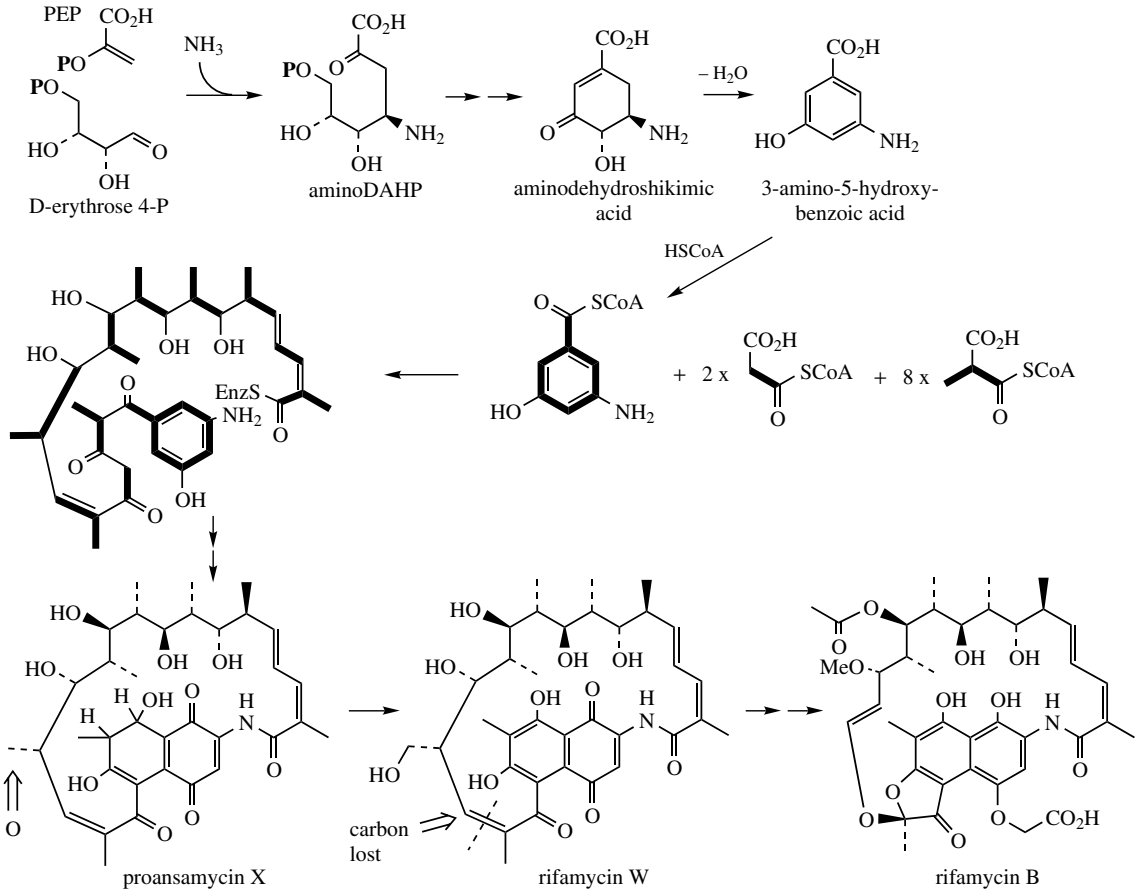

Figure 3.71

known to be constructed during, not after, chain assembly. **Rifamycin W** and then the antibiotic rifamycin B are the result of further modifications including cleavage of the double bond, loss of one carbon, then formation of the ketal. **Maytansine** (Figure 3.72) is a plant-derived ansa macrolide from *Maytenus serrata* (Celastraceae), though other esters of the parent alcohol, maytansinol, are produced by species of the fungus *Nocardia*. Maytansine has been extensively investigated for its potential antitumour activity.

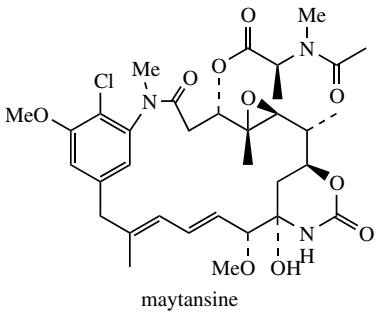

Figure 3.72

The macrolide systems described above are produced by formation of an intramolecular ester or amide linkage, utilizing appropriate functionalities in the growing polyketide chain. Macrolide formation does not always occur, and similar acetate–propionate precursors might also be expected to yield molecules which are essentially linear in nature. Good examples of such molecules are **lasalocid A** (Figure 3.74) from *Streptomyces lasaliensis* and **monensin A** (Figure 3.75) from *Streptomyces cinnamonensis*, representatives of a large group of compounds called **polyether antibiotics**. These, and other examples, are of value in veterinary medicine, being effective in preventing

Ansa Macrolides

Ansamycins are a class of macrocyclic compounds in which non-adjacent positions on an aromatic ring system are spanned by the long aliphatic bridge (Latin: *ansa* = handle). The aromatic portion may be a substituted naphthalene or naphthaquinone, or alternatively a substituted benzene ring. The macrocycle in the ansamycins is closed by an amide rather than an ester linkage, i.e. ansamycins are lactams. The only ansamycins currently used therapeutically are semi-synthetic naphthalene-based macrocycles produced from rifamycin B.

The rifamycins are ansamycin antibiotics produced by cultures of *Amycolatopsis mediterranei* (formerly *Nocardia mediterranei* or *Streptomyces mediterranei*). The crude antibiotic mixture was found to contain five closely related substances rifamycins A–E, but if the organism was cultured in the presence of sodium diethyl barbiturate (barbitone or barbital), the product was almost entirely rifamycin B (Figure 3.71). Rifamycin B has essentially no antibacterial activity, but on standing in aqueous solution in the presence of air, it is readily transformed by oxidation and intramolecular nucleophilic addition into rifamycin O, which

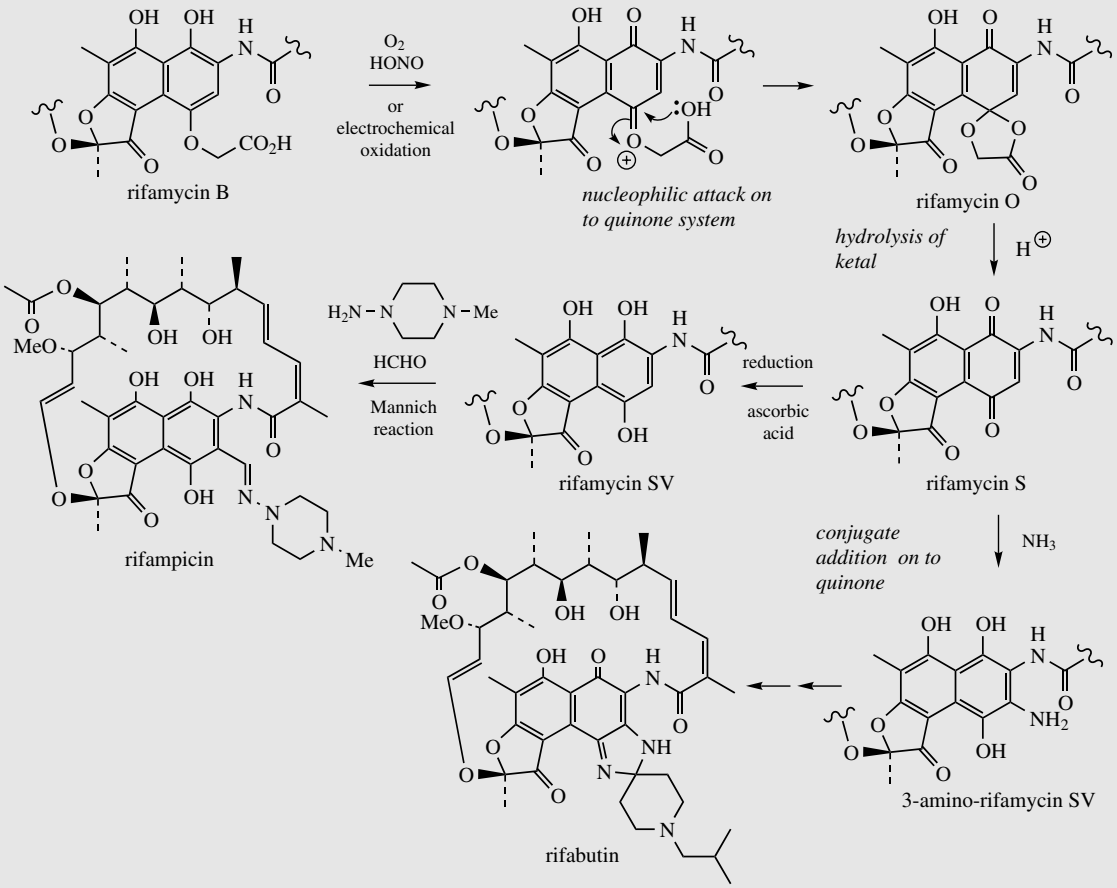

Figure 3.73

(*Continues*)

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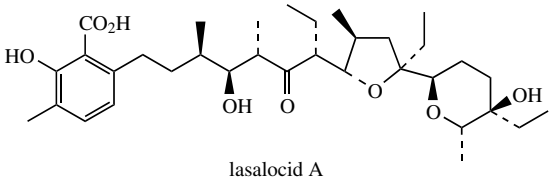
under acidic conditions then hydrolyses and gives rifamycin S, a highly active antibacterial agent (Figure 3.73). Chemical reduction of rifamycin S using ascorbic acid (vitamin C) converts the quinone into a quinol and provides a further antibacterial, rifamycin SV. Rifamycins O, S, and SV can all be obtained by fermentation using appropriate strains of *A. mediterranei*. Rifamycin SV is actually the immediate biosynthetic precursor of rifamycin B under normal conditions, so this conversion can be genetically blocked and lead to accumulation of rifamycin SV. Several other rifamycin analogues have also been characterized. Rifamycin O is usually produced by chemical or electrochemical oxidation of rifamycin B, and converted into rifamycin SV as in Figure 3.73.

The most useful rifamycin employed clinically is **rifampicin** (Figure 3.73), a semi-synthetic derivative produced from rifamycin SV via a Mannich reaction (see page 18) using formaldehyde and *N*-amino-*N′*-methylpiperazine. Rifampicin has a wide antibacterial spectrum, with high activity towards Gram-positive bacteria and a lower activity towards Gram-negative organisms. Its most valuable activity is towards *Mycobacterium tuberculosis* and rifampicin is a key agent in the treatment of tuberculosis, usually in combination with at least one other drug to reduce the chances for development of resistant bacterial strains. It is also useful in control of meningococcal meningitis and leprosy. Rifampicin's antibacterial activity arises from inhibition of RNA synthesis by binding to DNA-dependent RNA polymerase. RNA polymerase from mammalian cells does not contain the peptide sequence to which rifampicin binds, so RNA synthesis is not affected. In contrast to the natural rifamycins which tend to have poor absorption properties, rifampicin is absorbed satisfactorily after oral administration, and is also relatively free of toxic side-effects. The most serious side-effect is disturbance of liver function. A trivial, but to the patient potentially worrying, side-effect is discoloration of body fluids, including urine, saliva, sweat, and tears, to a red–orange colour, a consequence of the naphthalene/naphthoquinone chromophore in the rifamycins. **Rifamycin**, the sodium salt of rifamycin SV (Figure 3.73), has also been used clinically in the treatment of Gram-positive infections, and particularly against tuberculosis. **Rifabutin** (Figure 3.73) is a newly introduced derivative, synthesized via 3-amino-rifamycin SV, which also has good activity against the *Mycobacterium avium* complex frequently encountered in patients with AIDS.



lasalocid A

Figure 3.74

and controlling coccidiae and also having the ability to improve the efficiency of food conversion in ruminants. The polyether antibiotics are characterized by the presence of a number of tetrahydrofuran and/or tetrahydropyran rings along the basic chain. The polyether acts as an ionophore, increasing influx of sodium ions into the parasite, causing a resultant and fatal increase in osmotic pressure. Current thinking is that these ring systems arise via a cascade cyclization mechanism, probably involving epoxide intermediates. Thus, in the biosynthesis of **monensin A** (Figure 3.75), chain assembly from acetate, malonate, methylmalonate, and ethylmalonate precursors could produce the triene shown. If the triepoxide is then formed, a concerted stereospecific cyclization sequence initiated by a hydroxyl and involving carbonyls and epoxides could proceed as indicated.

Even more remarkable polyether structures are found in some toxins produced by marine dinoflagellates, which are in turn taken up by shellfish and pass on their toxicity to the shellfish. **Okadaic acid** (Figure 3.76) and related polyether structures from *Dinophysis* species are responsible for

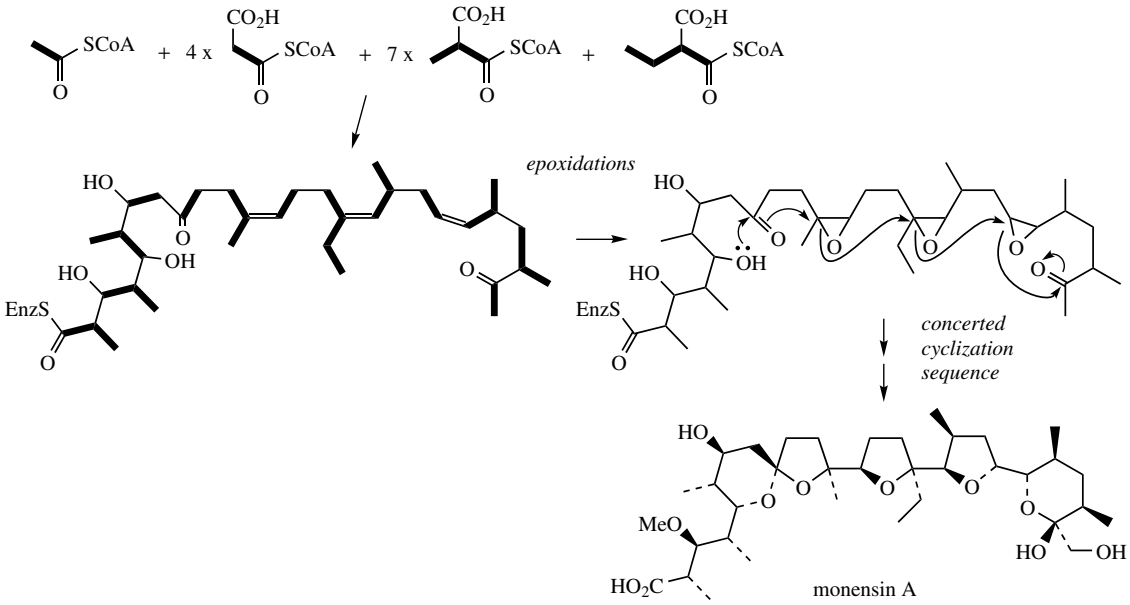


Figure 3.75

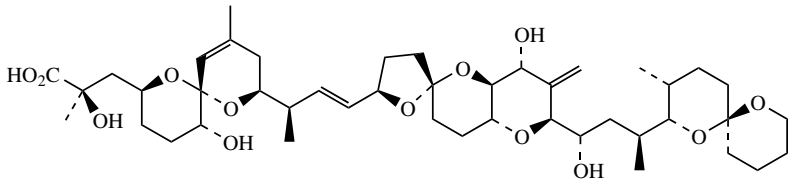


Figure 3.76

diarrhoeic shellfish poisoning in mussels, causing severe diarrhoea in consumers of contaminated shellfish in many parts of the world. **Brevetoxin A** (Figure 3.77) is an example of the toxins associated with 'red tide' blooms of dinoflagellates, which affect fishing and also tourism especially in Florida and the Gulf of Mexico. The red tide toxins are derived from *Gymnodimium breve* and are the causative agents of neurotoxic shellfish poisoning, leading to neurological disorders as well as gastrointestinal troubles. The toxins are known to bind to sodium channels, keeping them in an open state. Fatalities among marine life, e.g. fish, dolphins, whales, and in humans, are associated with these toxins synthesized by organisms at the base of the marine food chain. These compounds are postulated to be produced from a polyunsaturated fatty acid by epoxidation of the double bonds, and then a concerted sequence of epoxide ring openings leads to the extended polyether structure (Figure 3.77). The carbon skeleton does not conform to a simple polyketide chain, and biosynthetic studies have shown that fragments from the citric acid cycle and a four-carbon starter unit from mevalonate are also involved, and that some of the methyls originate from methionine. **Ciguatoxin** (Figure 3.78) is one of the most complex examples of a polyether structure found in nature. This is found in the moray eel (*Gymnothorax javanicus*) and in a variety of coral reef fish, such as red snapper (*Lutjanus bohar*). Ciguatoxin is remarkably toxic even at microgram levels, causing widespread food poisoning (ciguatera) in tropical and subtropical regions, characterized by vomiting, diarrhoea, and neurological problems. Most sufferers slowly recover, and few cases are fatal, due principally to the very low

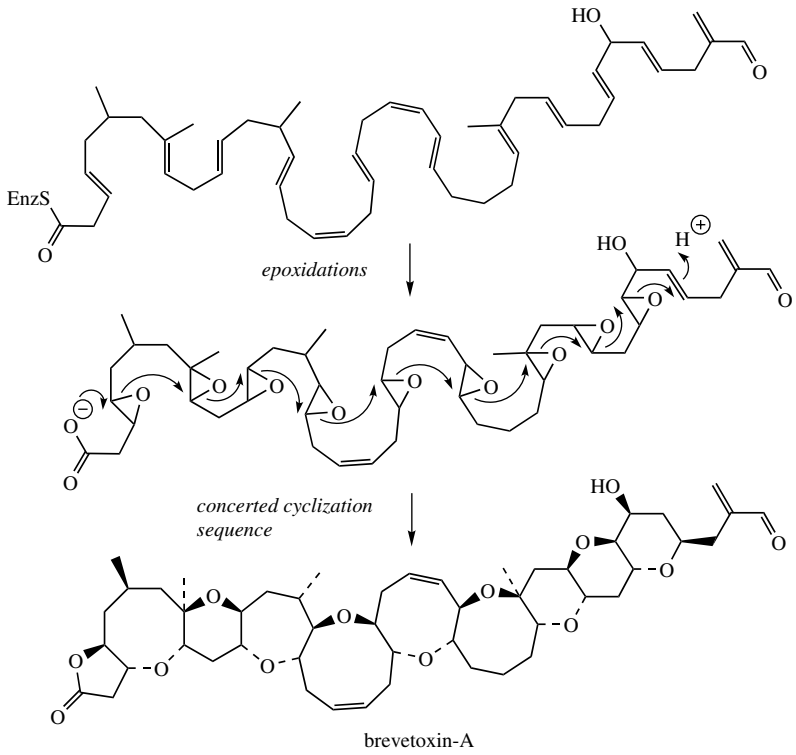


Figure 3.77

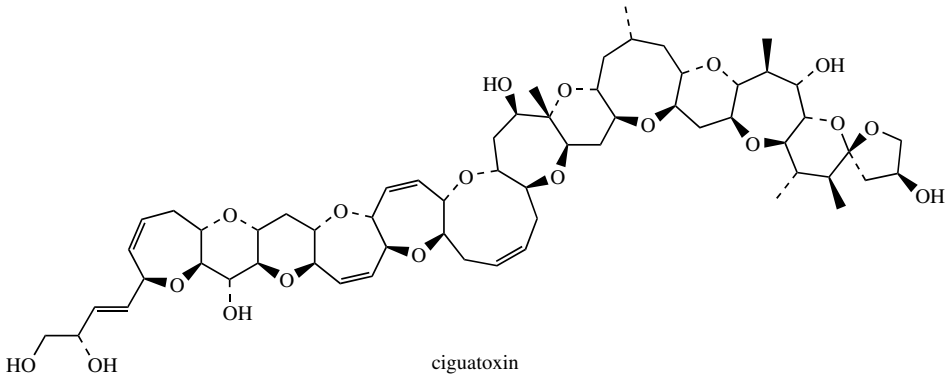


Figure 3.78

levels of toxin actually present in the fish. A dinoflagellate *Gambierdiscus toxicus* is ultimately responsible for polyether production, synthesizing a less toxic analogue, which is passed through the food chain and eventually modified into the very toxic ciguatoxin by the fish.

The **zaragozic acids** (**squalestatins**) are not macrolides, but they are primarily acetate derived, and the central ring system is suggested to be formed by an epoxide-initiated process resembling the polyether derivatives just described. Thus, **zaragozic acid A** (Figure 3.79) is known to be constructed from two acetate-derived chains and a C_4 unit such as the Krebs cycle intermediate oxaloacetate (see Figure 2.1). One chain has a benzoyl-CoA starter (from the shikimate pathway, see page 141), and both contain two methionine-derived side-chain substituents (Figure 3.79). The

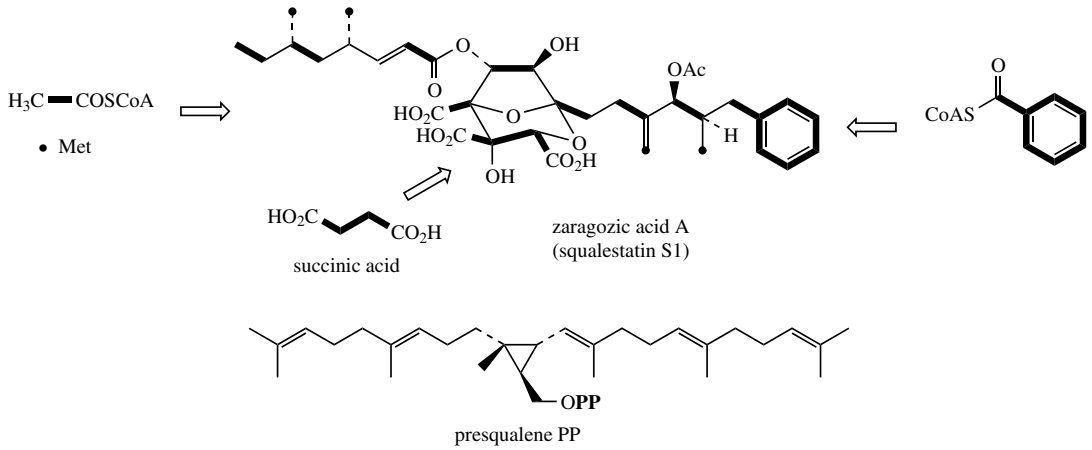


Figure 3.79

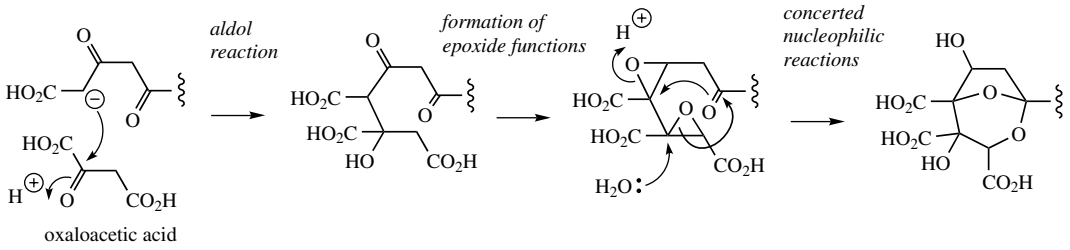
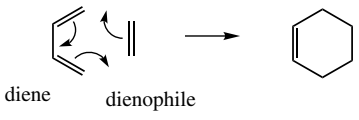


Figure 3.80

heterocyclic ring system can be envisaged as arising via nucleophilic attack on to oxaloacetic acid, formation of a diepoxide, then a concerted sequence of reactions as indicated (Figure 3.80). The zaragozic acids are produced by a number of fungi, including *Sporomiella intermedia* and *Leptodontium elatius*, and are attracting considerable interest since they are capable of reducing blood cholesterol levels in animals by acting as potent inhibitors of the enzyme squalene synthase (see page 212). This is achieved by mimicking the steroid precursor presqualene PP (Figure 3.79) and irreversibly inactivating the enzyme. They thus have considerable medical potential for reducing the incidence of coronary-related deaths (compare the statins, below).

CYCLIZATION THROUGH DIELS–ALDER REACTIONS

A number of cyclic structures, typically containing cyclohexane rings, are known to be formed via the acetate pathway, but experimental evidence supports cyclization processes different from the aldol and Claisen reactions seen in the biosynthesis of aromatic compounds. They can, however, be rationalized in terms of an enzymic Diels–Alder reaction, represented as the electrocyclic sequence shown in Figure 3.81. Thus, **lovastatin** can be formulated as arising from two polyketide chains with *C*-methylation as outlined in Figure 3.82, with relatively few of the oxygen functions being retained in the final product. Accordingly, it is possible that lovastatin is formed by cyclization of the trienoic acid (Figure 3.82), which is likely to arise by a variant of the macrolide



Diels–Alder reaction

Figure 3.81

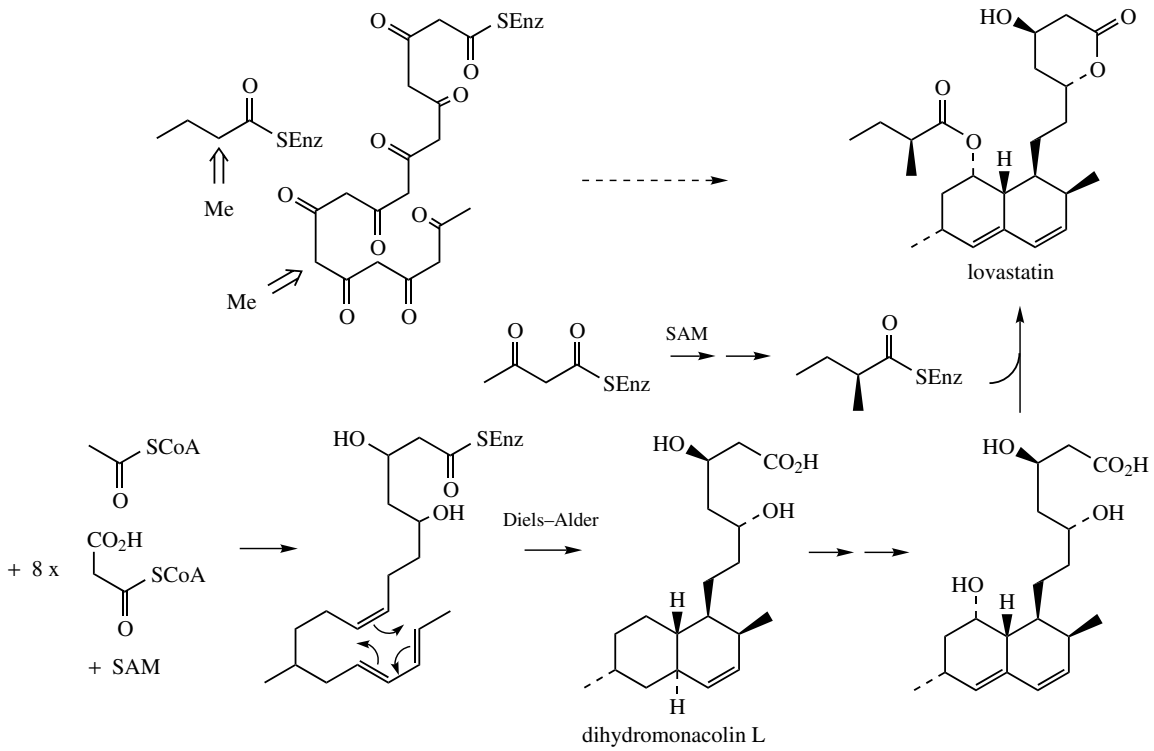


Figure 3.82

Mevastatin and other Statins

Mevastatin (formerly compactin) (Figure 3.83) is produced by cultures of *Penicillium citrinum* and *P. brevicompactum*, and was shown to be a reversible competitive inhibitor of HMG-CoA reductase, dramatically lowering sterol biosynthesis in mammalian cell cultures and animals, and reducing total and low density lipoprotein cholesterol levels (see page 236). Mevastatin in its ring-opened form (Figure 3.84) mimics the half-reduced substrate mevaldate hemithioacetal during the two-stage reduction of HMG-CoA to mevalonate (see page 170), and the affinity of this agent towards HMG-CoA reductase is 10 000-fold more than the normal substrate. High blood cholesterol levels contribute to the incidence of coronary heart disease (see page 236), so mevastatin, or analogues, are of potential value in treating high risk coronary patients, and some agents are already in use. Although lowering of cholesterol levels reduces the risk of heart attacks, there is evidence that the beneficial effects of statins may extend beyond simply cholesterol reduction.

Lovastatin (formerly called mevinolin or monacolin K) (Figure 3.83) is produced by *Monascus ruber* and *Aspergillus terreus* and is slightly more active than mevastatin, but has been superseded by more active agents. **Simvastatin** is obtained from lovastatin by ester hydrolysis and then re-esterification, and is two to three times as potent as lovastatin. **Pravastatin** is prepared from mevastatin by microbiological hydroxylation using *Streptomyces carbophilus* and is consequently more hydrophilic than the other drugs, with an activity similar to lovastatin. Lovastatin and simvastatin are both lactones, and are inactive until metabolized

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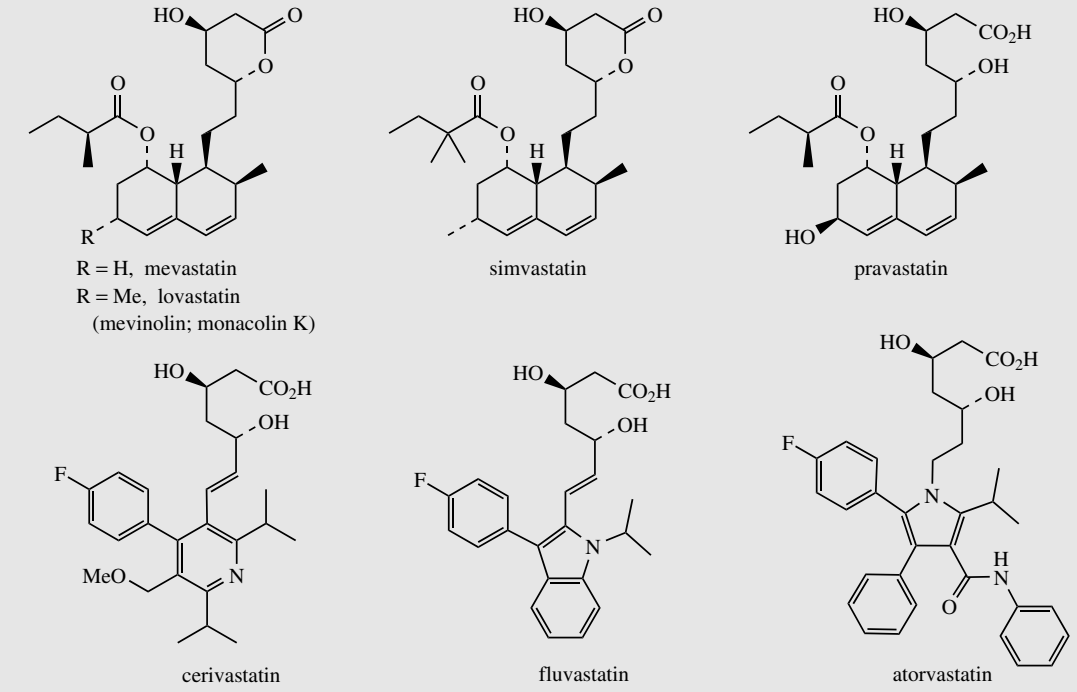

Figure 3.83

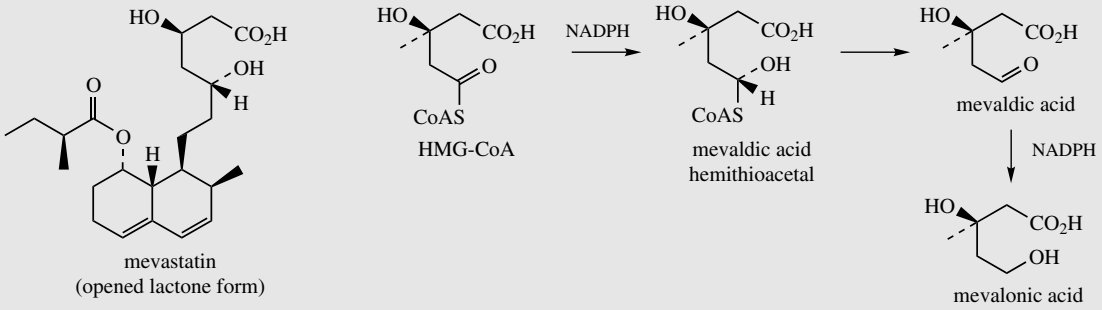

Figure 3.84

in the liver to the open-ring hydroxy acids typified by pravastatin. Other agents currently in use are synthetic, though they feature the same dihydroxycarboxylic acid side-chain as in pravastatin. **Atorvastatin**, **cerivastatin**, and **fluvastatin** have all been introduced recently.

biosynthetic processes, though *C*-methylation must occur during chain assembly whilst activating carbonyl groups are available. The Diels–Alder reaction can then account for formation of the decalin system and further reactions will allow the other functional groups in lovastatin to be produced. The ester side-chain is derived as a separate unit from two acetates with a methyl from methionine, again with *C*-methylation preceding reduction processes. Lovastatin was isolated from cultures of *Aspergillus terreus* and was found to be a potent inhibitor of hydroxymethylglutaryl-CoA

(HMG-CoA) reductase, a rate-limiting enzyme in the mevalonate pathway (see page 169). Analogues of lovastatin (statins*) (Figure 3.83) find drug use as HMG-CoA reductase inhibitors, thus lowering blood cholesterol levels in patients.

GENETIC MANIPULATION OF THE ACETATE PATHWAY

With only a few exceptions, the transformations in any particular biosynthetic pathway are catalysed by enzymes. These proteins facilitate the chemical modification of substrates by virtue of binding properties conferred by a particular combination of functional groups in the constituent amino acids. As a result, enzymes tend to demonstrate quite remarkable specificity towards their substrates, and usually catalyse only a single transformation. This specificity means enzymes do not accept alternative substrates, or, if they do, they convert a limited range of structurally similar substrates and usually much less efficiently. Any particular organism thus synthesizes a range of secondary metabolites dictated largely by its enzyme complement and the supply of substrate molecules. Occasionally, where enzymes do possess broader substrate specificities, it is possible to manipulate an organism's secondary metabolite pattern by supplying an alternative, but acceptable, substrate. A good example of this approach is in the directed biosynthesis of modified penicillins by the use of phenylacetic acid analogues in cultures of *Penicillium chrysogenum* (see page 437), but its scope is generally very limited. It has also been possible, particularly with microorganisms, to select natural mutants, or to generate mutants artificially, where the new strain synthesizes modified or substantially different products. For example, mutant strains of *Streptomyces aureofaciens* synthesize tetracycline or demeclocycline rather than chlortetracycline (see page 90). Such mutants are usually deficient in a single enzyme and are thus unable to carry out a single transformation, but the broader specificity of later enzymes in the sequence means subsequent modifications may still occur. However, as exemplified throughout this book, the vast bulk of modified natural products of medicinal importance are currently obtained by chemical synthesis or semi-synthesis.

Rapid advances in genetic engineering have now opened up tremendous scope for manipulating the processes of biosynthesis by providing an organism with, or depriving it of, specific enzymes. The genes encoding a particular protein (see page 407) can now be identified, synthesized, and inserted into a suitable organism for expression; to avoid complications with the normal biosynthetic machinery, this is usually different from the source organism. Specific genes can be damaged or deleted to prevent a particular enzyme being expressed. Genes from different organisms can be combined and expressed together so that an organism synthesizes abnormal combinations of enzyme activities, allowing production of modified products. Although the general approaches for genetic manipulation are essentially the same for all types of organism and/or natural product, it has proved possible to make best progress using the simpler organisms, especially bacteria, and in particular there have been some substantial achievements in the area of acetate-derived structures. Accordingly, some results from this group of compounds are used to exemplify how genetic manipulation may provide an extra dimension in the search for new medicinal agents. However, it is important that an organism is not viewed merely as a sackful of freely diffusible and always available enzymes; biosynthetic pathways are under sophisticated controls in which there may be restricted availability or localization of enzymes and/or substrates (see the different localizations of the mevalonate and deoxyxylulose phosphate pathways to terpenoids in plants, page 172). Enzymes involved in the biosynthesis of many important secondary metabolites are often grouped together as enzyme complexes, or may form part of a multifunctional protein.

A detailed study of amino acid sequences and mechanistic similarities in various **polyketide synthase** (PKS) enzymes has led to two main types being distinguished. **Type I enzymes** consist of one or more large multifunctional proteins that possess a distinct active site for every enzyme-catalysed step. On the other hand, **Type II enzymes** are multienzyme complexes that carry out a single set of repeating activities. Like fatty acid synthases, PKSs catalyse the condensation of coenzyme A esters of simple carboxylic acids. However, the variability at each step in

the biosynthetic pathway gives rise to much more structural diversity than encountered with fatty acids. The usual starter units employed are acetyl-CoA or propionyl-CoA, whilst malonyl-CoA or methylmalonyl-CoA are the main extender units. At each cycle of chain extension, Type I PKSs may retain the β-ketone, or modify it to a hydroxyl, methenyl, or methylene, according to the presence of ketoreductase, dehydratase, or enoylreductase activities (see page 95). The enzyme activities for each extension cycle with its subsequent modification is considered a 'module'. The linear sequence of modules in the enzyme corresponds to the generated sequence of extender units in the polyketide product. The β-ketone groups are predominantly left intact by Type II PKSs, and the highly reactive polyketide backbone undergoes further enzyme-catalysed intramolecular cyclization reactions, which are responsible for generating a range of aromatic structures (see page 61).

6-Deoxyerythronolide B synthase (DEBS) is a modular Type I PKS involved in **erythromycin** biosynthesis (see page 96) and its structure and function are illustrated in Figure 3.85. The enzyme contains three subunits (DEBS-1, 2, and 3), each encoded by a gene (*eryA*-I, II, and III). It has a linear organization of six modules, each of which contains the activities needed for one cycle of chain extension. A minimal module contains a β-ketoacyl synthase (KS), an acyltransferase (AT), and an acyl carrier protein (ACP), that together would catalyse a two-carbon chain extension. The specificity of the AT for either malonyl-CoA or an alkyl-malonyl-CoA determines which two-carbon chain extender is used. The starter unit used is similarly determined by the specificity of the AT in a loading domain in the first module. After each condensation reaction, the oxidation state of the β-carbon is determined by the presence of a β-ketoacyl reductase (KR), a KR + a dehydratase (DH), or a KR + DH + an enoylreductase (ER) in the appropriate module. The sequence is finally terminated by a thioesterase (TE) activity which releases the polyketide from the enzyme and allows cyclization. Thus in DEBS, module 3 lacks any β-carbon modifying domains, modules 1, 2, 5, and 6 contain KR domains and are responsible for hydroxy substituents, whereas module 4 contains the complete KR, DH, and ER set, and results in complete reduction to a methylene. Overall, the AT specificity and the catalytic domains on each module determine the structure and stereochemistry of each two-carbon extension unit, the order of the modules specifies the sequence of the units,

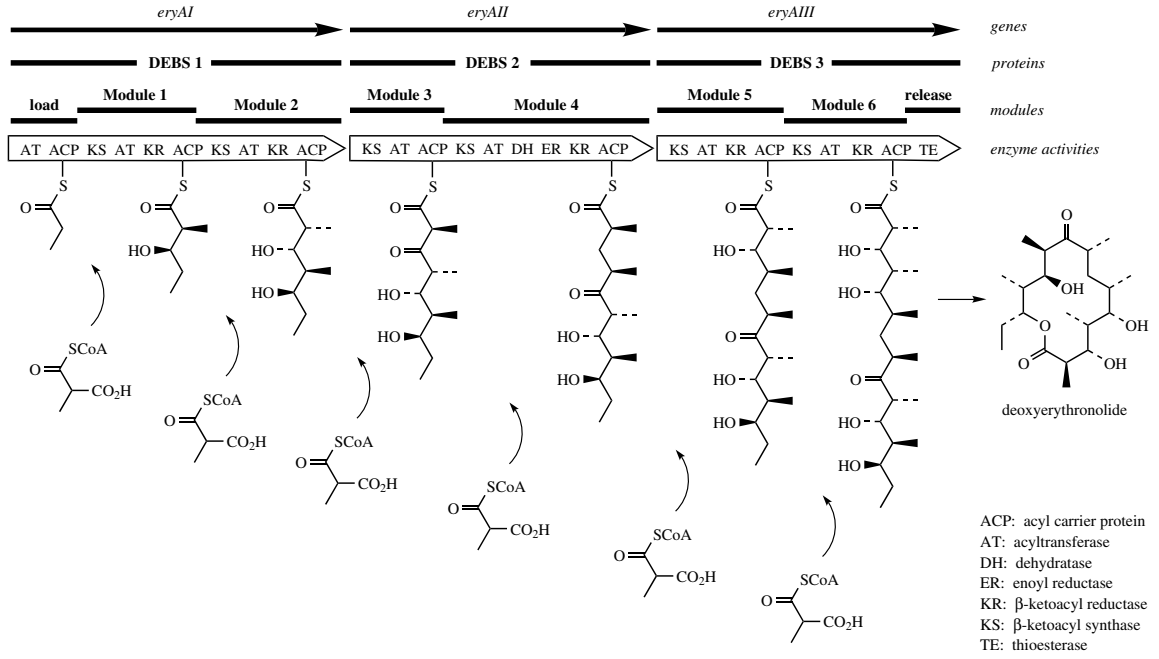

Figure 3.85

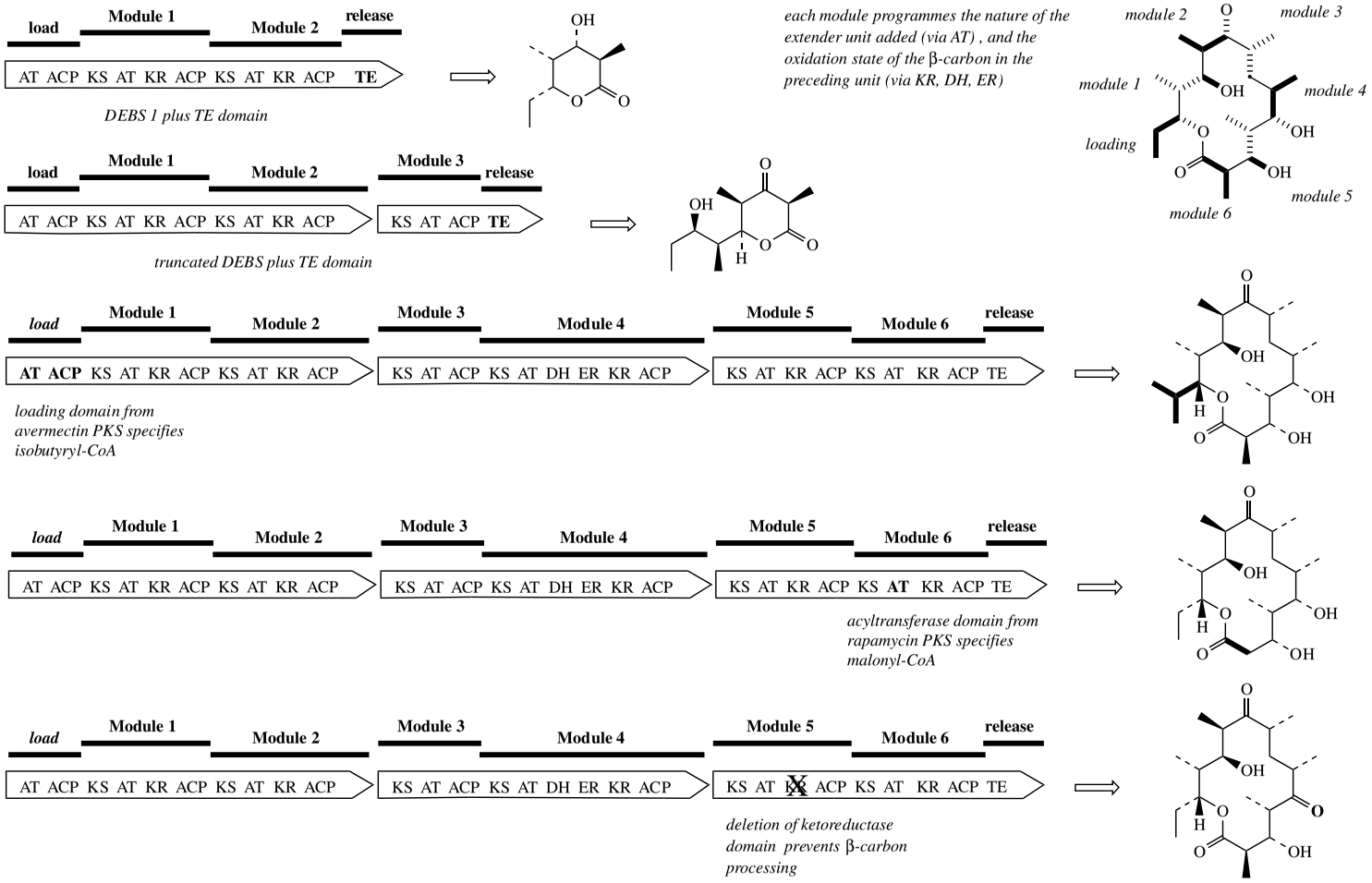
load
Module 1
Module 2
release
AT ACP KS AT KR ACP KS AT KR ACP TE
DEBS 1 plus TE domain
each module programmes the nature of the extender unit added (via AT) , and the oxidation state of the β-carbon in the preceding unit (via KR, DH, ER)
module 1
module 2
module 3
module 4
module 5
module 6
loading
load
Module 1
Module 2
Module 3
release
AT ACP KS AT KR ACP KS AT KR ACP
KS AT ACP TE
truncated DEBS plus TE domain
load
Module 1
Module 2
Module 3
Module 4
Module 5
Module 6
release
AT ACP KS AT KR ACP KS AT KR ACP
KS AT ACP KS AT DH ER KR ACP
KS AT KR ACP KS AT KR ACP TE
loading domain from avermectin PKS specifies isobutyryl-CoA
load
Module 1
Module 2
Module 3
Module 4
Module 5
Module 6
release
AT ACP KS AT KR ACP KS AT KR ACP
KS AT ACP KS AT DH ER KR ACP
KS AT KR ACP KS AT KR ACP TE
acyltransferase domain from rapamycin PKS specifies malonyl-CoA
load
Module 1
Module 2
Module 3
Module 4
Module 5
Module 6
release
AT ACP KS AT KR ACP KS AT KR ACP
KS AT ACP KS AT DH ER KR ACP
KS AT ACP KS AT KR ACP TE
deletion of ketoreductase domain prevents β-carbon processing

Figure 3.86

and the number of modules determines the size of the polyketide chain. The vast structural diversity of natural polyketides arises from combinatorial possibilities of arranging modules containing the various catalytic domains, the sequence and number of modules, and the post-PKS enzymes which subsequently modify the first-formed product, e.g. 6-deoxyerythronolide B → erythromycin (see page 96). Genetic engineering now offers vast opportunities for rational modification of the resultant polyketide structure.

A few representative examples of successful experiments leading to engineered polyketides are shown in Figure 3.86. Reducing the size of the gene sequence so that it encodes fewer modules results in the formation of smaller polyketides, characterized by the corresponding loss of extender units; in these examples the gene encoding the chain terminating thioesterase also has to be attached to complete the biosynthetic sequence. Replacing the loading domain of DEBS with that from another PKS, e.g. that producing avermectin (see page 97), alters the specificity of the enzyme for the starter unit. The loading module of the avermectin-producing PKS actually has a much broader specificity than that for DEBS; Figure 3.86 shows the utilization of isobutyryl-CoA as features in the natural biosynthesis of avermectin B_{1b}. Other examples include the replacement of an AT domain (in DEBS specifying a methylmalonyl extender) with a malonyl-specific AT domain from the rapamycin-producing PKS (see page 103), and deletion of a KR domain, thus stopping any β-carbon processing for that module with consequent retention of a carbonyl group. Not all experiments in gene modification are successful, and even when they are yields can be disappointingly lower than in the natural system. There is always a fundamental requirement that enzymes catalysing steps after the point of modification need to have sufficiently broad substrate specificities to accept and process the abnormal compounds being synthesized; this becomes more unlikely where two or more genetic changes have been made. Nevertheless, multiple modifications have been successful, and it has also been possible to exploit changes in a combinatorial fashion using different expression vectors for the individual subunits, thus creating a library of polyketides, which may then be screened for potential biological activity.

Non-ribosomal peptide synthases (see page 421) are also modular and lend themselves to similar genetic manipulation as the Type I PKSs. The production of modified aromatic polyketides by genetically engineered Type II PKSs is not quite so 'obvious' as with the modular Type I enzymes, but significant progress has been made in many systems. Each Type II PKS contains a minimal set of three protein subunits, two β-ketoacyl synthase (KS) subunits and an ACP to which the growing chain is attached. Additional subunits, including KRs, cyclases (CYC), and aromatases (ARO), are responsible for modification of the nascent chain to form the final cyclized structure. Novel polyketides have been generated by manipulating Type II PKSs, exchanging KS, CYC, and ARO subunits among different systems. However, because of the highly reactive nature of poly-β-keto chains, the cyclizations that occur with the modified gene product frequently vary from those in the original compound. Compared with Type I PKSs, the formation of new products with predictable molecular structure has proven less controllable.

The polyketide synthases responsible for chain extension of cinnamoyl-CoA starter units leading to flavonoids and stilbenes, and of anthraniloyl-CoA leading to quinoline and acridine alkaloids (see page 377) do not fall into either of the above categories and have now been termed **Type III PKSs**. These enzymes differ from the other examples in that they are homodimeric proteins, they utilize coenzyme A esters rather than acyl carrier proteins, and they employ a single active site to perform a series of decarboxylation, condensation, cyclization, and aromatization reactions.

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