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THE SHIKIMATE PATHWAY: AROMATIC AMINO ACIDS AND PHENYLPROPANOIDS

Shikimic acid and its role in the formation of aromatic amino acids, benzoic acids, and cinnamic acids is described, along with further modifications leading to lignans and lignin, phenylpropenes, and coumarins. Combinations of the shikimate pathway and the acetate pathway are responsible for the biosynthesis of styrylpyrones, flavonoids and stilbenes, flavonolignans, and isoflavonoids. Terpenoid quinones are formed by a combination of the shikimate pathway with the terpenoid pathway. Monograph topics giving more detailed information on medicinal agents include folic acid, chloramphenicol, podophyllum, volatile oils, dicoumarol and warfarin, psoralens, kava, *Silybum marianum*, phyto-oestrogens, derris and lonchocarpus, vitamin E, and vitamin K.

The shikimate pathway provides an alternative route to aromatic compounds, particularly the aromatic amino acids L-**phenylalanine**, L-**tyrosine** and L-**tryptophan**. This pathway is employed by microorganisms and plants, but not by animals, and accordingly the aromatic amino acids feature among those essential amino acids for man which have to be obtained in the diet. A central intermediate in the pathway is **shikimic acid** (Figure 4.1), a compound which had been isolated from plants of *Illicium* species (Japanese 'shikimi') many years before its role in metabolism had been discovered. Most of the intermediates in the pathway were identified by a careful study of a series of *Escherichia coli* mutants prepared by UV irradiation. Their nutritional requirements for growth, and any by-products formed, were then characterized. A mutant strain capable of growth usually differs from its parent in only a single gene, and the usual effect is the impaired synthesis of a single enzyme. Typically, a mutant blocked in the transformation of compound A into compound B will require B for growth whilst accumulating A in its culture medium. In this way, the pathway from phosphoenolpyruvate (from glycolysis) and D-erythrose 4-phosphate (from the pentose phosphate cycle) to the aromatic amino acids was broadly outlined. Phenylalanine and tyrosine form the basis of C_6C_3 phenylpropane units found in many natural products, e.g. cinnamic acids, coumarins, lignans, and flavonoids, and along with tryptophan are precursors of a wide range of alkaloid structures. In addition, it is found that many simple benzoic acid derivatives, e.g. gallic acid (Figure 4.1) and *p*-aminobenzoic acid (4-aminobenzoic acid) (Figure 4.4) are produced via branchpoints in the shikimate pathway.

AROMATIC AMINO ACIDS AND SIMPLE BENZOIC ACIDS

The shikimate pathway begins with a coupling of phosphoenolpyruvate (PEP) and D-erythrose 4-phosphate to give the seven-carbon 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) (Figure 4.1). This reaction, shown here as an aldol-type condensation, is known to be mechanistically more complex in the enzyme-catalysed version; several of the other transformations in the pathway have also been found to be surprisingly

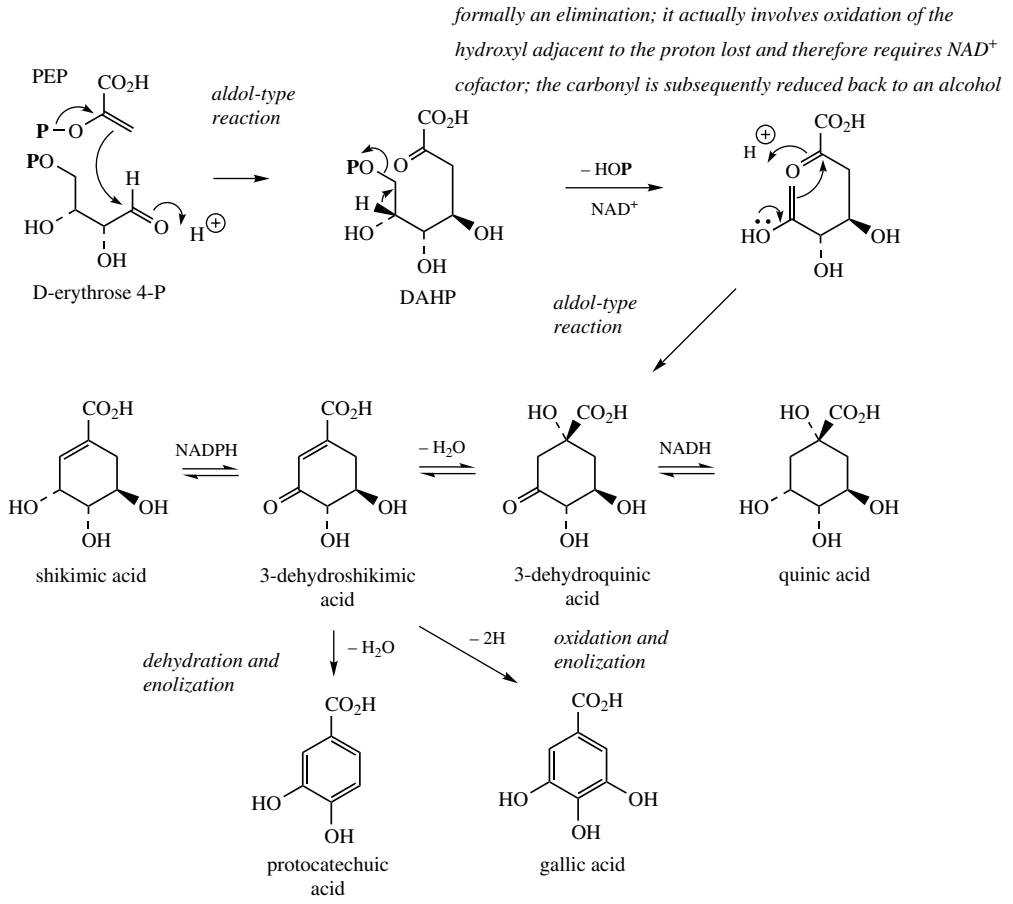

Figure 4.1

complex. Elimination of phosphoric acid from DAHP followed by an intramolecular aldol reaction generates the first carbocyclic intermediate **3-dehydroquinic acid**. However, this also represents an oversimplification. The elimination of phosphoric acid actually follows an NAD^+-dependent oxidation of the central hydroxyl, and this is then re-formed in an NADH-dependent reduction reaction on the intermediate carbonyl compound prior to the aldol reaction occurring. All these changes occur in the presence of a single enzyme. Reduction of 3-dehydroquinic acid leads to **quinic acid**, a fairly common natural product found in the free form, as esters, or in combination with alkaloids such as quinine (see page 362). **Shikimic acid** itself is formed from 3-dehydroquinic acid via **3-dehydroshikimic acid** by dehydration and reduction steps. The simple phenolic acids **protocatechuic acid** (3,4-dihydroxybenzoic acid) and **gallic acid** (3,4,5-trihydroxybenzoic acid) can be formed by branchpoint reactions from 3-dehydroshikimic acid, which involve dehydration and enolization, or, in the case of gallic acid, dehydrogenation and enolization. **Gallic acid** features as a component of many tannin materials (**gallotannins**), e.g. pentagalloylglucose (Figure 4.2), found in plants, materials which have been used for thousands of years in the tanning of animal hides to make leather, due to their ability to cross-link protein molecules. Tannins also contribute to the astringency of foods and beverages, especially tea, coffee and wines (see also condensed tannins, page 151).

A very important branchpoint compound in the shikimate pathway is **chorismic acid** (Figure 4.3), which has incorporated a further molecule of PEP as an enol ether side-chain. PEP combines with shikimic acid 3-phosphate produced in a

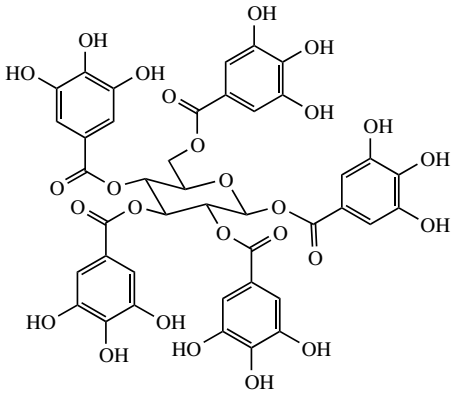

Figure 4.2

simple ATP-dependent phosphorylation reaction. This combines with PEP via an addition–elimination reaction giving **3-enolpyruvylshikimic acid 3-phosphate** (**EPSP**). This reaction is catalysed by the enzyme EPSP synthase. The synthetic *N*-(phosphonomethyl)glycine derivative **glyphosate** (Figure 4.2) is a powerful inhibitor of this enzyme, and is believed to bind to the PEP binding site on the enzyme. Glyphosate finds considerable use as a broad spectrum herbicide, a plant's subsequent inability to synthesize aromatic amino acids causing its death. The transformation of EPSP to **chorismic acid** (Figure 4.3) involves a 1,4-elimination of phosphoric acid, though this is probably not a concerted elimination.

4-hydroxybenzoic acid (Figure 4.4) is produced in bacteria from chorismic acid by an elimination reaction, losing the recently introduced enolpyruvic acid side-chain. However, in plants, this phenolic acid is formed by a branch much further on in the pathway via side-chain degradation of cinnamic acids (see page 141). The three phenolic acids so far encountered, 4-hydroxybenzoic, protocatechuic, and gallic acids, demonstrate some of the hydroxylation patterns characteristic of shikimic acid-derived metabolites, i.e. a single hydroxy *para* to the side-chain function, dihydroxy groups arranged *ortho* to each other, typically 3,4- to the side-chain, and trihydroxy groups also *ortho* to each other and 3,4,5- to the side-chain. The single *para*-hydroxylation and the *ortho*-polyhydroxylation patterns contrast with the typical *meta*-hydroxylation patterns characteristic of phenols derived via the acetate pathway (see page 62), and in most cases allow the biosynthetic origin (acetate or shikimate) of an aromatic ring to be deduced by inspection.

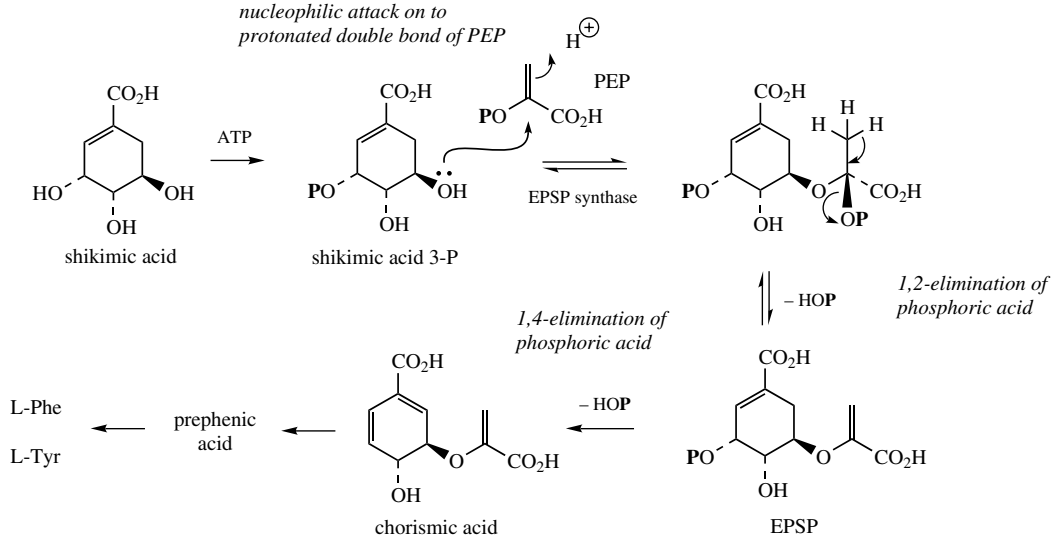

Figure 4.3

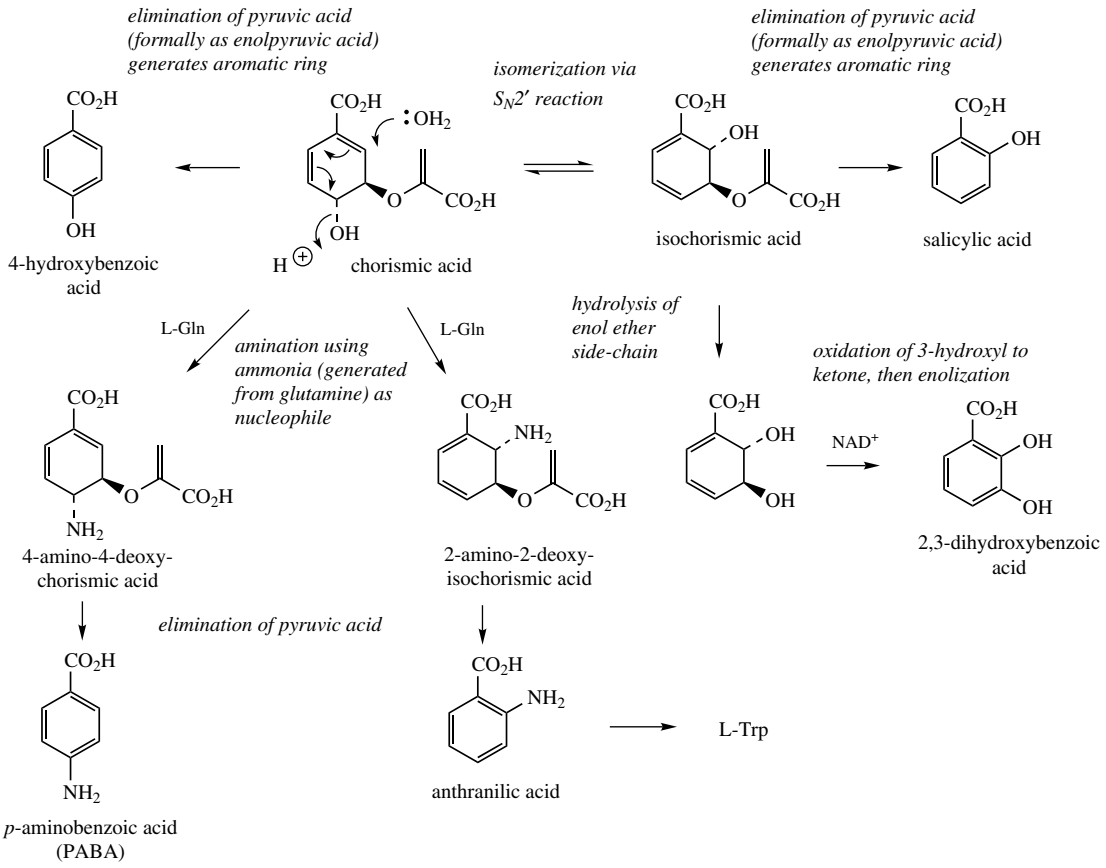

Figure 4.4

2,3-dihydroxybenzoic acid, and **salicylic acid** (2-hydroxybenzoic acid) (in microorganisms, but not in plants, see page 141), are derived from chorismic acid via its isomer **isochorismic acid** (Figure 4.4). The isomerization involves an S_N2'-type of reaction, an incoming water nucleophile attacking the diene system and displacing the hydroxyl. Salicyclic acid arises by an elimination reaction analogous to that producing 4-hydroxybenzoic acid from chorismic acid. In the formation of 2,3-dihydroxybenzoic acid, the side-chain of isochorismic acid is first lost by hydrolysis, then dehydrogenation of the 3-hydroxy to a 3-keto allows enolization and formation of the aromatic ring. 2,3-Dihydroxybenzoic acid is a component of the powerful iron chelator (siderophore) **enterobactin** (Figure 4.5) found in *Escherichia coli* and many other Gram-negative bacteria. Such compounds play an important role in bacterial growth by making available sufficient concentrations of essential iron. Enterobactin comprises three molecules of 2,3-dihydroxybenzoic acid and three of the amino acid L-serine, in cyclic triester form.

Simple amino analogues of the phenolic acids are produced from chorismic acid by related transformations in which ammonia, generated from glutamine, acts as a nucleophile (Figure 4.4). Chorismic acid can be aminated at C-4 to give 4-amino-4-deoxychorismic acid and then *p*-aminobenzoic (4-aminobenzoic) acid, or at C-2 to give the isochorismic acid analogue which will yield 2-aminobenzoic (anthranilic) acid. Amination at C-4 has been found to occur with retention of configuration, so perhaps a double inversion mechanism is involved. ***p*-Aminobenzoic acid** (**PABA**) forms part of the structure of **folic acid** (vitamin B_9)* (Figure 4.6). The folic acid structure is built up (Figure 4.6) from a dihydropterin diphosphate which reacts with *p*-aminobenzoic

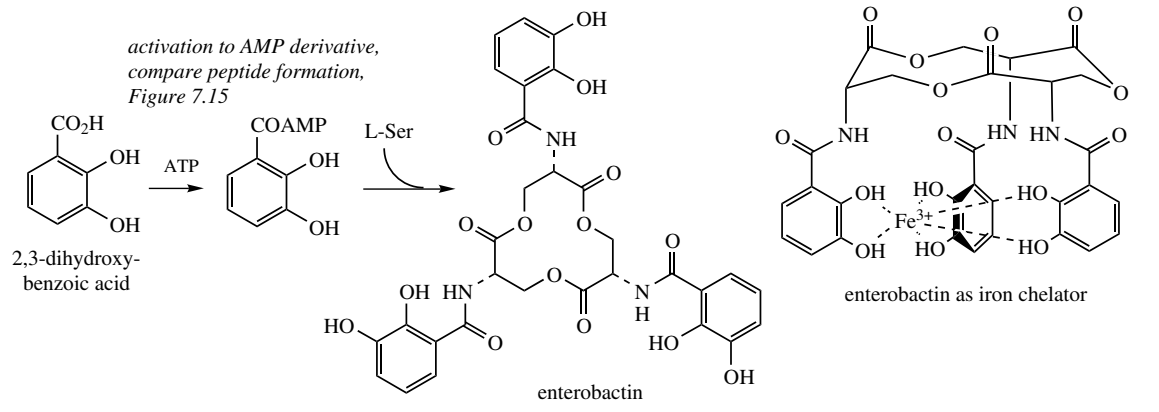

Figure 4.5

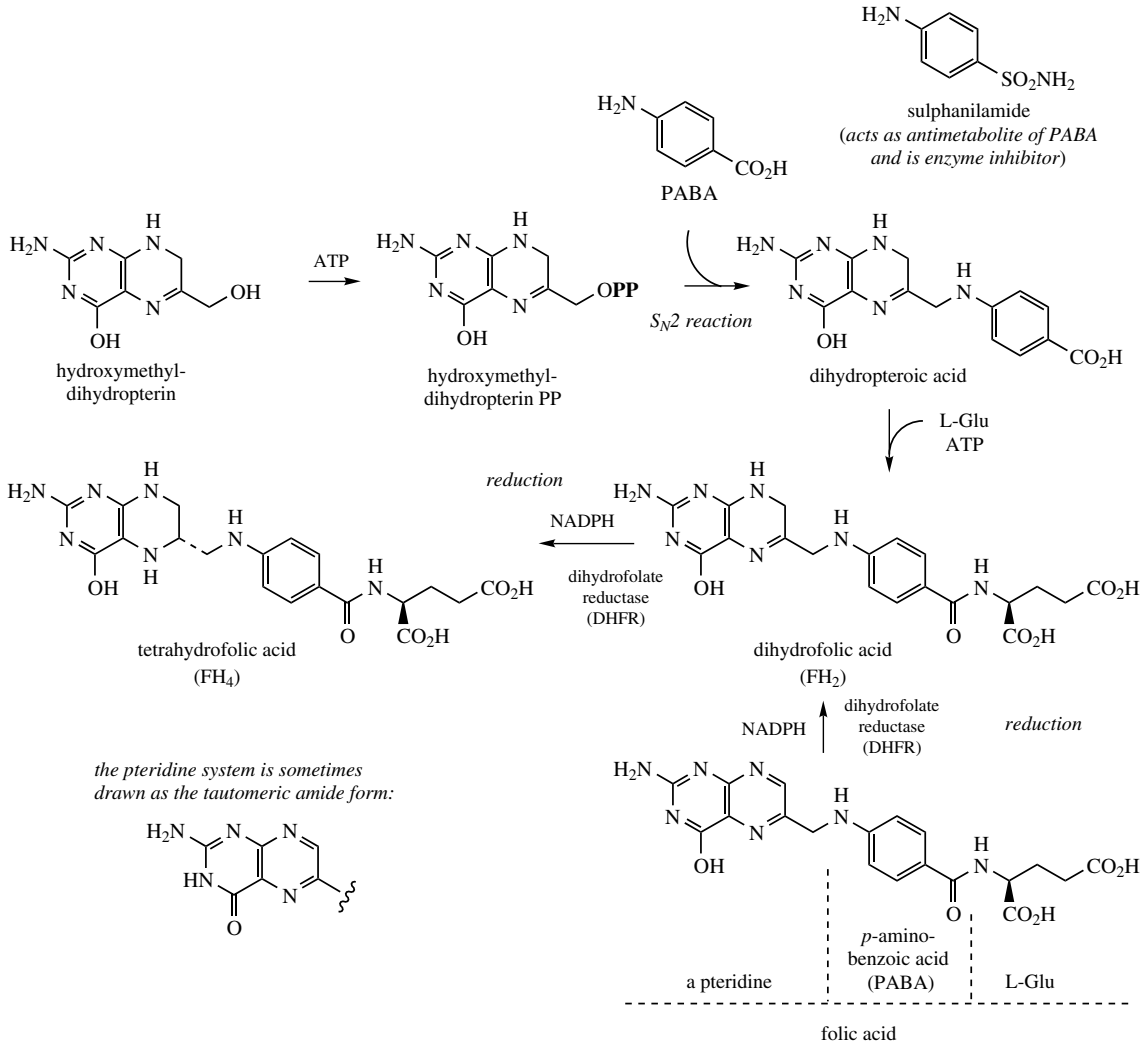

Figure 4.6

acid to give dihydropteroic acid, an enzymic step for which the sulphonamide antibiotics are inhibitors. **Dihydrofolic acid** is produced from the dihydropteroic acid by incorporating glutamic acid, and reduction yields **tetrahydrofolic acid**. This reduction step is also necessary for the continual regeneration of tetrahydrofolic acid, and forms an important site of action for some antibacterial, antimalarial, and anticancer drugs.

Anthranilic acid (Figure 4.4) is an intermediate in the biosynthetic pathway to the indole-containing aromatic amino acid L-**tryptophan** (Figure 4.10).

Folic Acid (Vitamin B_9)

Folic acid (vitamin B_9) (Figure 4.6) is a conjugate of a pteridine unit, *p*-aminobenzoic acid, and glutamic acid. It is found in yeast, liver, and green vegetables, though cooking may destroy up to 90% of the vitamin. Deficiency gives rise to anaemia, and supplementation is often necessary during pregnancy. Otherwise, deficiency is not normally encountered unless there is malabsorption, or chronic disease. Folic acid used for supplementation is usually synthetic, and it becomes sequentially reduced in the body by the enzyme dihydrofolate reductase to give dihydrofolic acid and then tetrahydrofolic acid (Figure 4.6). Tetrahydrofolic acid then functions as a carrier of one-carbon groups, which may be in the form of methyl, methylene, methenyl, or formyl groups, by the reactions outlined in Figure 4.7. These groups are involved in amino acid and nucleotide metabolism. Thus a methyl group is transferred in the regeneration of methionine from homocysteine, purine biosynthesis involves methenyl and formyl transfer, and pyrimidine biosynthesis utilizes methylene transfer. Tetrahydrofolate derivatives also serve as acceptors of one-carbon units in degradative pathways.

Mammals must obtain their tetrahydrofolate requirements from their diet, but microorganisms are able to synthesize this material. This offers scope for selective action and led to the use of sulphanilamide and other antibacterial sulpha drugs, compounds which competitively inhibit dihydropteroate synthase, the biosynthetic enzyme incorporating *p*-aminobenzoic acid into the structure. These sulpha drugs thus act as antimetabolites of *p*-aminobenzoate. Specific dihydrofolate reductase inhibitors have also become especially useful as antibacterials,

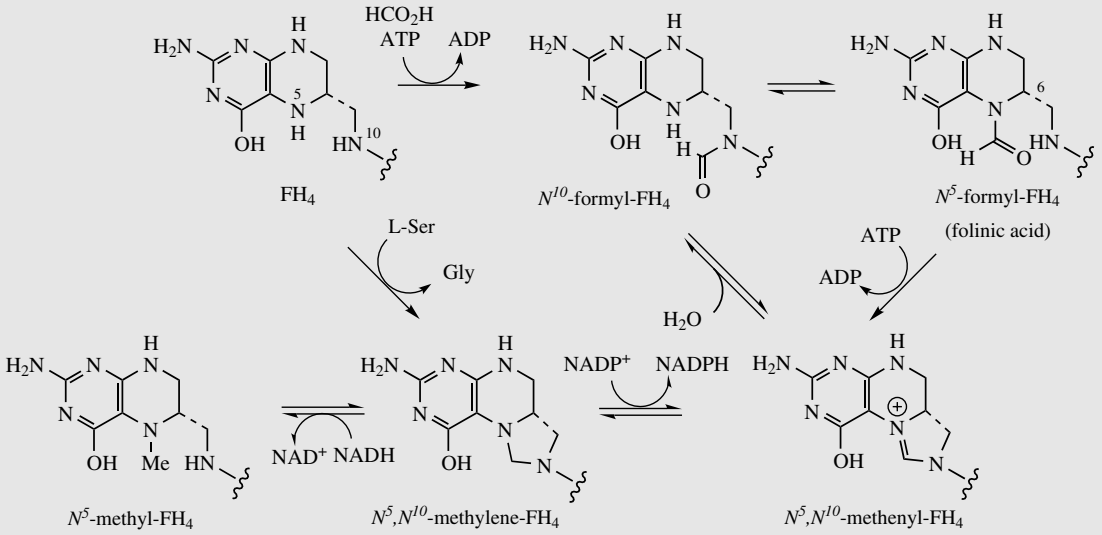

Figure 4.7

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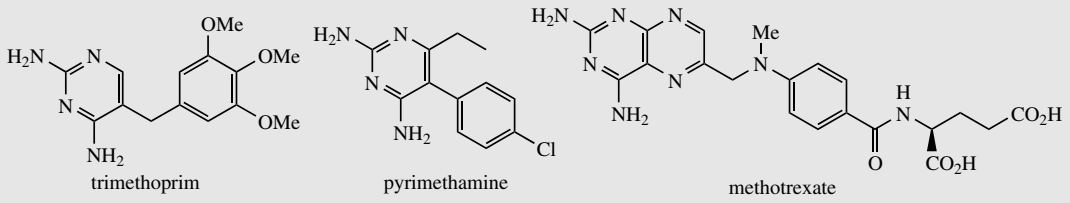


Figure 4.8

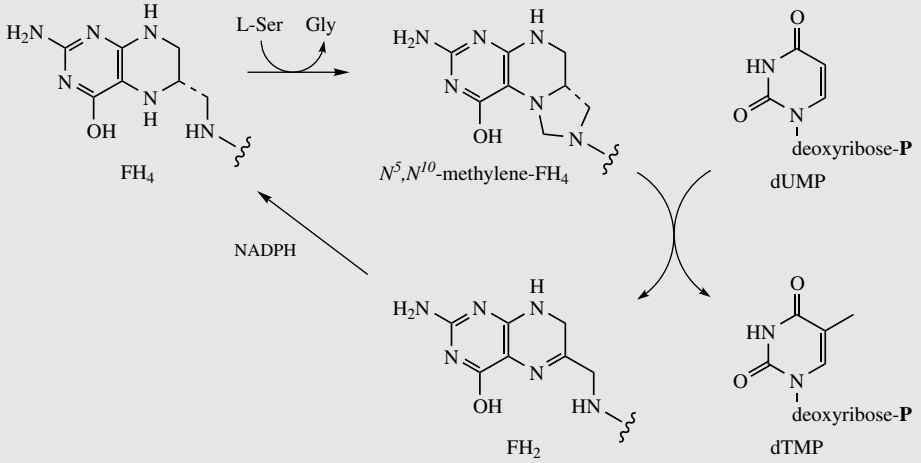


Figure 4.9

e.g. **trimethoprim** (Figure 4.8), and antimalarial drugs, e.g. **pyrimethamine**, relying on the differences in susceptibility between the enzymes in humans and in the infective organism. Anticancer agents based on folic acid, e.g. **methotrexate** (Figure 4.8), primarily block pyrimidine biosynthesis, but are less selective than the antimicrobial agents, and rely on a stronger binding to the enzyme than the natural substrate has. Regeneration of tetrahydrofolate from dihydrofolate is vital for DNA synthesis in rapidly proliferating cells. The methylation of deoxyuridylate (dUMP) to deoxythymidylate (dTMP) requires N^5,N^{10}-methylenetetrahydrofolate as the methyl donor, which is thereby transformed into dihydrofolate (Figure 4.9). N^5-Formyl-tetrahydrofolic acid (**folinic acid, leucovorin**) (Figure 4.7) is used to counteract the folate-antagonist action of anticancer agents like methotrexate. The natural 6*S* isomer is termed **levofolinic acid** (**levoleucovorin**); **folinic acid** in drug use is usually a mixture of the 6*R* and 6*S* isomers.

In a sequence of complex reactions, which will not be considered in detail, the indole ring system is formed by incorporating two carbons from phosphoribosyl diphosphate, with loss of the original anthranilate carboxyl. The remaining ribosyl carbons are then removed by a reverse aldol reaction, to be replaced on a bound form of indole by those from L-serine, which then becomes the side-chain of L-tryptophan. Although a precursor of L-tryptophan, anthranilic acid may also be produced by metabolism of tryptophan. Both compounds feature as building blocks for a variety of alkaloid structures (see Chapter 6).

Returning to the main course of the shikimate pathway, a singular rearrangement process occurs transforming **chorismic acid** into **prephenic acid**

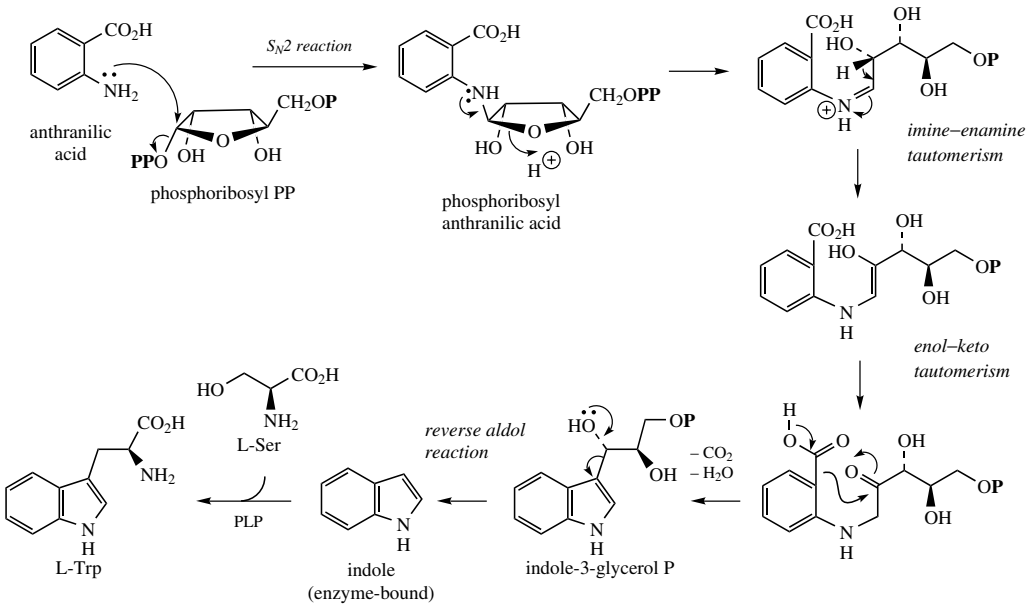


Figure 4.10

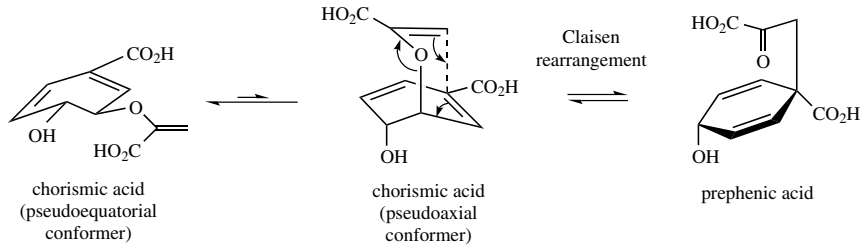


Figure 4.11

(Figure 4.11). This reaction, a Claisen rearrangement, transfers the PEP-derived side-chain so that it becomes directly bonded to the carbocycle, and so builds up the basic carbon skeleton of phenylalanine and tyrosine. The reaction is catalysed in nature by the enzyme chorismate mutase, and, although it can also occur thermally, the rate increases some 10^6-fold in the presence of the enzyme. The enzyme achieves this by binding the pseudoaxial conformer of chorismic acid, allowing a transition state with chairlike geometry to develop.

Pathways to the aromatic amino acids L-**phenylalanine** and L-**tyrosine** via prephenic acid may vary according to the organism, and often more than one route may operate in a particular species according to the enzyme activities that are available (Figure 4.12). In essence, only three reactions are involved, decarboxylative aromatization, transamination, and in the case of tyrosine biosynthesis an oxidation, but the order in which these reactions occur differentiates the routes. Decarboxylative aromatization of prephenic acid yields **phenylpyruvic acid**, and PLP-dependent transamination leads to L-phenylalanine. In the presence of an NAD^+-dependent dehydrogenase enzyme, decarboxylative aromatization occurs with retention of the hydroxyl function, though as yet there is no evidence that any intermediate carbonyl analogue of prephenic acid is involved. Transamination of the resultant **4-hydroxyphenylpyruvic acid** subsequently gives L-tyrosine. L-**Arogenic acid** is the result of transamination of prephenic acid occurring prior to the decarboxylative aromatization, and can be transformed into both L-phenylalanine and L-tyrosine depending on the absence or presence

of a suitable enzymic dehydrogenase activity. In some organisms, broad activity enzymes are known to be capable of accepting both prephenic acid and arogenic acid as substrates. In microorganisms and plants, L-phenylalanine and L-tyrosine tend to be synthesized separately as in Figure 4.12, but in animals, which lack the shikimate pathway, direct hydroxylation of L-phenylalanine to L-tyrosine, and of L-tyrosine to L-**DOPA** (dihydroxyphenylalanine), may be achieved (Figure 4.13). These reactions are catalysed by tetrahydropterin-dependent hydroxylase enzymes, the hydroxyl oxygen being derived from molecular oxygen. L-DOPA is a precursor of the **catecholamines**, e.g. the neurotransmitter noradrenaline and the hormone adrenaline (see page 316). Tyrosine and DOPA are also converted by oxidation reactions into a heterogeneous polymer **melanin**, the main pigment in mammalian skin, hair, and eyes. In this material, the indole system is not formed from tryptophan, but arises from DOPA by cyclization of DOPAquinone, the nitrogen of the side-chain then attacking the *ortho*-quinone (Figure 4.13).

Some organisms are capable of synthesizing an unusual variant of L-phenylalanine, the aminated derivative L-*p*-aminophenylalanine (L-PAPA) (Figure 4.14). This is known to occur by a series of reactions paralleling those in Figure 4.12, but utilizing the PABA precursor 4-amino-4-deoxychorismic acid (Figure 4.4) instead of chorismic acid. Thus, amino derivatives of prephenic acid and pyruvic acid are elaborated. One important metabolite known to be formed from L-PAPA is the antibiotic

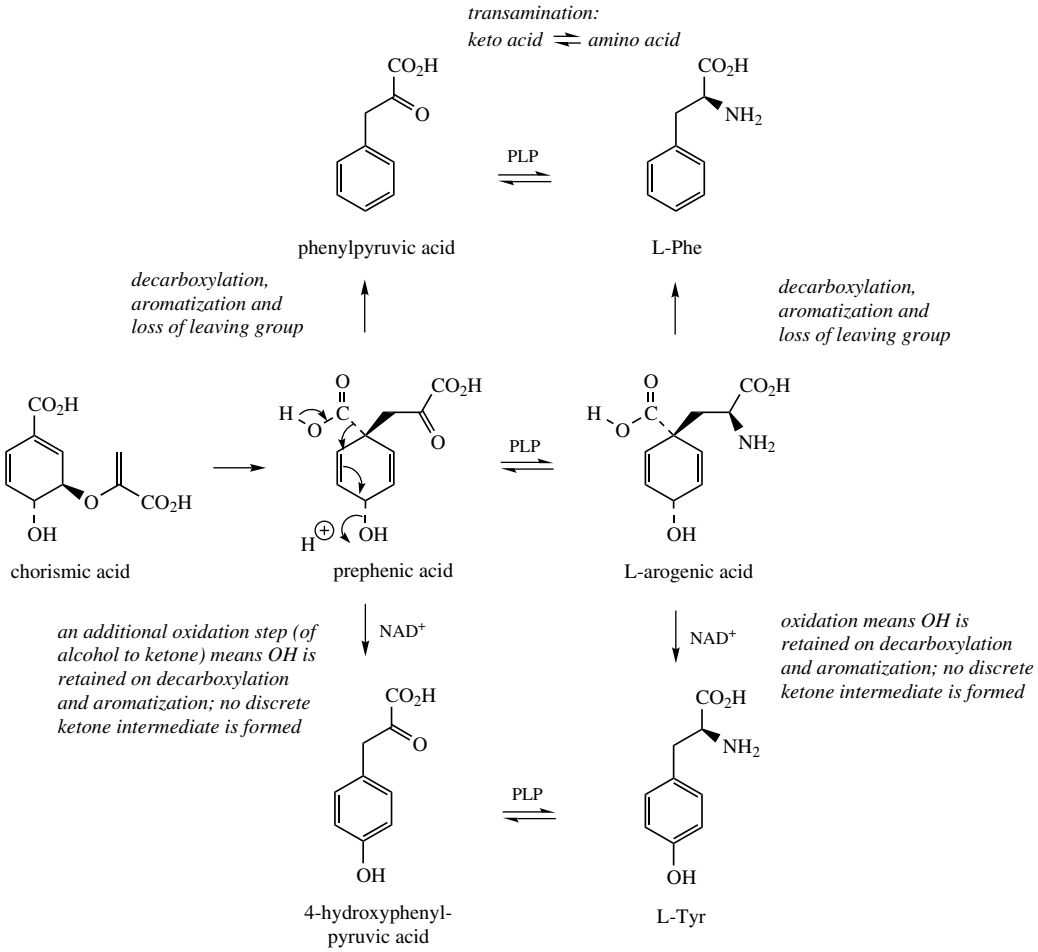


Figure 4.12

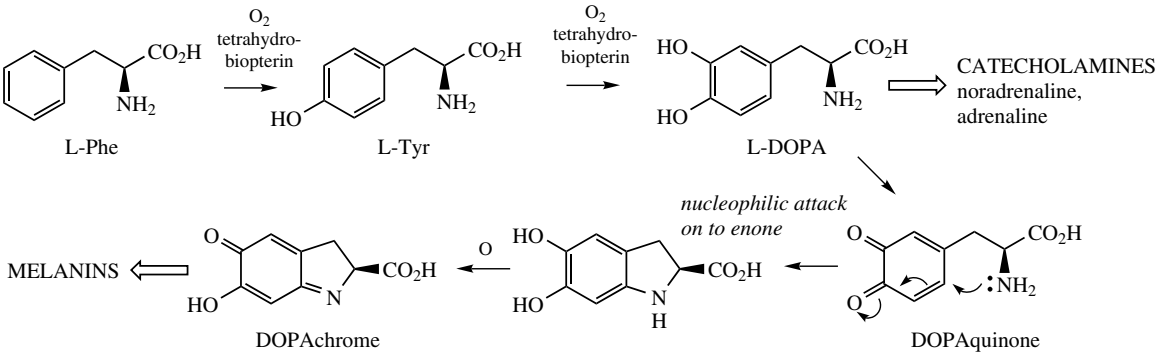


Figure 4.13

chloramphenicol*, produced by cultures of *Streptomyces venezuelae*. The late stages of the pathway (Figure 4.14) have been formulated to involve hydroxylation and *N*-acylation in the side-chain, the latter reaction probably requiring a coenzyme A ester of dichloroacetic acid. Following reduction of the carboxyl group, the final reaction is oxidation of the 4-amino group to a nitro, a fairly rare substituent in natural product structures.

CINNAMIC ACIDS

L-Phenylalanine and L-tyrosine, as C_6C_3 building blocks, are precursors for a wide range of natural products. In plants, a frequent first step is the elimination of ammonia from the side-chain to generate the appropriate *trans* (*E*) cinnamic acid. In the case of phenylalanine, this would give **cinnamic acid**, whilst tyrosine could yield **4-coumaric acid** (*p*-coumaric acid) (Figure 4.15). All plants appear to have the ability to deaminate phenylalanine via the enzyme phenylalanine ammonia lyase (PAL), but the corresponding transformation of tyrosine is more restricted, being mainly limited to members of the grass family (the Graminae/Poaceae). Whether a separate enzyme tyrosine ammonia lyase (TAL) exists, or whether grasses merely have a broad specificity PAL also

Chloramphenicol

Chloramphenicol (chloromycetin) (Figure 4.14) was initially isolated from cultures of *Streptomyces venezuelae*, but is now obtained for drug use by chemical synthesis. It was one of the first broad spectrum antibiotics to be developed, and exerts its antibacterial action by inhibiting protein biosynthesis. It binds reversibly to the 50S subunit of the bacterial ribosome, and in so doing disrupts peptidyl transferase, the enzyme that catalyses peptide bond formation (see page 408). This reversible binding means that bacterial cells not destroyed may resume protein biosynthesis when no longer exposed to the antibiotic. Some microorganisms have developed resistance to chloramphenicol by an inactivation process involving enzymic acetylation of the primary alcohol group in the antibiotic. The acetate binds only very weakly to the ribosomes, so has little antibiotic activity. The value of chloramphenicol as an antibacterial agent has been severely limited by some serious side-effects. It can cause blood disorders including irreversible aplastic anaemia in certain individuals, and these can lead to leukaemia and perhaps prove fatal. Nevertheless, it is still the drug of choice for some life-threatening infections such as typhoid fever and bacterial meningitis. The blood constitution must be monitored regularly during treatment to detect any abnormalities or adverse changes. The drug is orally active, but may also be injected. Eye-drops are useful for the treatment of bacterial conjunctivitis.

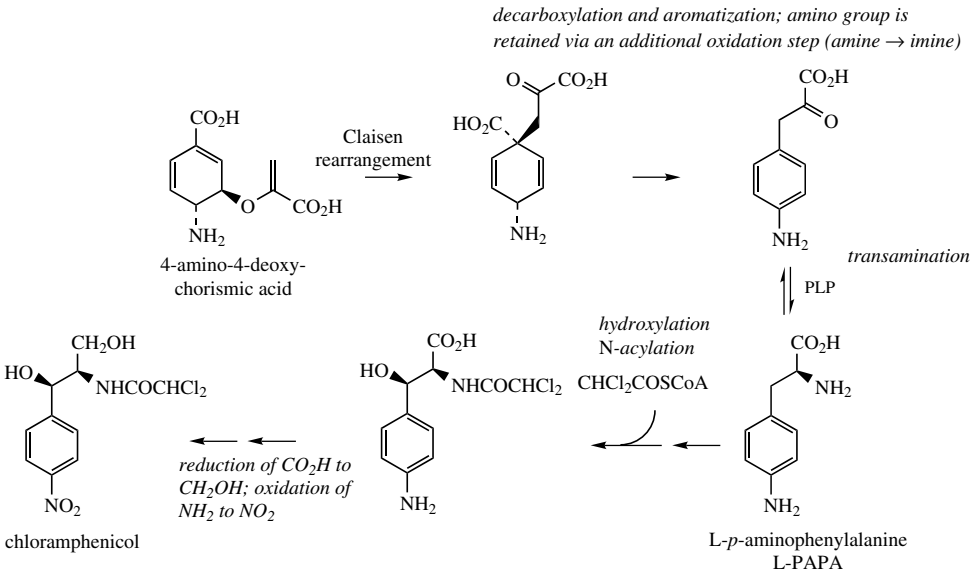


Figure 4.14

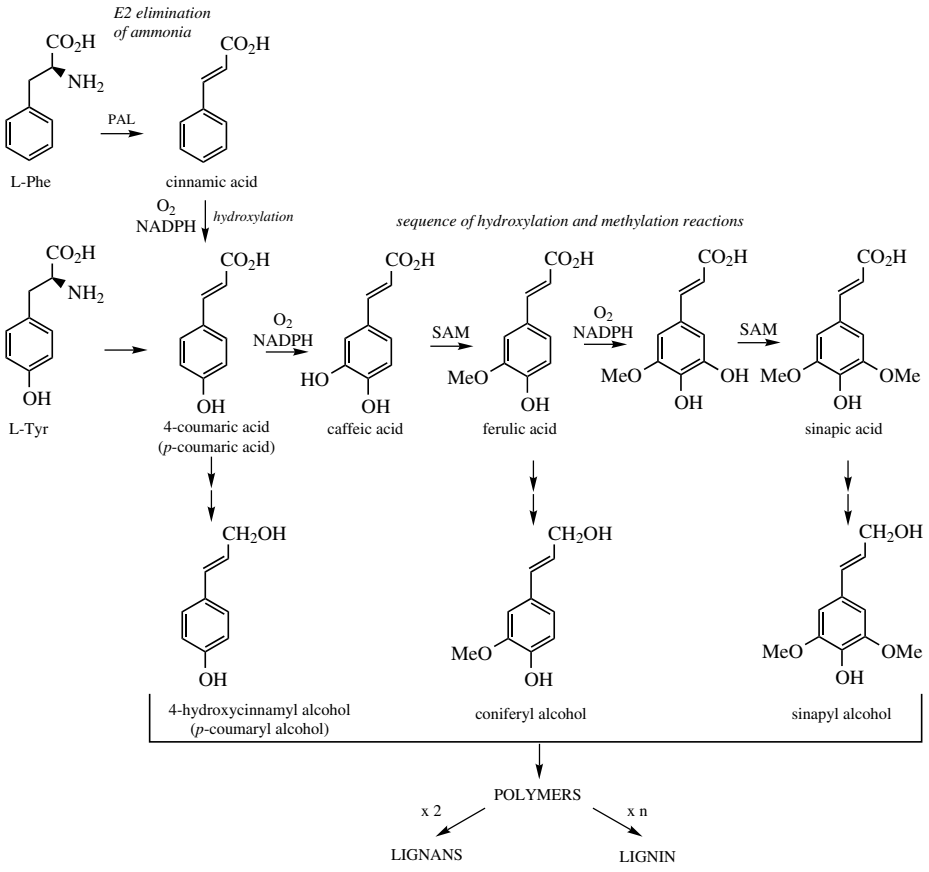
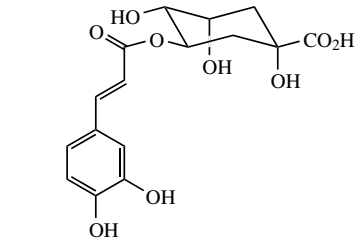
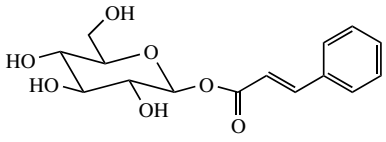


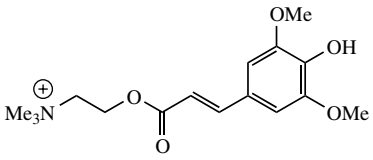
Figure 4.15



chlorogenic acid
(5-*O*-caffeoylquinic acid)



1-*O*-cinnamoylglucose



sinapine

Figure 4.16

capable of deaminating tyrosine, is still debated. Those species that do not transform tyrosine synthesize 4-coumaric acid by direct hydroxylation of cinnamic acid, in a cytochrome P-450-dependent reaction, and tyrosine is often channelled instead into other secondary metabolites, e.g. alkaloids. Other cinnamic acids are obtained by further hydroxylation and methylation reactions, sequentially building up substitution patterns typical of shikimate pathway metabolites, i.e. an *ortho* oxygenation pattern (see page 123). Some of the more common natural cinnamic acids are 4-coumaric, **caffeic, ferulic**, and **sinapic acids** (Figure 4.15). These can be found in plants in free form and in a range of esterified forms, e.g. with quinic acid as in **chlorogenic acid** (5-*O*-caffeoylquinic acid) (see coffee, page 395), with glucose as in **1-*O*-cinnamoylglucose**, and with choline as in **sinapine** (Figure 4.16).

LIGNANS AND LIGNIN

The cinnamic acids also feature in the pathways to other metabolites based on C_6C_3 building blocks. Pre-eminent amongst these, certainly as far as nature is concerned, is the plant polymer **lignin**, a strengthening material for the plant cell wall which acts as a matrix for cellulose microfibrils (see page 473). Lignin represents a vast reservoir of aromatic materials, mainly untapped because of the difficulties associated with release of these metabolites. The action of wood-rotting fungi offers the most effective way of making these useful products more accessible. Lignin is formed by phenolic oxidative coupling of hydroxycinnamyl alcohol monomers, brought about by peroxidase enzymes (see page 28). The most important of these monomers are **4-hydroxycinnamyl alcohol** (*p*-coumaryl alcohol), **coniferyl alcohol**, and **sinapyl alcohol** (Figure 4.15), though the monomers used vary according to the plant type. Gymnosperms polymerize mainly coniferyl alcohol, dicotyledonous plants coniferyl alcohol and sinapyl alcohol, whilst monocotyledons use all three alcohols. The alcohols are derived by reduction of cinnamic acids via coenzyme A esters and aldehydes (Figure 4.17), though the substitution patterns are not necessarily elaborated completely at the cinnamic acid stage, and coenzyme A esters and aldehydes may also be substrates for aromatic hydroxylation and methylation. Formation of the coenzyme A ester facilitates the first reduction step by introducing a better leaving group ($CoAS^-$) for the NADPH-dependent reaction. The second reduction step, aldehyde to alcohol, utilizes a further molecule of NADPH and is reversible. The peroxidase enzyme then achieves one-electron oxidation of the phenol group. One-electron oxidation of a simple phenol allows delocalization of the unpaired electron, giving resonance forms in which the free electron resides at positions *ortho* and *para* to the oxygen function (see page 29). With cinnamic acid derivatives, conjugation allows the unpaired electron to be delocalized also into the side-chain (Figure 4.18). Radical pairing of resonance structures can then provide a range of dimeric systems containing reactive quinonemethides, which are susceptible to nucleophilic attack from hydroxyl groups in the same system, or by external water molecules. Thus, **coniferyl alcohol** monomers can couple, generating linkages as exemplified by **guaiacylglycerol β-coniferyl ether** (β-arylether linkage), **dehydrodiconiferyl alcohol** (phenylcoumaran linkage), and

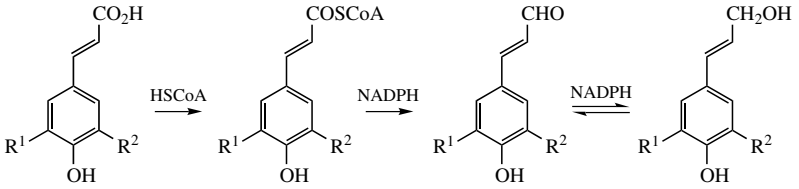
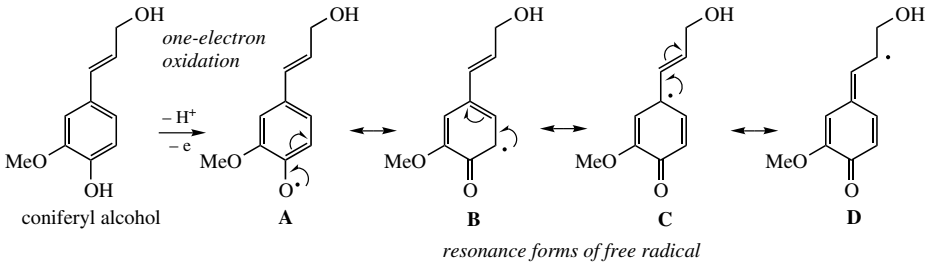


Figure 4.17



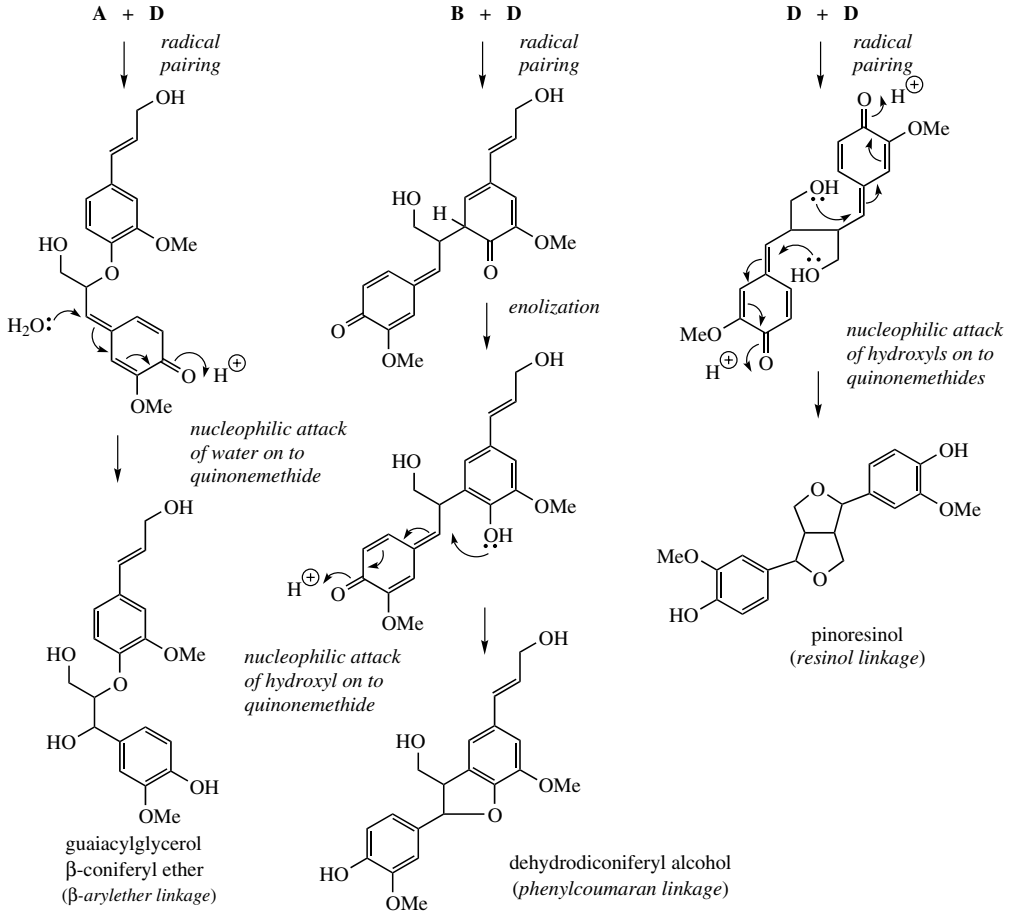


Figure 4.18

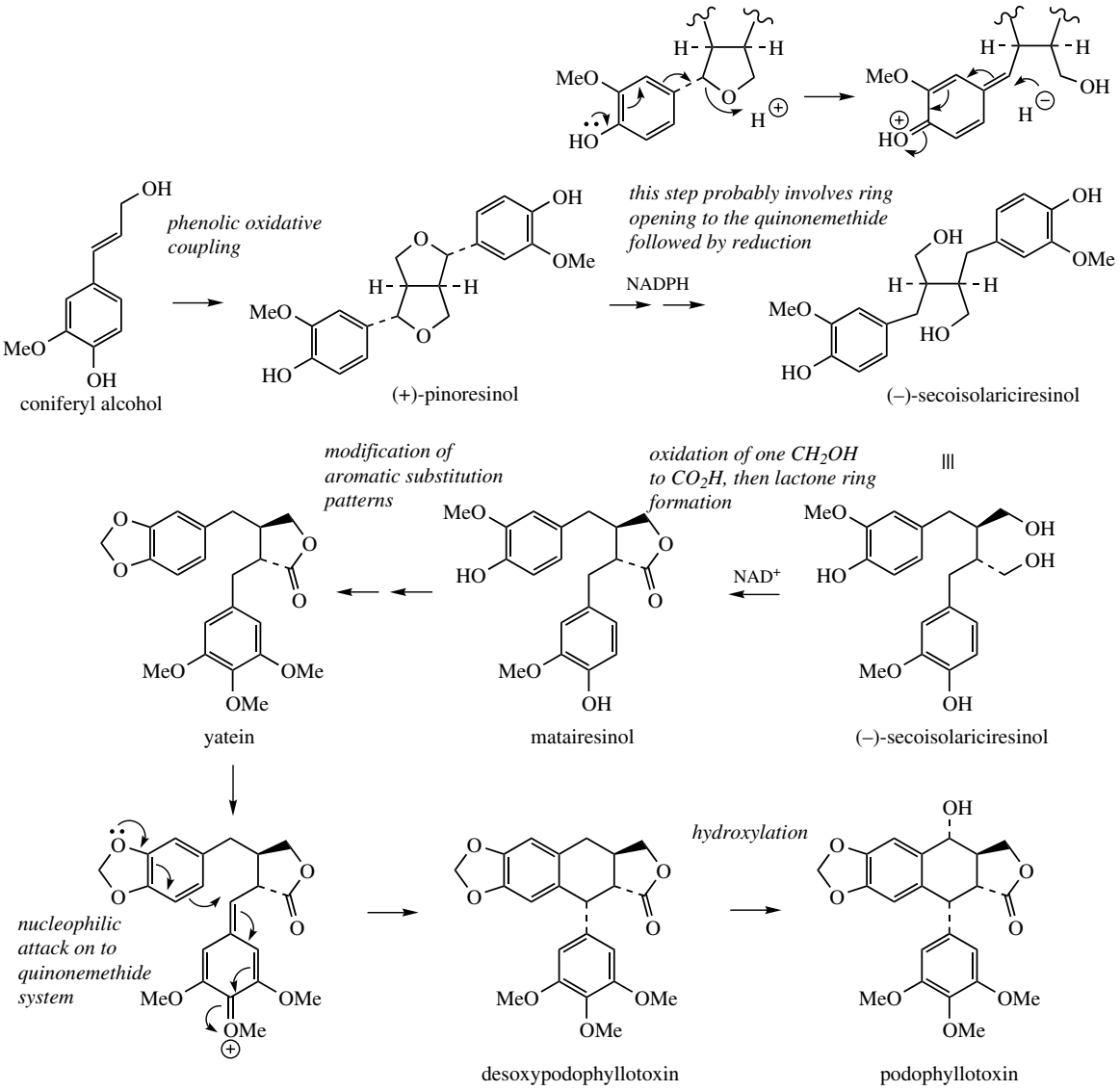


Figure 4.19

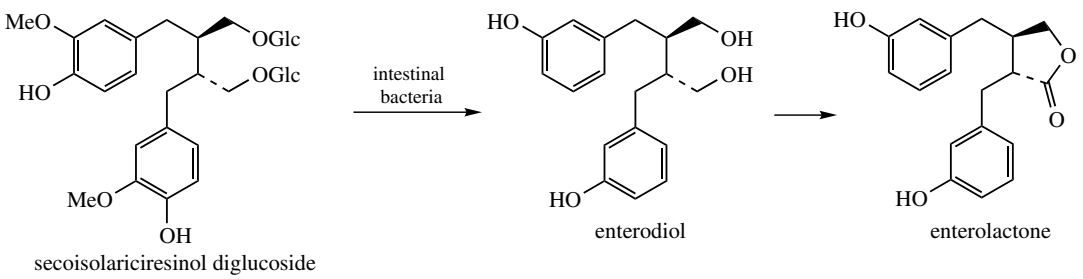


Figure 4.20

pinoresinol (resinol linkage). These dimers can react further by similar mechanisms to produce a lignin polymer containing a heterogeneous series of inter-molecular bondings as seen in the various dimers. In contrast to most other natural polymeric materials, lignin appears to be devoid of ordered repeating units, though some 50–70% of the linkages are of the β-arylether type. The dimeric materials are also found in nature and are called **lignans**. Some authorities like to restrict the term lignan specifically to molecules in which the two phenylpropane units are coupled at the central carbon of the side-chain, e.g. pinoresinol, whilst compounds containing other types of coupling, e.g. as in guaiacylglycerol β-coniferyl ether and dehydrodiconiferyl alcohol, are then referred to as **neolignans**. Lignan/neolignan formation and lignin biosynthesis are catalysed by different enzymes, and a consequence is that natural lignans/neolignans are normally enantiomerically pure because they arise from stereochemically controlled coupling. The control mechanisms for lignin biosynthesis are less well defined, but the enzymes appear to generate products lacking optical activity.

Further cyclization and other modifications can create a wide range of lignans of very different structural types. One of the most important of the natural lignans having useful biological activity is the aryltetralin lactone **podophyllotoxin** (Figure 4.19), which is derived from coniferyl alcohol via the dibenzylbutyrolactones **matairesinol** and **yatein**, cyclization probably occurring as shown in Figure 4.19. Matairesinol is known to arise by reductive opening of the furan rings of **pinoresinol**, followed by oxidation of a primary alcohol to the acid and then lactonization. The substitution pattern in the two aromatic rings is built up further during the pathway, i.e. matairesinol → yatein, and does not arise by initial coupling of two different cinnamyl alcohol residues. The methylenedioxy ring system, as found in many shikimate-derived natural products, is formed by an oxidative reaction on an *ortho*-hydroxymethoxy pattern (see page 27). Podophyllotoxin and related lignans are found in the roots of *Podophyllum** species (Berberidaceae), and have clinically useful cytotoxic and anticancer activity. The lignans **enterolactone** and **enterodiol** (Figure 4.20) were discovered in human urine, but were subsequently shown to be derived from dietary plant lignans, especially secoisolariciresinol diglucoside, by the action of intestinal microflora. Enterolactone and enterodiol have oestrogenic activity and have been implicated as contributing to lower levels of breast cancer amongst vegetarians (see phyto-oestrogens, page 156).

PHENYLPROPENES

The reductive sequence from an appropriate cinnamic acid to the corresponding cinnamyl alcohol is not restricted to lignin and lignan biosynthesis, and is utilized for the production of various phenylpropene derivatives. Thus **cinnamaldehyde** (Figure 4.23) is the principal component in the

Podophyllum

Podophyllum consists of the dried rhizome and roots of *Podophyllum hexandrum* (*P. emodi*) or *P. peltatum* (Berberidaceae). *Podophyllum hexandrum* is found in India, China, and the Himalayas and yields Indian podophyllum, whilst *P. peltatum* (May apple or American mandrake) comes from North America and is the source of American podophyllum. Plants are collected from the wild. Both plants are large-leafed perennial herbs with edible fruits, though other parts of the plant are toxic. The roots contain cytotoxic lignans and their glucosides, *P. hexandrum* containing about 5%, and *P. peltatum* about 1%. A concentrated form of the active principles is obtained by pouring an ethanolic extract of the root into water, and drying the precipitated podophyllum resin or 'podophyllin'. Indian podophyllum yields about 6–12% of resin containing 50–60% lignans, and American podophyllum 2–8% of resin containing 14–18% lignans.

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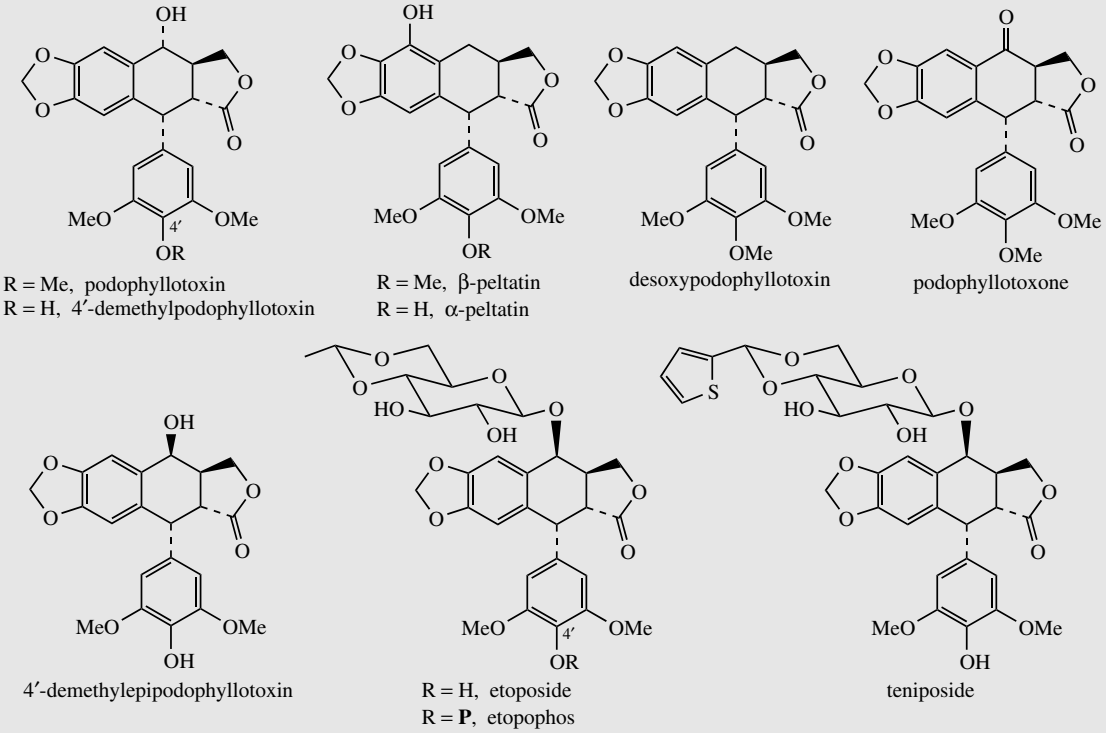

Figure 4.21

The lignan constituents of the two roots are the same, but the proportions are markedly different. The Indian root contains chiefly podophyllotoxin (Figure 4.21) (about 4%) and 4′-demethylpodophyllotoxin (about 0.45%). The main components in the American root are podophyllotoxin (about 0.25%), β-peltatin (about 0.33%) and α-peltatin (about 0.25%). Desoxypodophyllotoxin and podophyllotoxone are also present in both plants, as are the glucosides of podophyllotoxin, 4′-demethylpodophyllotoxin, and the peltatins, though preparation of the resin results in considerable losses of the water-soluble glucosides.

Podophyllum resin has long been used as a purgative, but the discovery of the cytotoxic properties of podophyllotoxin and related compounds has now made podophyllum a commercially important drug plant. Preparations of **podophyllum resin** (the Indian resin is preferred) are effective treatments for warts, and pure **podophyllotoxin** is available as a paint for venereal warts, a condition which can be sexually transmitted. The antimitotic effect of podophyllotoxin and the other lignans is by binding to the protein tubulin in the mitotic spindle, preventing polymerization and assembly into microtubules (compare vincristine, page 356, and colchicine, page 343). During mitosis, the chromosomes separate with the assistance of these microtubules, and after cell division the microtubules are transformed back to tubulin. Podophyllotoxin and other *Podophyllum* lignans were found to be unsuitable for clinical use as anticancer agents due to toxic side-effects, but the semi-synthetic derivatives etoposide and teniposide (Figure 4.21), which are manufactured from natural podophyllotoxin, have proved excellent antitumour agents. They were developed as modified forms (acetals) of the natural

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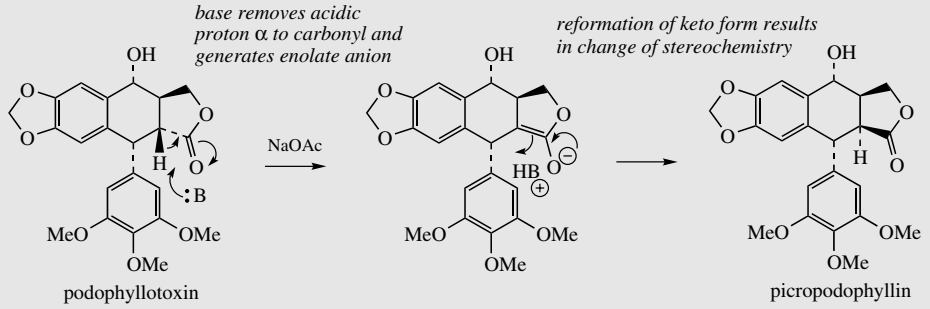

Figure 4.22

4′-demethylpodophyllotoxin glucoside. Attempted synthesis of the glucoside inverted the stereochemistry at the sugar–aglycone linkage, and these agents are thus derivatives of 4′-demethylepipodophyllotoxin (Figure 4.21). **Etoposide** is a very effective anticancer agent, and is used in the treatment of small cell lung cancer, testicular cancer and lymphomas, usually in combination therapies with other anticancer drugs. It may be given orally or intravenously. The water-soluble pro-drug **etopophos** (etoposide 4′-phosphate) is also available. **Teniposide** has similar anticancer properties, and, though not as widely used as etoposide, has value in paediatric neuroblastoma.

Remarkably, the 4′-demethylepipodophyllotoxin series of lignans do not act via a tubulin-binding mechanism as does podophyllotoxin. Instead, these drugs inhibit the enzyme topoisomerase II, thus preventing DNA synthesis and replication. Topoisomerases are responsible for cleavage and resealing of the DNA strands during the replication process, and are classified as type I or II according to their ability to cleave one or both strands. Camptothecin (see page 365) is an inhibitor of topoisomerase I. Etoposide is believed to inhibit strand-rejoining ability by stabilizing the topoisomerase II–DNA complex in a cleavage state, leading to double-strand breaks and cell death. Development of other topoisomerase inhibitors based on podophyllotoxin-related lignans is an active area of research. Biological activity in this series of compounds is very dependent on the presence of the *trans*-fused five-membered lactone ring, this type of fusion producing a highly-strained system. Ring strain is markedly reduced in the corresponding *cis*-fused system, and the natural compounds are easily and rapidly converted into these *cis*-fused lactones by treatment with very mild bases, via enol tautomers or enolate anions (Figure 4.22). Picropodophyllin is almost devoid of cytotoxic properties.

Podophyllotoxin is also found in significant amounts in the roots of other *Podophyllum* species, and in closely related genera such as *Diphylleia* (Berberidaceae).

oil from the bark of cinnamon (*Cinnamomum zeylanicum*; Lauraceae), widely used as a spice and flavouring. Fresh bark is known to contain high levels of **cinnamyl acetate**, and cinnamaldehyde is released from this by fermentation processes which are part of commercial preparation of the bark, presumably by enzymic hydrolysis and participation of the reversible aldehyde–alcohol oxidoreductase. Cinnamon leaf, on the other hand, contains large amounts of **eugenol** (Figure 4.23) and much smaller amounts of cinnamaldehyde. Eugenol is also the principal constituent in oil from cloves (*Syzygium aromaticum*; Myrtaceae), used for many years as a dental anaesthetic, as well as for flavouring. The side-chain of eugenol is derived from that of the cinnamyl alcohols by reduction, but differs in the location of the double bond. This change is accounted for by resonance

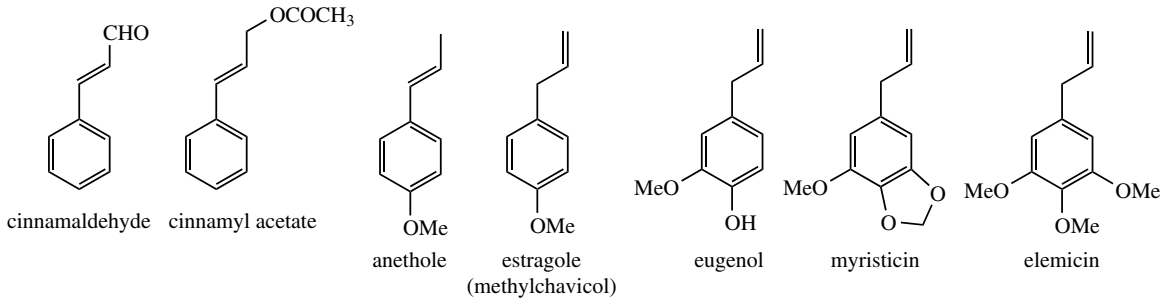

Figure 4.23

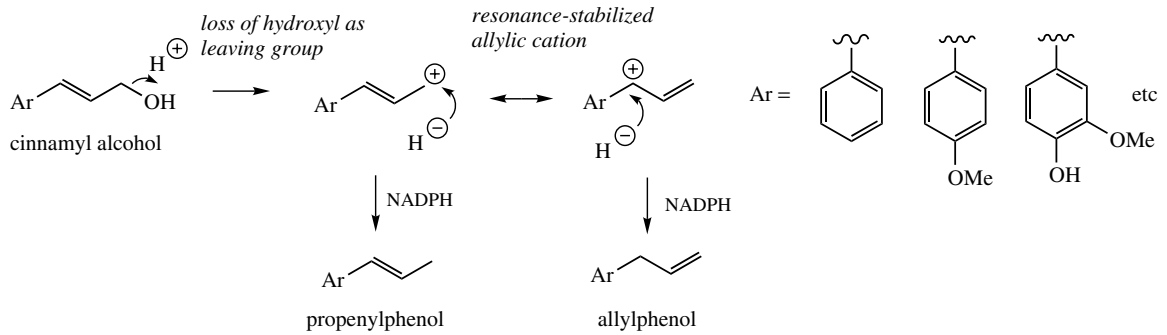

Figure 4.24

forms of the allylic cation (Figure 4.24), and addition of hydride (from NADPH) can generate either allylphenols, e.g. eugenol, or propenylphenols, e.g. **anethole** (Figure 4.23). Loss of hydroxyl from a cinnamyl alcohol may be facilitated by protonation, or perhaps even phosphorylation, though there is no evidence for the latter. **Myristicin** (Figure 4.23) from nutmeg (*Myristica fragrans*; Myristicaceae) is a further example of an allylphenol found in flavouring materials. Myristicin also has a history of being employed as a mild hallucinogen via ingestion of ground nutmeg. Myristicin is probably metabolized in the body via an amination reaction to give an amfetamine-like derivative (see page 385). **Anethole** is the main component in oils from aniseed (*Pimpinella anisum*; Umbelliferae/Apiaceae), star anise (*Illicium verum*; Illiciaceae), and fennel (*Foeniculum vulgare*; Umbelliferae/Apiaceae). The propenyl components of flavouring materials such as cinnamon, star anise, nutmeg, and sassafras (*Sassafras albidum*; Lauraceae) have reduced their commercial use somewhat since these constituents have been shown to be weak carcinogens in laboratory tests on animals. In the case of **safrole** (Figure 4.25), the main component of sassafras oil, this has been shown to arise from hydroxylation in the side-chain followed by sulphation, giving an agent which binds to cellular macromolecules. Further data on volatile oils containing aromatic constituents isolated from these and other plant materials are given in Table 4.1. Volatile oils in which the main components are terpenoid in nature are listed in Table 5.1, page 177.

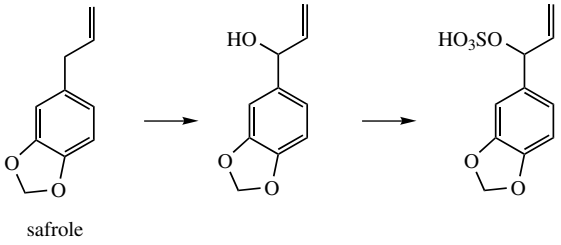

Figure 4.25

Table 4.1 Volatile oils containing principally aromatic compounds

Volatile or essential oils are usually obtained from the appropriate plant material by steam distillation, though if certain components are unstable at these temperatures, other less harsh techniques such as expression or solvent extraction may be employed. These oils, which typically contain a complex mixture of low boiling components, are widely used in flavouring, perfumery, and aromatherapy. Only a small number of oils have useful therapeutic properties, e.g. clove and dill, though a wide range of oils is now exploited for aromatherapy. Most of those employed in medicines are simply added for flavouring purposes. Some of the materials are commercially important as sources of chemicals used industrially, e.g. turpentine.

For convenience, the major oils listed are divided into two groups. Those which contain principally chemicals which are aromatic in nature and which are derived by the shikimate pathway are given in Table 4.1 below. Those oils which are composed predominantly of terpenoid compounds are listed in Table 5.1 on page 177, since they are derived via the deoxyxylulose phosphate pathway. It must be appreciated that many oils may contain aromatic and terpenoid components, but usually one group predominates. The oil yields, and the exact composition of any sample of oil will be variable, depending on the particular plant material used in its preparation. The quality of an oil and its commercial value is dependent on the proportion of the various components.

Oil	Plant source	Plant part used	Oil content (%)	Major constituents with typical (%) composition	Uses, notes
Aniseed (Anise)	*Pimpinella anisum* (Umbelliferae/ Apiaceae)	ripe fruit	2–3	anethole (80–90) estragole (1–6)	flavour, carminative, aromatherapy
Star anise	*Illicium verum* (Illiciaceae)	ripe fruit	5–8	anethole (80–90) estragole (1–6)	flavour, carminative fruits contain substantial amounts of shikimic and quinic acids
Cassia	*Cinnamomum cassia* (Lauraceae)	dried bark, or leaves and twigs	1–2	cinnamaldehyde (70–90) 2-methoxycinnamal-dehyde (12)	flavour, carminative known as cinnamon oil in USA
Cinnamon bark	*Cinnamomum zeylanicum* (Lauraceae)	dried bark	1–2	cinnamaldehyde (70–80) eugenol (1–13) cinnamyl acetate (3–4)	flavour, carminative, aromatherapy
Cinnamon leaf	*Cinnamomum zeylanicum* (Lauraceae)	leaf	0.5–0.7	eugenol (70–95)	flavour

(*Continued overleaf*)

Table 4.1 (*Continued*)

Oil	Plant source	Plant part used	Oil content (%)	Major constituents with typical (%) composition	Uses, notes
Clove	*Syzygium aromaticum* (*Eugenia caryophyllus*) (Myrtaceae)	dried flower buds	15–20	eugenol (75–90) eugenyl acetate (10–15) β-caryophyllene (3)	flavour, aromatherapy, antiseptic
Fennel	*Foeniculum vulgare* (Umbelliferae/ Apiaceae)	ripe fruit	2–5	anethole (50–70) fenchone (10–20) estragole (3–20)	flavour, carminative, aromatherapy
Nutmeg	*Myristica fragrans* (Myristicaceae)	seed	5–16	sabinene (17–28) α-pinene (14–22) β-pinene (9–15) terpinen-4-ol (6–9) myristicin (4–8) elemicin (2)	flavour, carminative, aromatherapy although the main constituents are terpenoids, most of the flavour comes from the minor aromatic constituents, myristicin, elemicin, etc myristicin is hallucinogenic (see page 385)
Wintergreen	*Gaultheria procumbens* (Ericacae)	leaves	0.7–1.5	methyl salicylate (98%)	flavour, antiseptic, antirheumatic
	or *Betula lenta* (Betulaceae)	bark	0.2–0.6		prior to distillation, plant material is macerated with water to allow enzymic hydrolysis of glycosides methyl salicylate is now produced synthetically

BENZOIC ACIDS FROM C_6C_3 COMPOUNDS

Some of the simple hydroxybenzoic acids (C_6C_1 compounds) such as 4-hydroxybenzoic acid and gallic acid can be formed directly from intermediates early in the shikimate pathway, e.g. 3-dehydroshikimic acid or chorismic acid (see page 121), but alternative routes exist in which cinnamic acid derivatives (C_6C_3 compounds) are cleaved at the double bond and lose two carbons from the side-chain. Thus, 4-coumaric acid may act as a precursor of **4-hydroxybenzoic acid**, and ferulic acid may give **vanillic acid** (4-hydroxy-3-methoxybenzoic acid) (Figure 4.26). A sequence analogous to that involved in the β-oxidation of fatty acids (see page 18) is possible, so that the double bond in the coenzyme A ester would be hydrated, the hydroxyl group oxidized to a ketone, and the β-ketoester would then lose acetyl-CoA by a reverse Claisen reaction, giving the coenzyme A ester of 4-hydroxybenzoic acid. Whilst this sequence has been generally accepted, newer evidence supports another side-chain cleavage mechanism, which is different from the fatty acid β-oxidation pathway (Figure 4.26). Coenzyme A esters are not involved, and though a similar hydration of the double bond occurs, chain shortening features a reverse aldol reaction, generating the appropriate aromatic aldehyde. The corresponding acid is then formed via an NAD^+-dependent oxidation step. Thus, aromatic aldehydes such as **vanillin**, the main flavour compound in vanilla (pods of the orchid *Vanilla planiflora*; Orchidaceae) would be formed from the correspondingly substituted cinnamic acid without proceeding through intermediate benzoic acids or esters. Whilst the substitution pattern in these C_6C_1 derivatives is generally built up at the C_6C_3 cinnamic acid stage, prior to chain shortening, there exists the possibility of further hydroxylations and/or methylations occurring at the C_6C_1 level, and this is known in certain examples. **Salicylic acid** (Figure 4.27) is synthesized in microorganisms directly from isochorismic acid (see page 124), but can arise in plants by two other mechanisms. It can be produced by hydroxylation of benzoic acid, or by side-chain cleavage of 2-coumaric acid, which itself is formed by an *ortho*-hydroxylation of cinnamic acid. **Methyl salicylate** is the principal component of oil of wintergreen from *Gaultheria procumbens* (Ericaceae), used for many years for pain relief. It is derived by

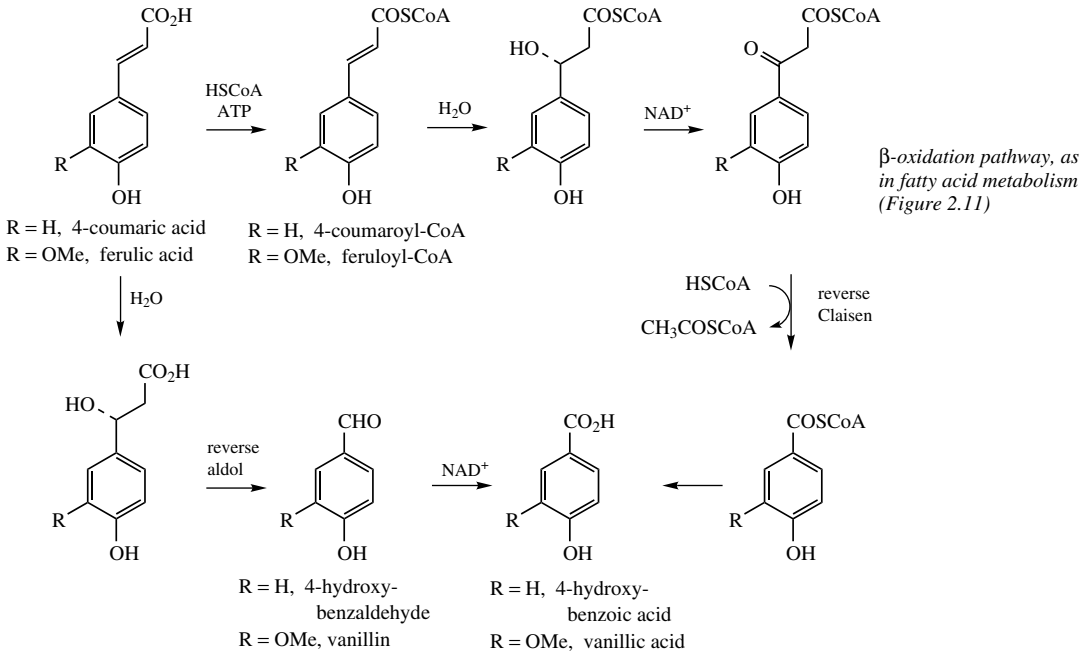

Figure 4.26

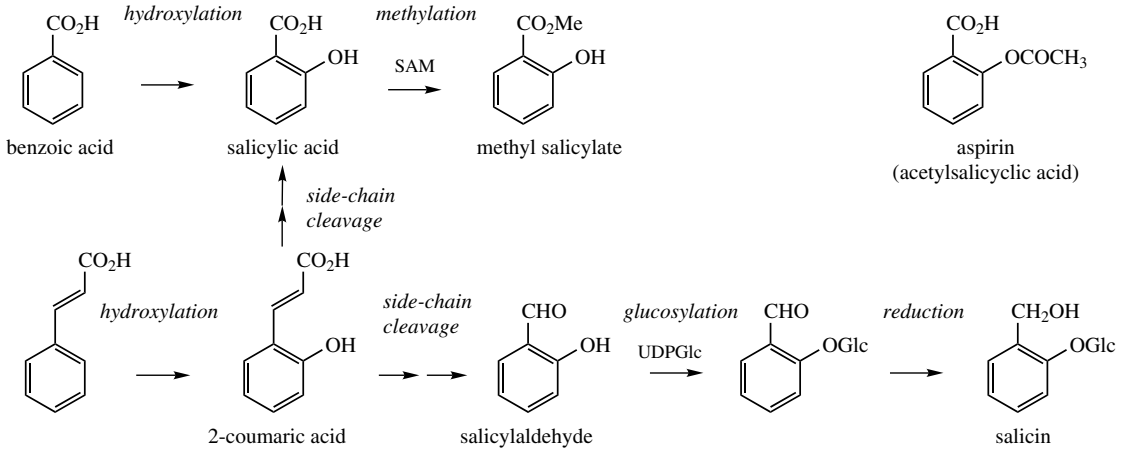

Figure 4.27

SAM-dependent methylation of salicylic acid. The salicyl alcohol derivative **salicin**, found in many species of willow (*Salix* species; Salicaceae), is not derived from salicylic acid, but probably via glucosylation of salicylaldehyde and then reduction of the carbonyl (Figure 4.27). Salicin is responsible for the analgesic and antipyretic effects of willow barks, widely used for centuries, and the template for synthesis of acetylsalicylic acid (**aspirin**) (Figure 4.27) as a more effective analogue.

COUMARINS

The hydroxylation of cinnamic acids *ortho* to the side-chain as seen in the biosynthesis of salicylic acid is a crucial step in the formation of a group of cinnamic acid lactone derivatives, the **coumarins**. Whilst the direct hydroxylation of the aromatic ring of the cinnamic acids is common, hydroxylation generally involves initially the 4-position *para* to the side-chain, and subsequent hydroxylations then proceed *ortho* to this substituent (see page 132). In contrast, for the coumarins, hydroxylation of cinnamic acid or 4-coumaric acid can occur *ortho* to the side-chain (Figure 4.28). In the latter case, the 2,4-dihydroxycinnamic acid produced confusingly seems to possess the *meta* hydroxylation pattern characteristic of phenols derived via the acetate pathway. Recognition of the C_6C_3 skeleton should help to avoid this confusion. The two 2-hydroxycinnamic acids then suffer a change in configuration in the side-chain, from the *trans* (*E*) to the less stable *cis* (*Z*) form. Whilst *trans–cis* isomerization would be unfavourable in the case of a single isolated double bond, in the cinnamic acids the fully conjugated system allows this process to occur quite readily, and UV irradiation, e.g. daylight, is sufficient to produce equilibrium mixtures which can be separated (Figure 4.29). The absorption of energy promotes an electron from the π-orbital to a higher energy state, the π^*-orbital, thus temporarily destroying the double bond character and allowing rotation. Loss of the absorbed energy then results in reformation of the double bond, but in the *cis*-configuration. In conjugated systems, the $\pi-\pi^*$ energy difference is considerably less than with a non-conjugated double bond. Chemical lactonization can occur on treatment with acid. Both the *trans–cis* isomerization and the lactonization are enzyme-mediated in nature, and light is not necessary for coumarin biosynthesis. Thus, cinnamic acid and 4-coumaric acid give rise to the coumarins **coumarin** and **umbelliferone** (Figure 4.28). Other coumarins with additional oxygen substituents on the aromatic ring, e.g. **aesculetin** and **scopoletin**, appear to be derived by modification of umbelliferone, rather than by a general cinnamic acid to coumarin pathway. This indicates that the hydroxylation *meta* to the existing hydroxyl, discussed above, is a rather uncommon occurrence.

Coumarins are widely distributed in plants, and are commonly found in families such as the Umbelliferae/Apiaceae and Rutaceae, both in the free form and as glycosides. **Coumarin** itself is

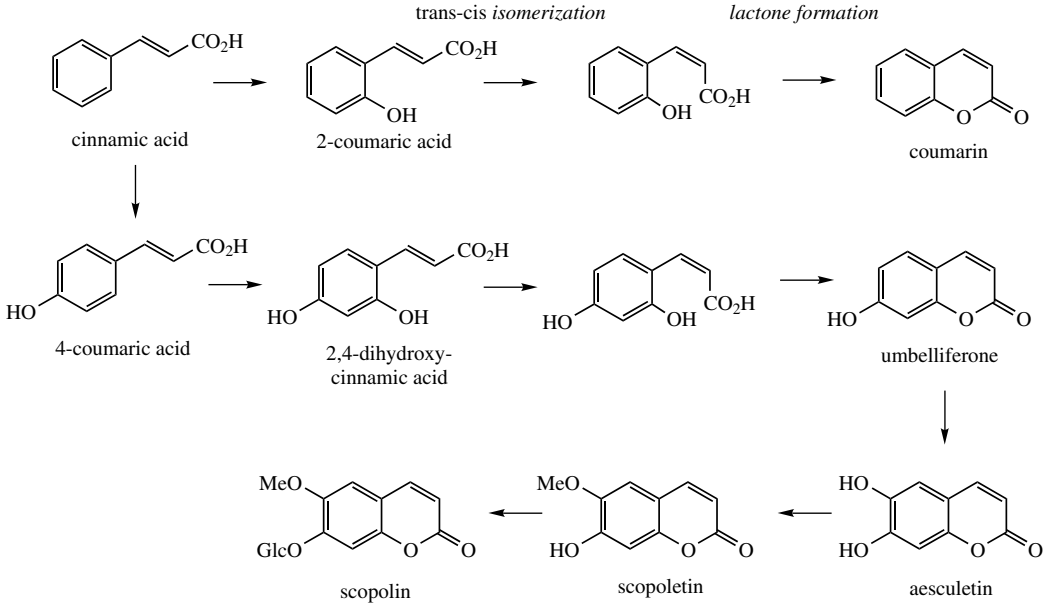

Figure 4.28

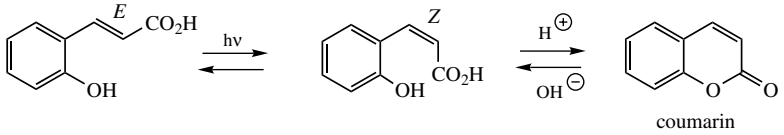

Figure 4.29

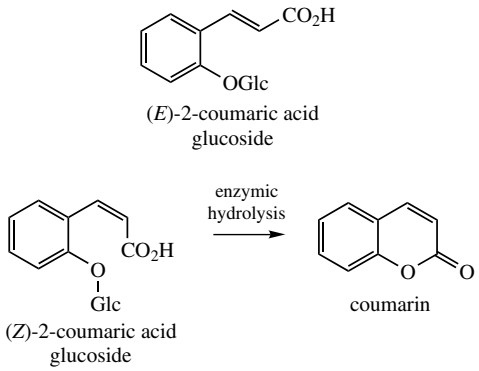

Figure 4.30

found in sweet clover (*Melilotus* species; Leguminosae/Fabaceae) and contributes to the smell of new-mown hay, though there is evidence that the plants actually contain the glucosides of (*E*)- and (*Z*)-2-coumaric acid (Figure 4.30), and coumarin is only liberated as a result of enzymic hydrolysis and lactonization through damage to the plant tissues during harvesting and processing (Figure 4.30). If sweet clover is allowed to ferment, 4-hydroxycoumarin is produced by the action of microorganisms on 2-coumaric acid (Figure 4.31) and this can react with formaldehyde, which is usually present due to microbial degradative reactions, combining to give **dicoumarol**. Dicoumarol* is a compound with pronounced blood anticoagulant properties, which can cause the deaths of livestock by internal bleeding, and is the forerunner of the warfarin* group of medicinal anticoagulants.

Many other natural coumarins have a more complex carbon framework and incorporate extra carbons derived from an isoprene unit (Figure 4.33). The aromatic ring in umbelliferone is activated at positions *ortho* to the hydroxyl group and can thus be alkylated by a suitable alkylating agent, in this case dimethylallyl diphosphate. The newly introduced dimethylallyl

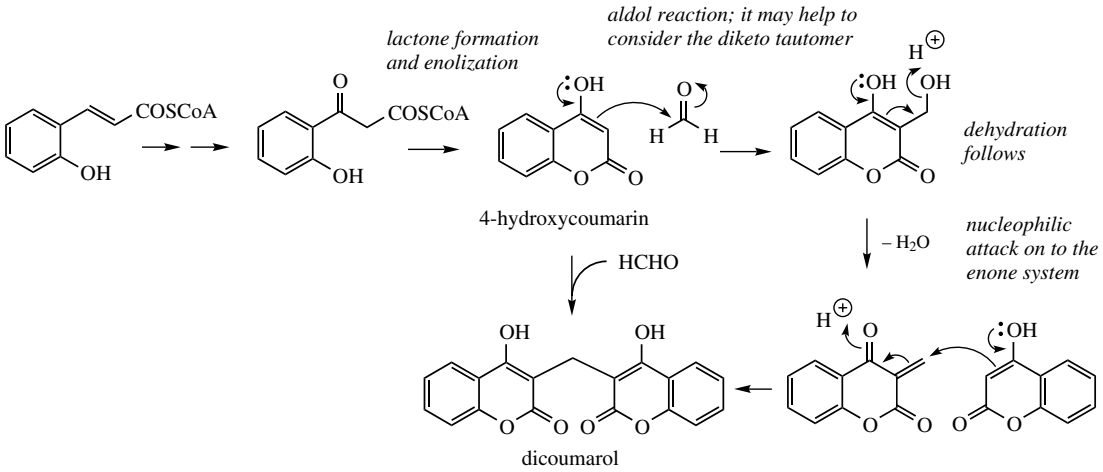

Figure 4.31

Dicoumarol and Warfarin

The cause of fatal haemorrhages in animals fed spoiled sweet clover (*Melilotus officinalis*; Leguminosae/Fabaceae) was traced to dicoumarol (bishydroxycoumarin) (Figure 4.31). This agent interferes with the effects of vitamin K in blood coagulation (see page 163), the blood loses its ability to clot, and thus minor injuries can lead to severe internal bleeding. Synthetic dicoumarol has been used as an oral blood anticoagulant in the treatment of thrombosis, where the risk of blood clots becomes life threatening. It has been superseded by salts of **warfarin** and **acenocoumarol** (**nicoumalone**) (Figure 4.32), which are synthetic developments from the natural product. An overdose of warfarin may be countered by injection of vitamin K_1.

Warfarin was initially developed as a rodenticide, and has been widely employed for many years as the first choice agent, particularly for destruction of rats. After

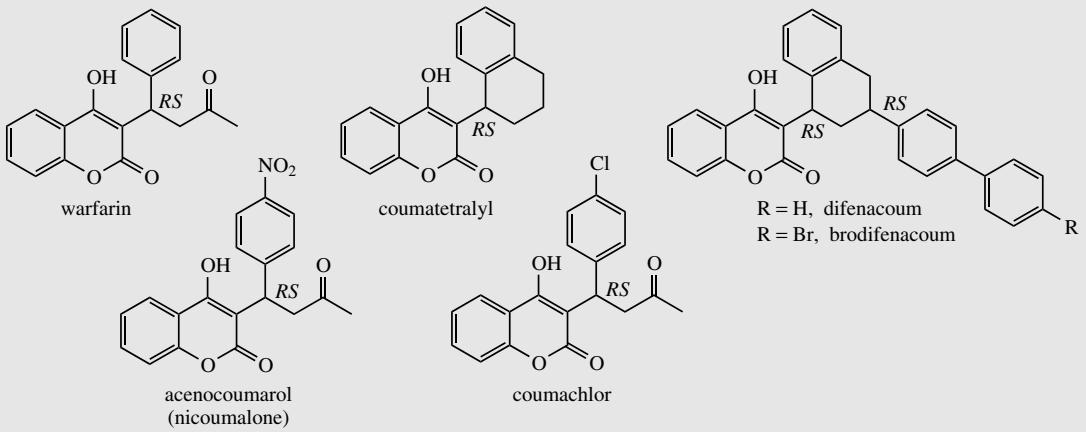

Figure 4.32

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consumption of warfarin-treated bait, rats die from internal haemorrhage. Other coumarin derivatives employed as rodenticides include **coumachlor** and **coumatetralyl** (Figure 4.32). In an increasing number of cases, rodents are becoming resistant to warfarin, an ability which has been traced to elevated production of vitamin K by their intestinal microflora. Modified structures **defenacoum** and **brodifenacoum** have been found to be more potent than warfarin, and are also effective against rodents that have become resistant to warfarin.

group in **demethylsuberosin** is then able to cyclize with the phenol group giving **marmesin**. This transformation is catalysed by a cytochrome P-450-dependent mono-oxygenase, and requires cofactors NADPH and molecular oxygen. For many years, the cyclization had been postulated to involve an intermediate epoxide, so that nucleophilic attack of the phenol on to the epoxide group might lead to formation of either five-membered furan or six-membered pyran heterocycles as commonly encountered in natural products (Figure 4.34). Although the reactions of Figure 4.34 offer a convenient rationalization for cyclization, epoxide intermediates have not been demonstrated in any of the enzymic systems so far investigated, and therefore some direct oxidative cyclization mechanism must operate. A second cytochrome P-450-dependent mono-oxygenase enzyme then cleaves off the hydroxyisopropyl fragment (as acetone) from **marmesin** giving the furocoumarin **psoralen** (Figure 4.35). This does not involve any hydroxylated intermediate, and cleavage is believed to be initiated by a radical abstraction process. Psoralen can act as a precursor for the further substituted furocoumarins **bergapten, xanthotoxin**, and **isopimpinellin** (Figure 4.33), such modifications occurring late in the biosynthetic sequence rather than at the cinnamic acid stage. Psoralen, bergapten, etc are termed 'linear' furocoumarins. 'Angular' furocoumarins, e.g. **angelicin** (Figure 4.33), can arise by a similar sequence of reactions, but these involve dimethylallylation at the alternative position *ortho* to the phenol. An isoprene-derived furan ring system has already been noted in the formation of khellin (see page 74), though the

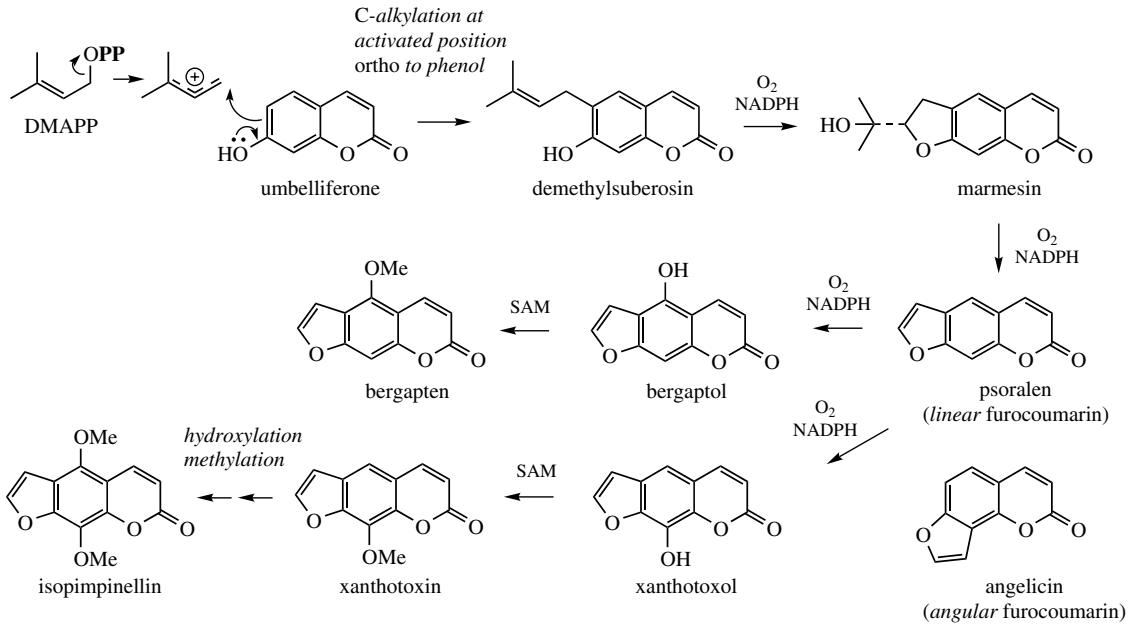

Figure 4.33

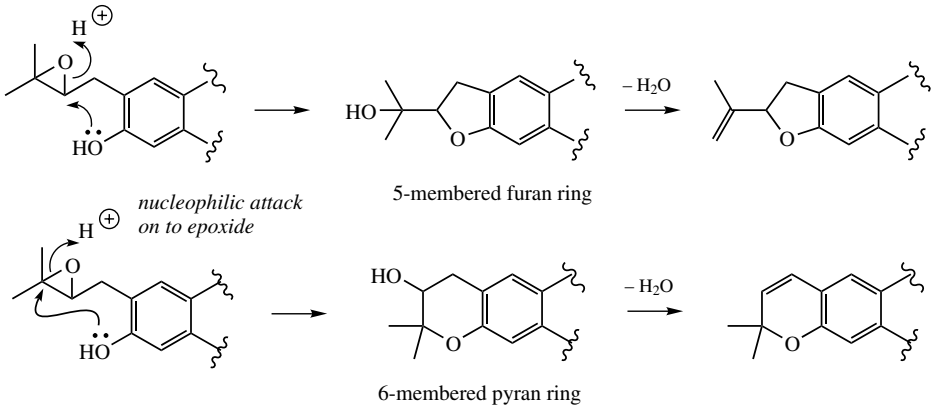

Figure 4.34

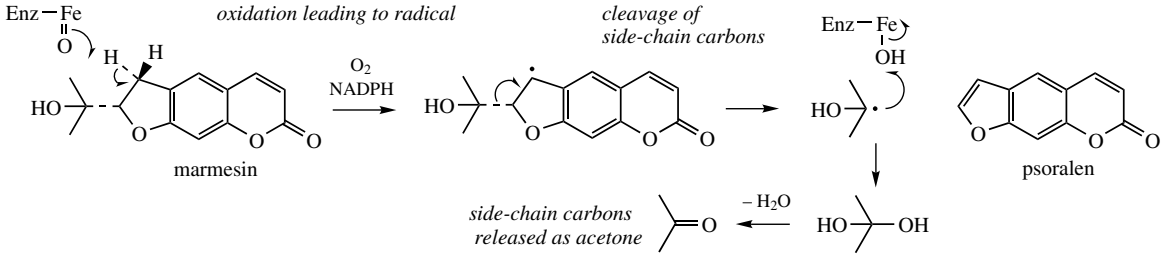

Figure 4.35

aromatic ring to which it was fused was in that case a product of the acetate pathway. Linear furocoumarins (**psoralens**)* can be troublesome to humans since they can cause photosensitization towards UV light, resulting in sunburn or serious blistering. Used medicinally, this effect may be valuable in promoting skin pigmentation and treating psoriasis.

Psoralens

Psoralens are linear furocoumarins which are widely distributed in plants, but are particularly abundant in the Umbelliferae/Apiaceae and Rutaceae. The most common examples are psoralen, bergapten, xanthotoxin, and isopimpinellin (Figure 4.33). Plants containing psoralens have been used internally and externally to promote skin pigmentation and sun-tanning. Bergamot oil obtained from the peel of *Citrus aurantium* ssp. *bergamia* (Rutaceae) (see page 179) can contain up to 5% bergapten, and is frequently used in external suntan preparations. The psoralen, because of its extended chromophore, absorbs in the near UV and allows this radiation to stimulate formation of melanin pigments (see page 129).

Methoxsalen (xanthotoxin; 8-methoxypsoralen) (Figure 4.36), a constituent of the fruits of *Ammi majus* (Umbelliferae/Apiaceae), is used medically to facilitate skin repigmentation where severe blemishes exist (vitiligo). An oral dose of methoxsalen is followed by long wave UV irradiation, though such treatments must be very carefully regulated to

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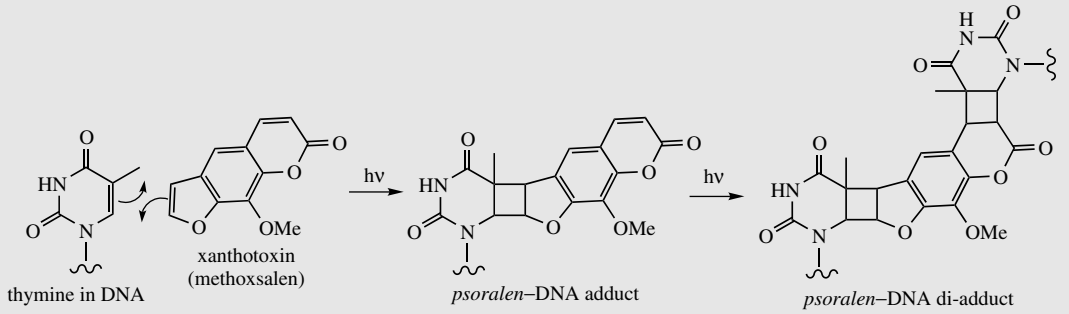


Figure 4.36

minimize the risk of burning, cataract formation, and the possibility of causing skin cancer. The treatment is often referred to as PUVA (psoralen + UV-A). PUVA is also of value in the treatment of psoriasis, a widespread condition characterized by proliferation of skin cells. Similarly, methoxsalen is taken orally, prior to UV treatment. Reaction with psoralens inhibits DNA replication and reduces the rate of cell division. Because of their planar nature, psoralens intercalate into DNA, and this enables a UV-initiated cycloaddition reaction between pyrimidine bases (primarily thymine) in DNA and the furan ring of psoralens (Figure 4.36). In some cases, di-adducts can form involving further cycloaddition via the pyrone ring, thus cross-linking the nucleic acid.

A troublesome extension of these effects can arise from the handling of plants that contain significant levels of furocoumarins. Celery (*Apium graveolens*; Umbelliferae/Apiaceae) is normally free of such compounds, but fungal infection with the natural parasite *Sclerotinia sclerotiorum* induces the synthesis of furocoumarins (xanthotoxin and others) as a response to the infections. Some field workers handling these infected plants have become very sensitive to UV light and suffer from a form of sunburn termed photophytodermatitis. Infected parsley (*Petroselinum crispum*) can give similar effects. Handling of rue (*Ruta graveolens*; Rutaceae) or giant hogweed (*Heracleum mantegazzianum*; Umbelliferae/Apiaceae), which naturally contain significant amounts of psoralen, bergapten, and xanthotoxin, can cause similar unpleasant reactions, or more commonly rapid blistering by direct contact with the sap. The giant hogweed can be particularly dangerous. Individuals vary in their sensitivity towards furocoumarins; some are unaffected whilst others tend to become sensitized by an initial exposure and then develop the allergic response on subsequent exposures.

STYRYLPYRONES

Cinnamic acids, as their coenzyme A esters, may also function as starter units for chain extension with malonyl-CoA units, thus combining elements of the shikimate and acetate pathways (see page 80). Most commonly, three C_2 units are added via malonate giving rise to flavonoids and stilbenes, as described in the next section (page 149). However, there are several examples of products formed from a cinnamoyl-CoA starter plus one or two C_2 units from malonyl-CoA. The short poly-β-keto chain frequently cyclizes to form a lactone derivative (compare triacetic acid lactone, page 62). Thus, Figure 4.37 shows the proposed derivation of **yangonin** via cyclization of the di-enol tautomer of the polyketide formed from 4-hydroxycinnamoyl-CoA and two malonyl-CoA extender units. Two methylation reactions complete the sequence. Yangonin and a series of related structures form the active principles of kava root (*Piper methysticum*; Piperaceae), a herbal remedy popular for its anxiolytic activity.

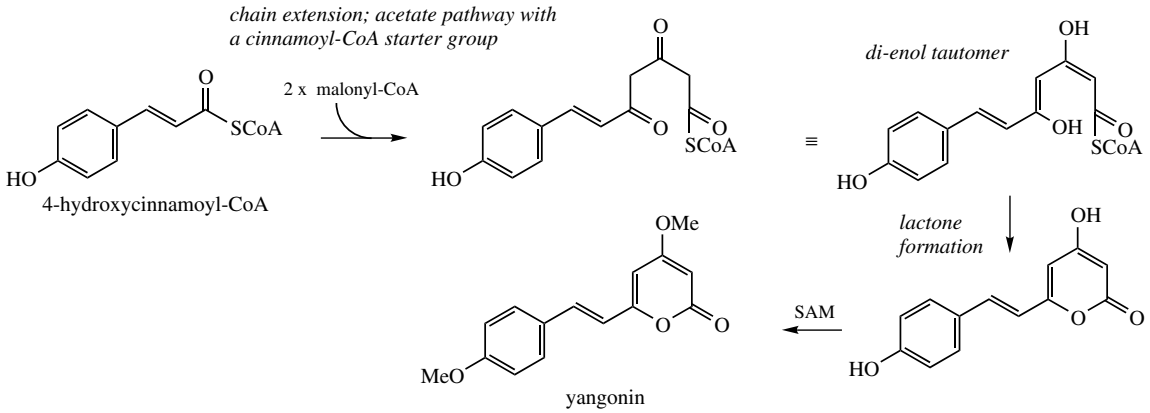


Figure 4.37

Kava

Aqueous extracts from the root and rhizome of *Piper methysticum* (Piperaceae) have long been consumed as an intoxicating beverage by the peoples of Pacific islands comprising Polynesia, Melanesia and Micronesia, and the name kava or kava-kava referred to this drink. In herbal medicine, the dried root and rhizome is now described as kava, and it is used for the treatment of anxiety, nervous tension, agitation and insomnia. The pharmacological activity is associated with a group of styrylpyrone derivatives termed kavapyrones or kavalactones, good quality roots containing 5–8% kavapyrones. At least 18 kavapyrones have been characterized, the six major ones being the enolides kawain, methysticin, and their dihydro derivatives reduced in the cinnamoyl side-chain, and the dienolides yangonin and demethoxyyangonin (Figure 4.38). Compared with the dienolides, the enolides have a reduced pyrone ring and a chiral centre. Clinical trials have indicated kava extracts to be effective as an anxiolytic, the kavapyrones also displaying anticonvulsive, analgesic, and central muscle relaxing action. Several of these compounds have been shown to have an effect on neurotransmitter systems including those involving glutamate, GABA, dopamine, and serotonin.

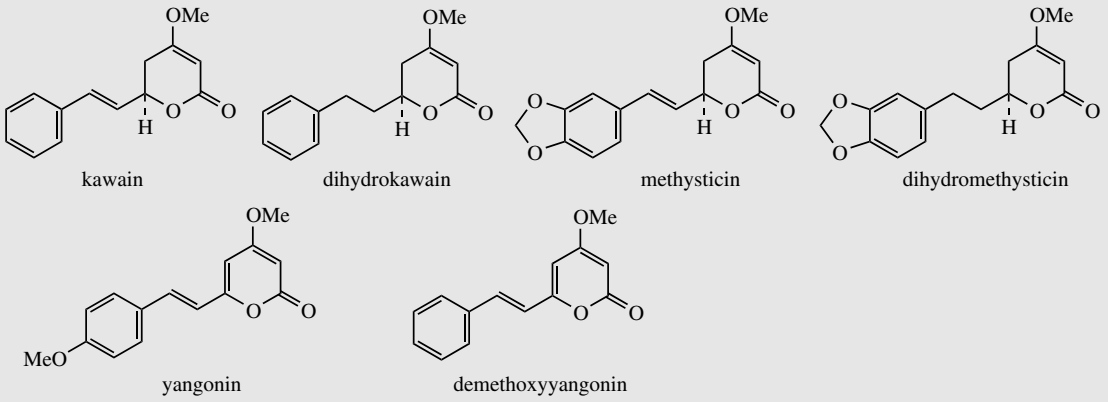


Figure 4.38

FLAVONOIDS AND STILBENES

Flavonoids and stilbenes are products from a cinnamoyl-CoA starter unit, with chain extension using three molecules of malonyl-CoA. This initially gives a polyketide (Figure 4.39), which, according to the nature of the enzyme responsible, can be folded in two different ways. These allow aldol or Claisen-like reactions to occur, generating aromatic rings as already seen in Chapter 3 (see page 80). Enzymes stilbene synthase and chalcone synthase couple a cinnamoyl-CoA unit with three malonyl-CoA units giving stilbenes, e.g. **resveratrol** or chalcones, e.g. **naringenin-chalcone** respectively. Both structures nicely illustrate the different characteristic oxygenation patterns in aromatic rings derived from the acetate or shikimate pathways. With the **stilbenes**, it is noted that the terminal ester function is no longer present, and therefore hydrolysis and decarboxylation have also taken place during this transformation. No intermediates, e.g. carboxylated stilbenes, have been detected, and the transformation from cinnamoyl-CoA/malonyl-CoA to stilbene is catalysed by the single enzyme. **Resveratrol** has assumed greater relevance in recent years as a constituent of grapes and wine, as well as other food products, with antioxidant, anti-inflammatory, anti-platelet, and cancer preventative properties. Coupled with

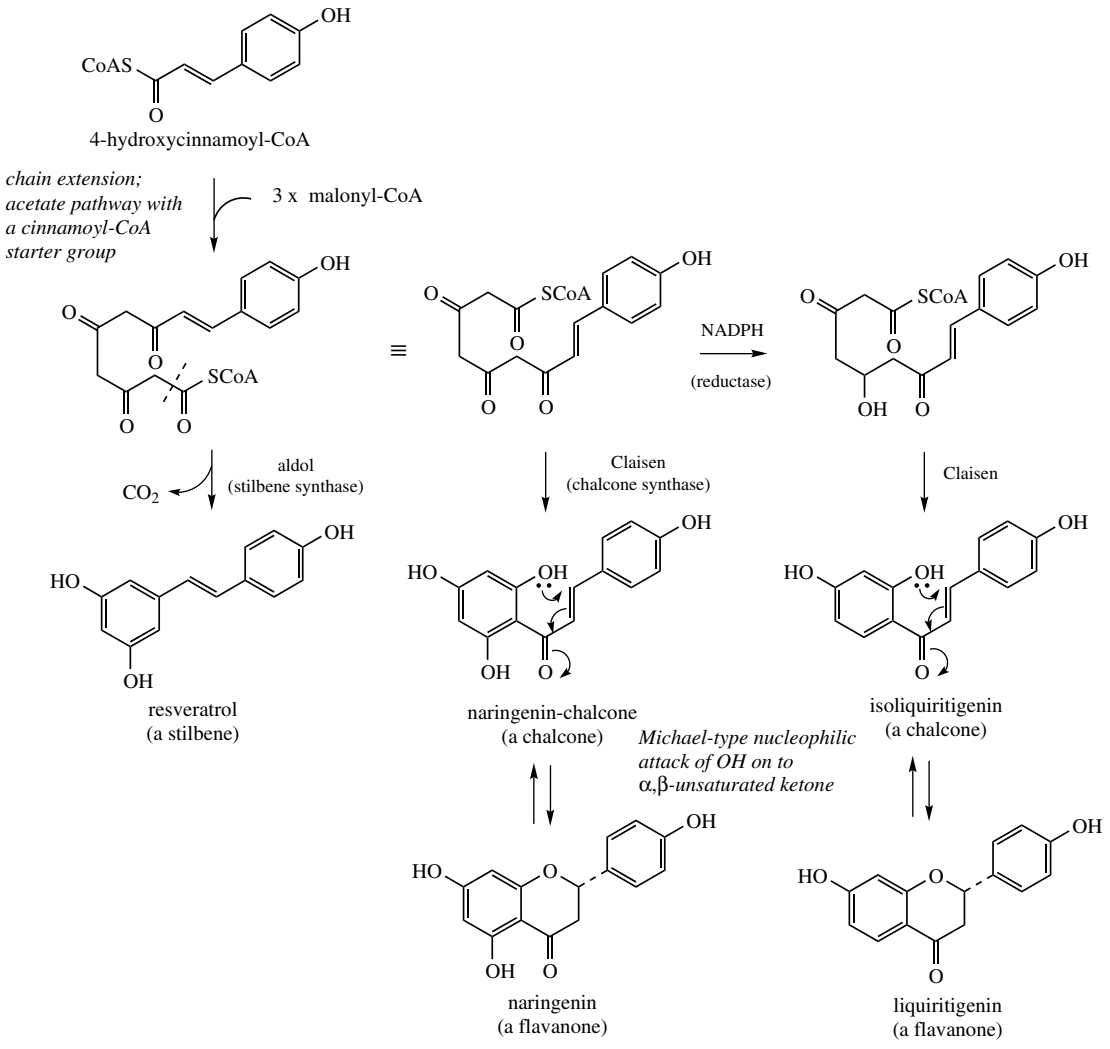


Figure 4.39

the cardiovascular benefits of moderate amounts of alcohol, and the beneficial antioxidant effects of flavonoids (see page 151), red wine has now emerged as an unlikely but most acceptable medicinal agent.

Chalcones act as precursors for a vast range of **flavonoid** derivatives found throughout the plant kingdom. Most contain a six-membered heterocyclic ring, formed by Michael-type nucleophilic attack of a phenol group on to the unsaturated ketone giving a **flavanone**, e.g. **naringenin** (Figure 4.39). This isomerization can occur chemically, acid conditions favouring the flavanone and basic conditions the chalcone, but in nature the reaction is enzyme catalysed and stereospecific, resulting in formation of a single flavanone enantiomer. Many flavonoid structures, e.g. **liquiritigenin**, have lost one of the hydroxyl groups, so that the acetate-derived aromatic ring has a resorcinol oxygenation pattern rather than the phloroglucinol system. This modification has been tracked down to the action of a reductase enzyme concomitant with the chalcone synthase, and thus **isoliquiritigenin** is produced rather than naringenin-chalcone. Flavanones can then give rise to many variants on this basic skeleton, e.g. **flavones, flavonols, anthocyanidins**, and **catechins** (Figure 4.40). Modifications to the hydroxylation patterns in the two aromatic rings may occur, generally at the flavanone or dihydroflavonol stage, and methylation, glycosylation, and dimethylallylation are also possible, increasing the range of compounds enormously. A high proportion of flavonoids occur naturally as water-soluble glycosides. Considerable quantities of flavonoids are consumed daily in our vegetable diet, so adverse biological effects on man are not particularly intense. Indeed, there is growing belief that some

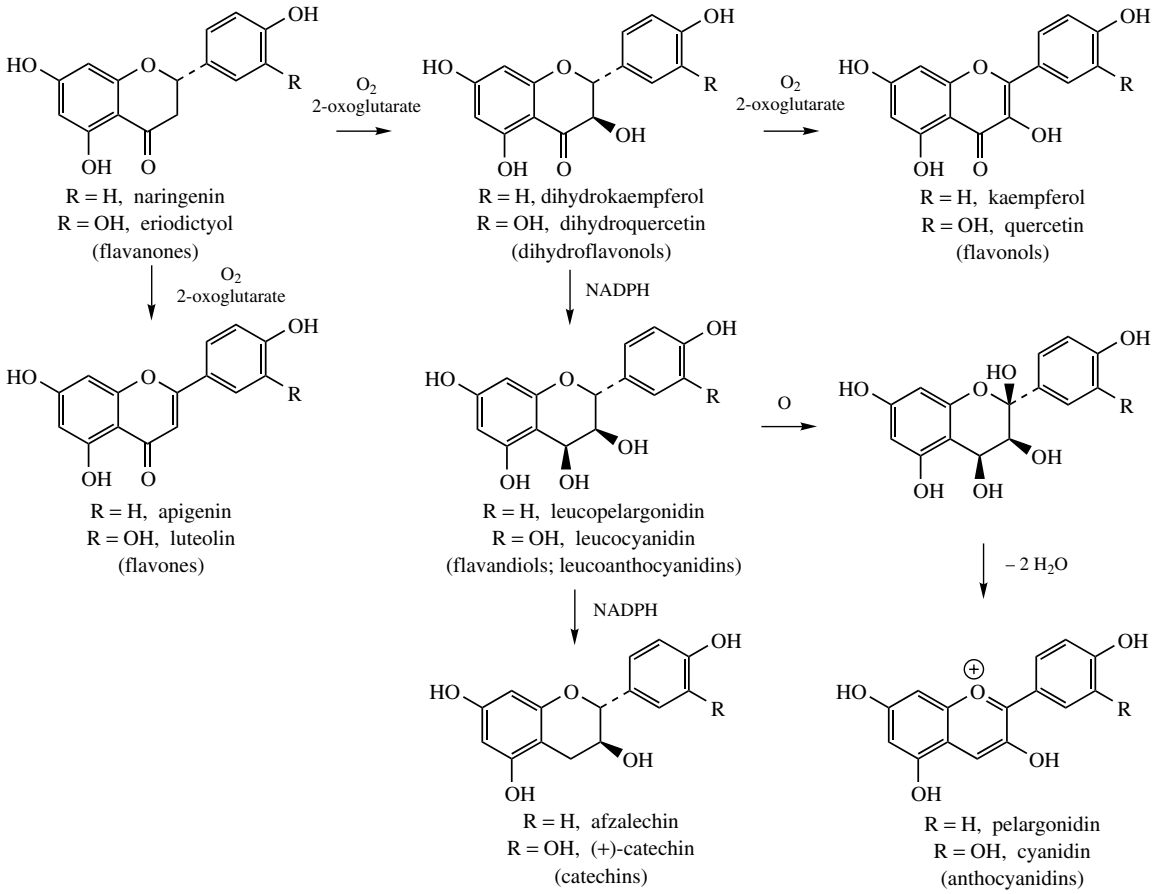
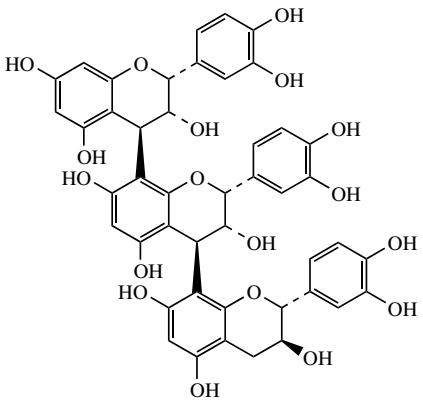


Figure 4.40



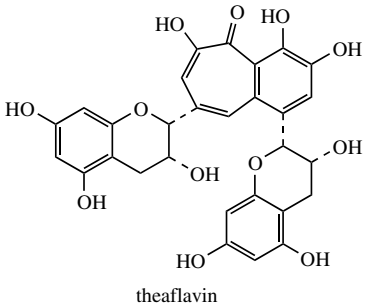


Figure 4.41

flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardiovascular disease, certain forms of cancer, and, it is claimed, age-related degeneration of cell components. Their polyphenolic nature enables them to scavenge injurious free radicals such as superoxide and hydroxyl radicals. **Quercetin** in particular is almost always present in substantial amounts in plant tissues, and is a powerful antioxidant, chelating metals, scavenging free radicals, and preventing oxidation of low density lipoprotein. Flavonoids in red wine (**quercetin, kaempferol**, and anthocyanidins) and in tea (**catechins** and catechin gallate esters) are also demonstrated to be effective antioxidants. Flavonoids contribute to plant colours, yellows from chalcones and flavonols, and reds, blues, and violets from anthocyanidins. Even the colourless materials, e.g. flavones, absorb strongly in the UV and are detectable by insects, probably aiding flower pollination. Catechins form small polymers (oligomers), the **condensed tannins**, e.g. the epicatechin trimer (Figure 4.41) which contribute astringency to our foods and drinks, as do the simpler gallotannins (see page 122), and are commercially important for tanning leather. **Theaflavins**, antioxidants found in fermented tea (see page 395), are dimeric catechin structures in which oxidative processes have led to formation of a seven-membered tropolone ring.

The flavonol glycoside **rutin** (Figure 4.42) from buckwheat (*Fagopyrum esculentum*; Polygonaceae) and rue (*Ruta graveolens*; Rutaceae), and the flavanone glycoside **hesperidin** from *Citrus* peels have been included in dietary supplements as vitamin P, and claimed to be of benefit in treating conditions characterized by capillary bleeding, but their therapeutic efficacy is far from conclusive. **Neohesperidin** (Figure 4.42) from bitter orange (*Citrus aurantium*; Rutaceae) and **naringin** from grapefruit peel (*Citrus paradisi*) are intensely bitter flavanone glycosides. It has been found that conversion of these compounds into **dihydrochalcones** by hydrogenation in alkaline solution (Figure 4.43) produces a remarkable change to their taste, and the products are now intensely sweet, being some 300–1000 times as sweet as sucrose. These and other dihydrochalcones have been investigated as non-sugar sweetening agents.

FLAVONOLIGNANS

An interesting combination of flavonoid and lignan structures is found in a group of compounds called **flavonolignans**. They arise by oxidative coupling processes between a flavonoid and a phenylpropanoid, usually coniferyl alcohol. Thus, the dihydroflavonol **taxifolin** through one-electron oxidation may provide a free radical, which may combine with the free radical generated from **coniferyl alcohol** (Figure 4.44). This would lead to an adduct, which could cyclize by attack of the phenol nucleophile on to the quinone methide system provided by coniferyl alcohol. The product would be **silybin**, found in *Silybum marianum* (Compositae/Asteraceae) as a mixture of two *trans* diastereoisomers, reflecting a lack of stereospecificity for the original radical coupling. In addition,

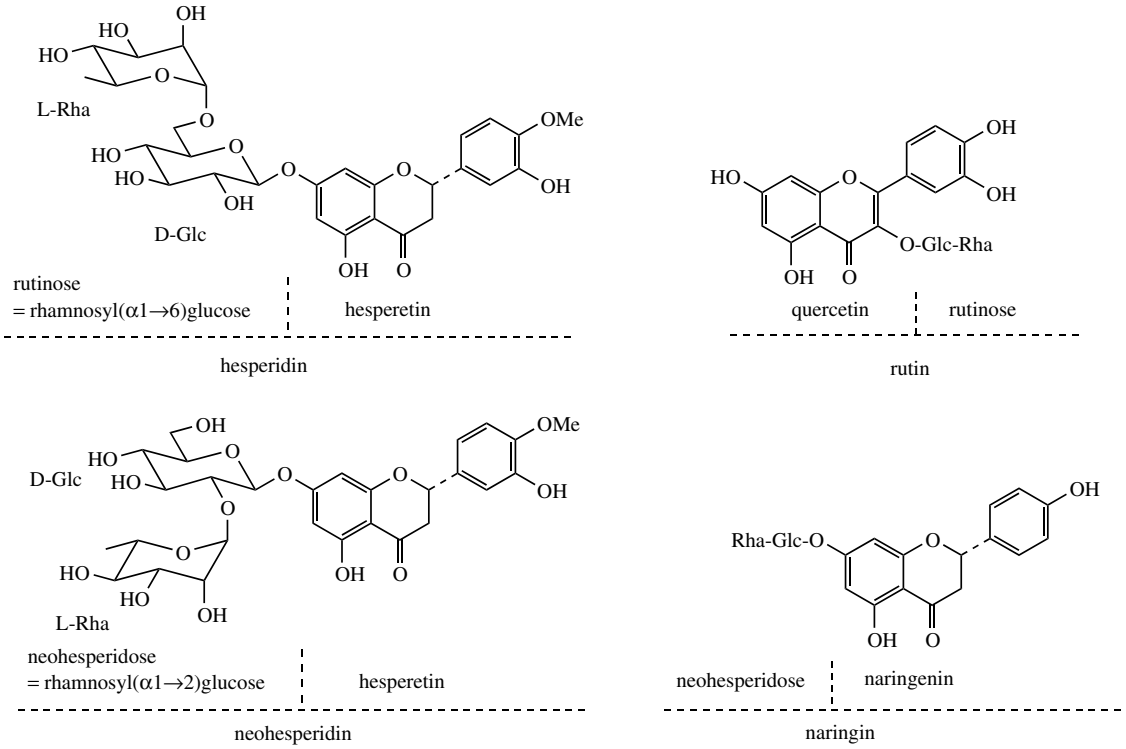


Figure 4.42

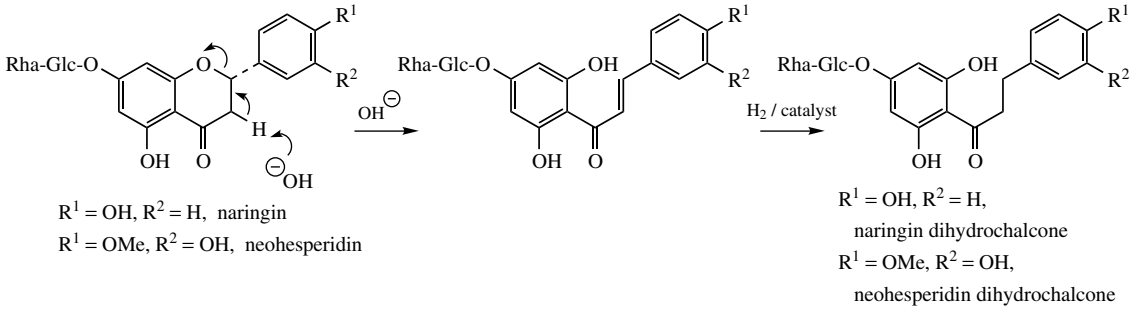


Figure 4.43

the regioisomer **isosilybin** (Figure 4.45), again a mixture of *trans* diastereoisomers, is also found in *Silybum*. **Silychristin** (Figure 4.45) demonstrates a further structural variant which can be seen to originate from a mesomer of the taxifolin-derived free radical, in which the unpaired electron is localized on the carbon *ortho* to the original 4-hydroxyl function. The more complex structure in **silydianin** is accounted for by the mechanism shown in Figure 4.46, in which the initial coupling product cyclizes further by intramolecular attack of an enolate nucleophile on to the quinonemethide. Hemiketal formation finishes the process. The flavonolignans from *Silybum** (milk thistle) have valuable antihepatotoxic properties, and can provide protection against liver-damaging agents. Coumarinolignans, which are products arising by a similar oxidative coupling mechanism which combines a coumarin with a cinnamyl alcohol, may be found in other plants. The benzodioxane ring as seen in silybin and isosilybin is a characteristic feature of many such compounds.

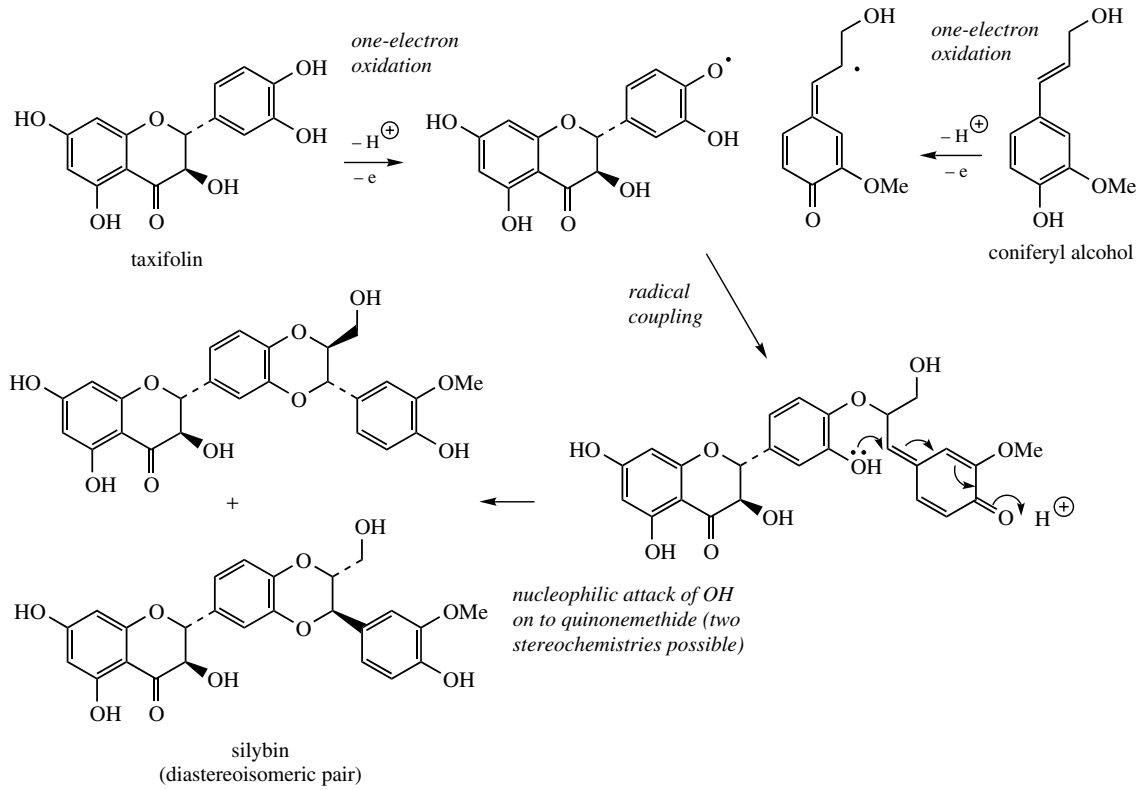


Figure 4.44

Silybum marianum

Silybum marianum (Compositae/Asteraceae) is a biennial thistle-like plant (milk thistle) common in the Mediterranean area of Europe. The seeds yield 1.5–3% of flavonolignans collectively termed silymarin. This mixture contains mainly silybin (Figure 4.44), together with silychristin (Figure 4.45), silydianin (Figure 4.46), and small amounts of isosilybin (Figure 4.45). Both silybin and isosilybin are equimolar mixtures of two *trans* diastereoisomers. *Silybum marianum* is widely used in traditional European medicine, the fruits being used to treat a variety of hepatic and other disorders. Silymarin has been shown to protect animal livers against the damaging effects of carbon tetrachloride, thioacetamide, drugs such as paracetamol, and the toxins α-amanitin and phalloin found in the death cap fungus (*Amanita phalloides*) (see page 433). Silymarin may be used in many cases of liver disease and injury, though it still remains peripheral to mainstream medicine. It can offer particular benefit in the treatment of poisoning by the death cap fungus. These agents appear to have two main modes of action. They act on the cellular membrane of hepatocytes inhibiting absorption of toxins, and secondly, because of their phenolic nature, they can act as antioxidants and scavengers for free radicals which cause liver damage originating from liver detoxification of foreign chemicals. Derivatives of silybin with improved water-solubility and/or bioavailability have been developed, e.g. the bis-hemisuccinate and a phosphatidylcholine complex.

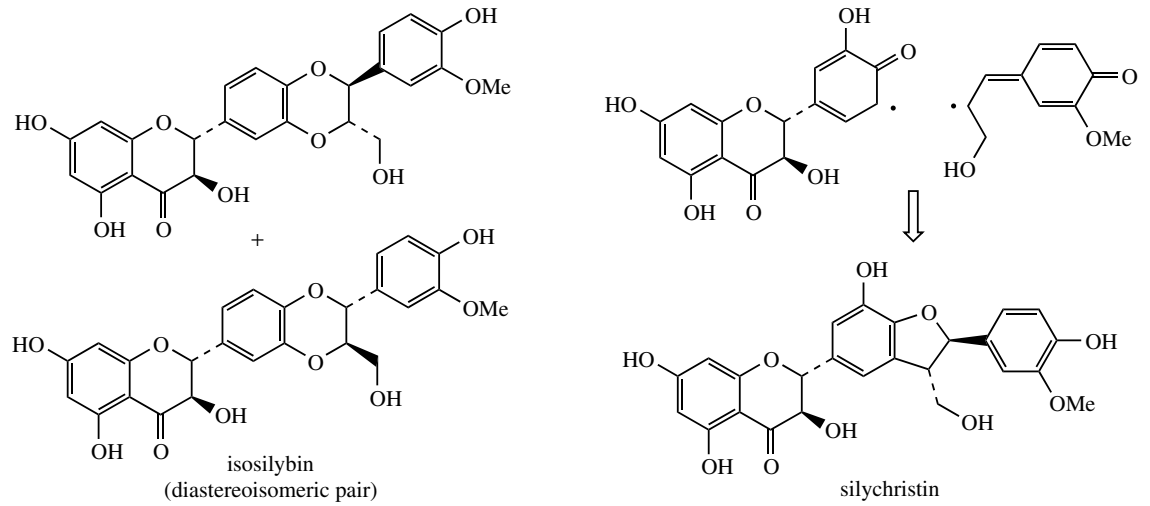


Figure 4.45

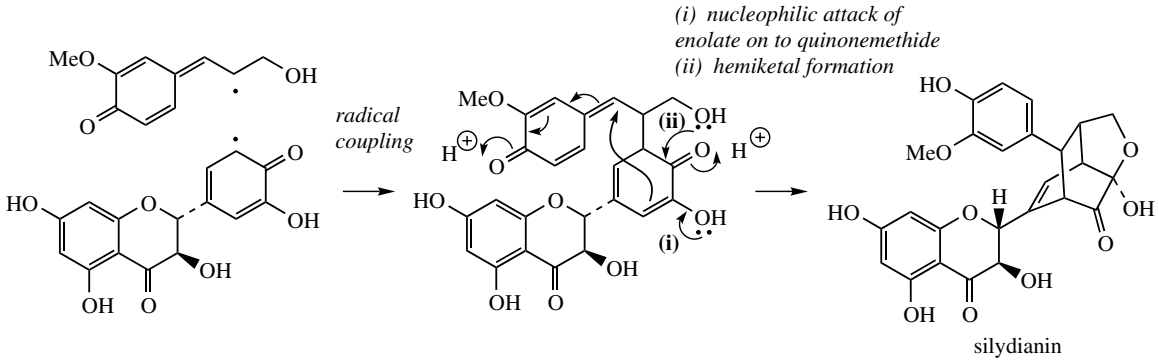


Figure 4.46

ISOFLAVONOIDS

The **isoflavonoids** form a quite distinct subclass of flavonoid compound, being structural variants in which the shikimate-derived aromatic ring has migrated to the adjacent carbon of the heterocycle. This rearrangement process is brought about by a cytochrome P-450-dependent enzyme requiring NADPH and O_2 cofactors, which transforms the flavanones **liquiritigenin** or **naringenin** into the isoflavones **daidzein** or **genistein** respectively via intermediate hydroxyisoflavanones (Figure 4.47). A radical mechanism has been proposed. This rearrangement is quite rare in nature, and isoflavonoids are almost entirely restricted to the plant family the Leguminosae/Fabaceae. Nevertheless, many hundreds of different isoflavonoids have been identified, and structural complexity is brought about by hydroxylation and alkylation reactions, varying the oxidation level of the heterocyclic ring, or forming additional heterocyclic rings. Some of the many variants are shown in Figure 4.48. **Pterocarpans**, e.g. **medicarpin** from lucerne (*Medicago sativa*), and **pisatin** from pea (*Pisum sativum*), have antifungal activity and form part of these plants' natural defence mechanism against fungal attack. Simple **isoflavones** such as **daidzein** and **coumestans** such as **coumestrol** from lucerne and clovers (*Trifolium* species), have sufficient oestrogenic activity to seriously affect the reproduction of grazing animals, and are termed **phyto-oestrogens***. These planar molecules undoubtedly mimic the shape and polarity of the steroid hormone estradiol

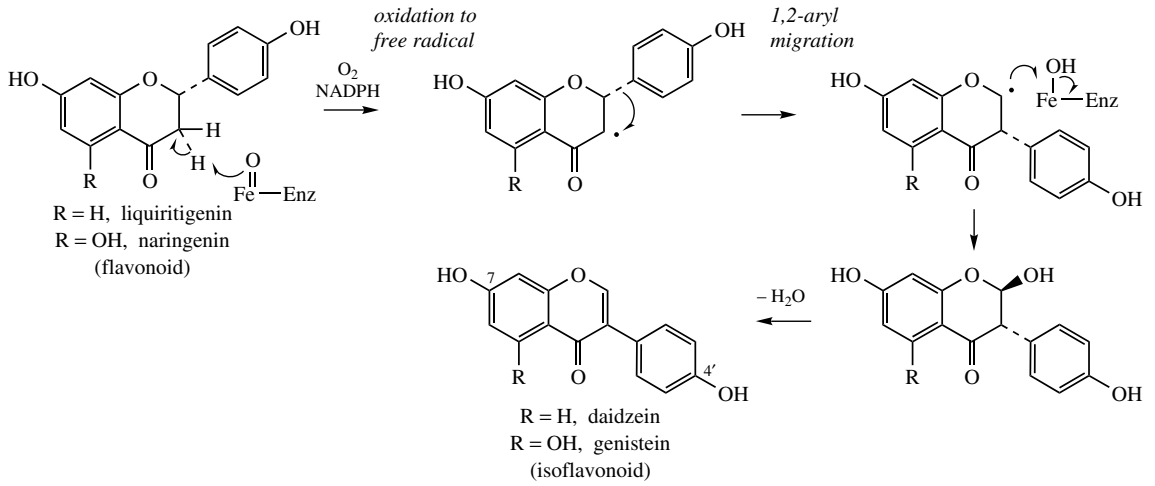


Figure 4.47

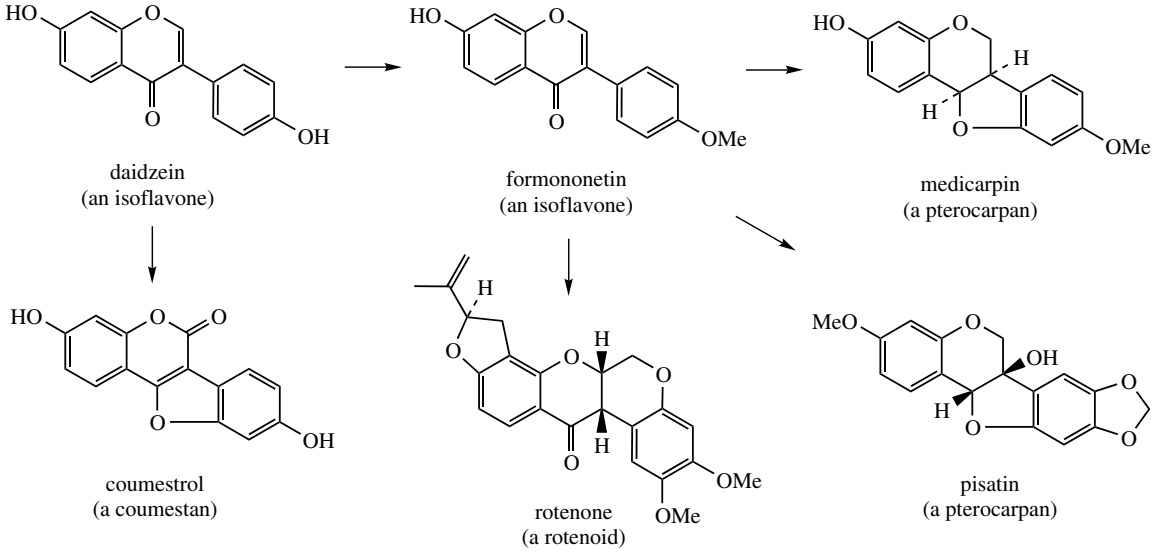


Figure 4.48

(see page 276). The consumption of legume fodder crops by animals must therefore be restricted, or low isoflavonoid producing strains have to be selected. Isoflavonoids in the human diet, e.g. from soya (*Glycine max*) products, are believed to give some protection against oestrogen-dependent cancers such as breast cancer, by restricting the availability of the natural hormone. In addition, they can feature as dietary oestrogen supplements in the reduction of menopausal symptoms, in a similar way to hormone replacement therapy (see page 279). The **rotenoids** take their name from the first known example **rotenone**, and are formed by ring cyclization of a methoxyisoflavone (Figure 4.49). Rotenone itself contains a C_5 isoprene unit (as do virtually all the natural rotenoids) introduced via dimethylallylation of **demethylmunduserone**. The isopropenylfurano system of rotenone, and the dimethylpyrano of **deguelin**, are formed via rotenonic acid (Figure 4.49) without any detectable epoxide or hydroxy intermediates (compare furocoumarins, page 145). Rotenone

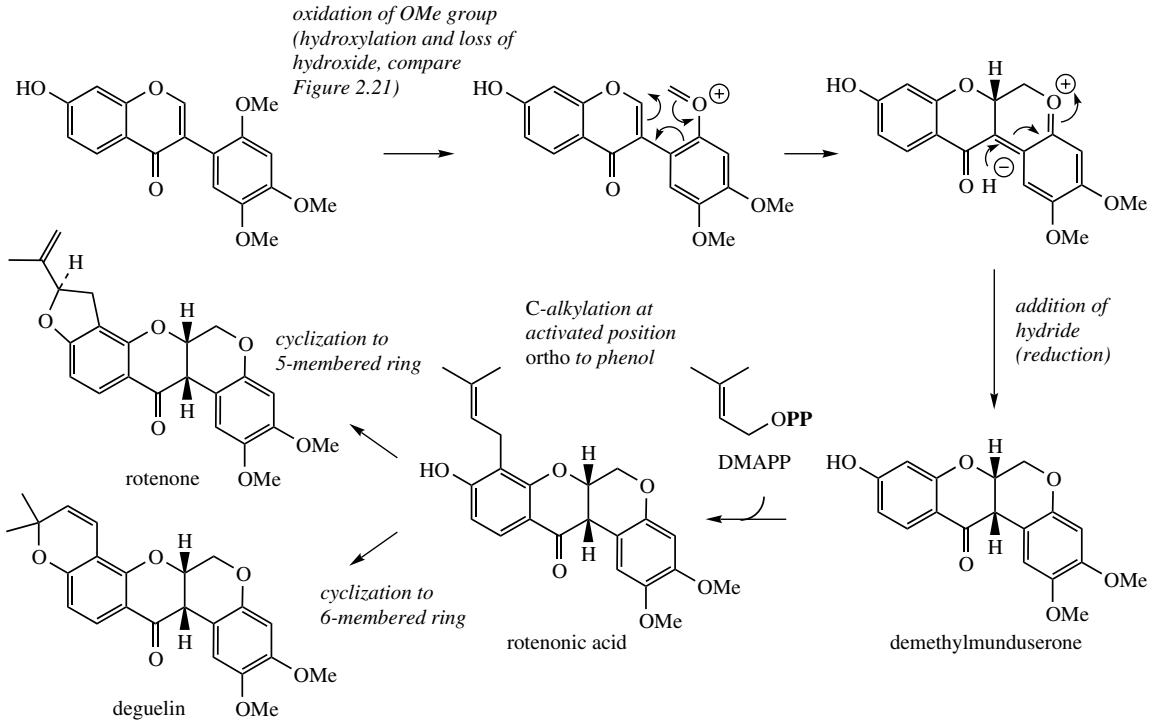


Figure 4.49

and other rotenoids are powerful insecticidal and piscicidal (fish poison) agents, interfering with oxidative phosphorylation. They are relatively harmless to mammals unless they enter the blood stream, being metabolized rapidly upon ingestion. Rotenone thus provides an excellent biodegradable insecticide, and is used as such either in pure or powdered plant form. Roots of *Derris elliptica** or *Lonchocarpus** species are rich sources of rotenone.

Phyto-oestrogens

Phyto-oestrogen (phytoestrogen) is a term applied to non-steroidal plant materials displaying oestrogenic properties. Pre-eminent amongst these are isoflavonoids. These planar molecules mimic the shape and polarity of the steroid hormone estradiol (see page 279), and are able to bind to an oestrogen receptor, though their activity is less than that of estradiol. In some tissues, they stimulate an oestrogenic response, whilst in others they can antagonize the effect of oestrogens. Such materials taken as part of the diet therefore influence overall oestrogenic activity in the body by adding their effects to normal levels of steroidal oestrogens (see page 282). Foods rich in isoflavonoids are valuable in countering some of the side-effects of the menopause in women, such as hot flushes, tiredness, and mood swings. In addition, there is mounting evidence that phyto-oestrogens also provide a range of other beneficial effects, helping to prevent heart attacks and other cardiovascular diseases, protecting against osteoporosis, lessening the risk of breast and uterine cancer, and in addition displaying significant antioxidant activity which may reduce the risk of Alzheimer's disease. Whilst some of these benefits may be obtained by the use of steroidal

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oestrogens, particularly via hormone replacement therapy (HRT; see page 279), phyto-oestrogens offer a dietary alternative.

The main food source of isoflavonoids is the soya bean (*Glycine max*; Leguminosae/Fabaceae) (see also page 256), which contains significant levels of the isoflavones daidzein, and genistein (Figure 4.47), in free form and as their 7-*O*-glucosides. Total isoflavone levels fall in the range 0.1–0.4%, according to variety. Soya products such as soya milk, soya flour, tofu, and soya-based textured vegetable protein may all be used in the diet for their isoflavonoid content. Breads in which wheat flour is replaced by soya flour are also popular. Extracts from red clover (*Trifolium pratense*; Leguminosae/Fabaceae) are also used as a dietary supplement. Red clover isoflavones are predominantly formononetin (Figure 4.48) and daidzein, together with their 7-*O*-glucosides.

The lignans enterodiol and enterolactone (see page 135) are also regarded as phyto-oestrogens. These compounds are produced by the action of intestinal microflora on lignans such as secoisolariciresinol or matairesinol ingested in the diet. A particularly important precursor is secoisolariciresinol diglucoside from flaxseed (*Linum usitatissimum*; Linaceae), and flaxseed may be incorporated into foodstuffs along with soya products. Enterolactone and enterodiol were first detected in human urine, and their origins were traced back to dietary fibre-rich foods. Levels in the urine were much higher in vegetarians, and have been related to a lower incidence of breast cancer in vegetarians.

Derris and Lonchocarpus

Species of *Derris* (e.g. *D. elliptica*, *D. malaccensis*) and *Lonchocarpus* (e.g. *L. utilis, L. urucu*) (Leguminosae/Fabaceae) have provided useful insecticides for many years. Roots of these plants have been employed as a dusting powder, or extracts have been formulated for sprays. *Derris* plants are small shrubs cultivated in Malaysia and Indonesia, whilst *Lonchocarpus* includes shrubs and trees, with commercial material coming from Peru and Brazil. The insecticidal principles are usually supplied as a black, resinous extract. Both *Derris* and *Lonchocarpus* roots contain 3–10% of rotenone (Figure 4.49) and smaller amounts of other rotenoids, e.g. deguelin (Figure 4.49). The resin may contain rotenone (about 45%) and deguelin (about 20%).

Rotenone and other rotenoids interfere with oxidative phosphorylation, blocking transfer of electrons to ubiquinone (see page 159) by complexing with NADH:ubiquinone oxidoreductase of the respiratory electron transport chain. However, they are relatively innocuous to mammals unless they enter the blood stream, being metabolized rapidly upon ingestion. Insects and also fish seem to lack this rapid detoxification. The fish poison effect has been exploited for centuries in a number of tropical countries, allowing lazy fishing by the scattering of powdered plant material on the water. The dead fish were collected, and when subsequently eaten produced no ill effects on the consumers. More recently, rotenoids have been used in fish management programmes to eradicate undesirable fish species prior to restocking with other species. As insecticides, the rotenoids still find modest use, and are valuable for their selectivity and rapid biodegradability. However, they are perhaps inactivated too rapidly in the presence of light and air to compete effectively with other insecticides such as the modern pyrethrin derivatives (see page 188).

TERPENOID QUINONES

Quinones are potentially derivable by oxidation of suitable phenolic compounds, catechols (1,2-dihydroxybenzenes) giving rise to *ortho*-quinones and quinols (1,4-dihydroxybenzenes) yielding *para*-quinones (see page 25). Accordingly, quinones can be formed from phenolic systems generated by either the acetate or shikimate pathways, provided a catechol or quinol

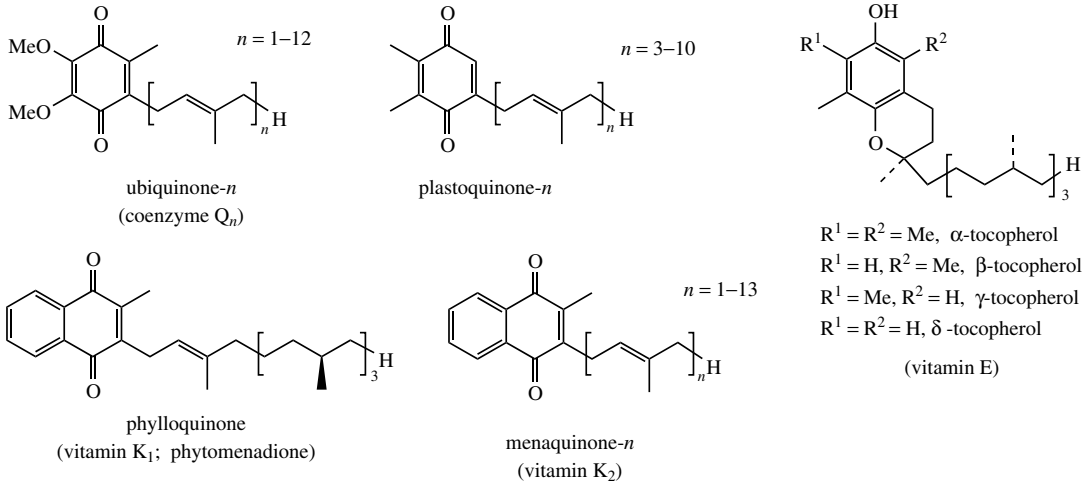

Figure 4.50

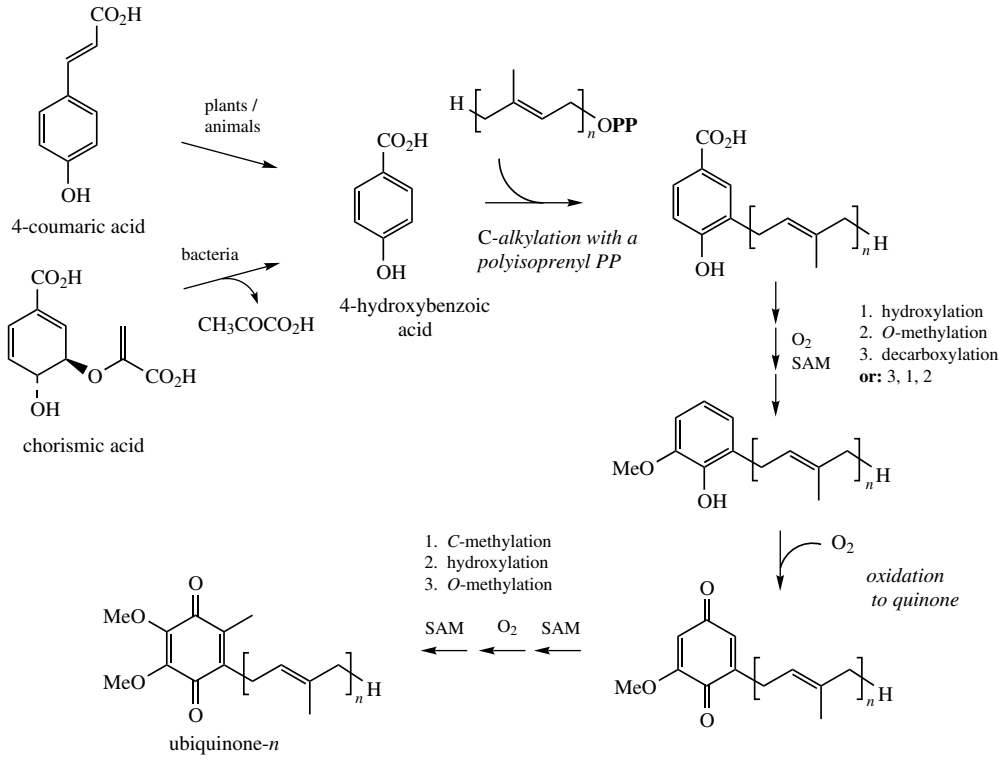

Figure 4.51

system has been elaborated, and many examples are found in nature. A range of quinone derivatives and related structures containing a terpenoid fragment as well as a shikimate-derived portion are also widely distributed. Many of these have important biochemical functions in electron transport systems for respiration or photosynthesis, and some examples are shown in Figure 4.50.

Ubiquinones (coenzyme Q) (Figure 4.50) are found in almost all organisms and function as electron carriers for the electron transport chain in mitochondria. The length of the terpenoid chain is variable ($n = 1-12$), and dependent on species, but most organisms synthesize a range of compounds, of which those where $n = 7-10$ usually predominate. The human redox carrier is coenzyme Q_{10}. They are derived from **4-hydroxybenzoic acid** (Figure 4.51), though the origin of this compound varies according to organism (see pages 123, 141). Thus, bacteria are known to transform chorismic acid by enzymic elimination of pyruvic acid, whereas plants and animals utilize a route from phenylalanine or tyrosine via 4-hydroxycinnamic acid (Figure 4.51). 4-Hydroxybenzoic acid is the substrate for *C*-alkylation *ortho* to the phenol group with a polyisoprenyl diphosphate of appropriate chain length (see page 231). The product then undergoes further elaboration, the exact sequence of modifications, i.e. hydroxylation, *O*-methylation, and decarboxylation, varying in eukaryotes and prokaryotes. Quinone formation follows in an O_2-dependent combined hydroxylation–oxidation process, and ubiquinone production then involves further hydroxylation, and *O*- and *C*-methylation reactions.

Plastoquinones (Figure 4.50) bear considerable structural similarity to ubiquinones, but are not derived from 4-hydroxybenzoic acid. Instead, they are produced from **homogentisic acid**, a phenylacetic acid derivative formed from **4-hydroxyphenylpyruvic acid** by a complex reaction involving decarboxylation, O_2-dependent hydroxylation, and subsequent migration of the $-CH_2CO_2H$ side-chain to the adjacent position on the aromatic ring (Figure 4.52). *C*-Alkylation of homogentisic acid *ortho* to a phenol group follows, and involves a polyisoprenyl diphosphate with $n = 3-10$, but most commonly with $n = 9$, i.e. **solanesyl diphosphate**. However, during the alkylation reaction, the $-CH_2CO_2H$ side-chain of homogentisic acid suffers decarboxylation, and the product is thus an alkyl methyl *p*-quinol derivative. Further aromatic methylation (via *S*-adenosylmethionine) and oxidation of the *p*-quinol to a quinone follow to yield the plastoquinone. Thus, only one of the two methyl groups on the quinone ring of the plastoquinone is derived from SAM. Plastoquinones are involved in the photosynthetic electron transport chain in plants.

Tocopherols are also frequently found in the chloroplasts and constitute members of the vitamin E* group. Their biosynthesis shares many of the features of plastoquinone biosynthesis, with an additional cyclization reaction involving the *p*-quinol and the terpenoid side-chain to give a chroman ring (Figure 4.52). Thus, the tocopherols, e.g. **α-tocopherol** and **γ-tocopherol**, are not in fact quinones, but are indeed structurally related to plastoquinones. The isoprenoid side-chain added, from **phytyl diphosphate**, contains only four isoprene units, and three of the expected double bonds have suffered reduction. Again, decarboxylation of homogentisic acid cooccurs with the alkylation reaction. *C*-Methylation steps using SAM, and the cyclization of the *p*-quinol to γ-tocopherol, have been established as in Figure 4.52. Note once again that one of the nuclear methyls is homogentisate-derived, whilst the others are supplied by SAM.

The **phylloquinones** (vitamin K_1) and **menaquinones** (vitamin K_2) are shikimate-derived naphthoquinone derivatives found in plants and algae (vitamin K_1*) or bacteria and fungi (vitamin K_2). The most common phylloquinone structure (Figure 4.50) has a diterpenoid side-chain, whereas the range of menaquinone structures tends to be rather wider with 1–13 isoprene units. These quinones are derived from chorismic acid via its isomer **isochorismic acid** (Figure 4.55). Additional carbons for the naphthoquinone skeleton are provided by 2-oxoglutaric acid, which is incorporated by a mechanism involving the coenzyme thiamine diphosphate (TPP). 2-Oxoglutaric acid is decarboxylated in the presence of TPP to give the TPP anion of succinic semialdehyde, which attacks isochorismic acid in a Michael-type reaction. Loss of the thiamine cofactor, elimination of pyruvic acid, and then dehydration yield the intermediate ***o*-succinylbenzoic acid** (OSB). This is activated by formation of a coenzyme A ester, and a Dieckmann-like condensation allows ring formation. The dihydroxynaphthoic acid is the

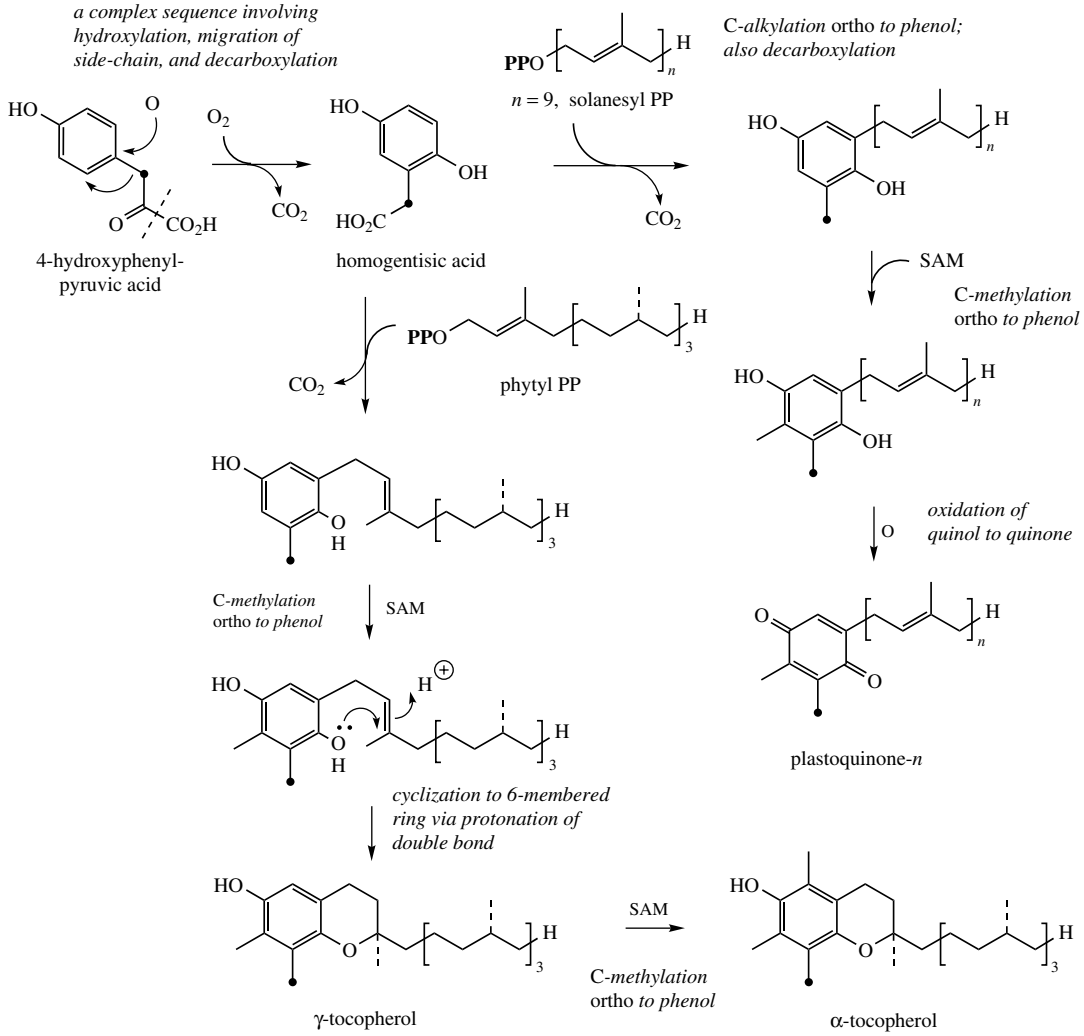

Figure 4.52

Vitamin E

Vitamin E refers to a group of fat-soluble vitamins, the tocopherols, e.g. α-, β-, γ-, and δ-tocopherols (Figure 4.53), which are widely distributed in plants, with high levels in cereal seeds such as wheat, barley, and rye. Wheat germ oil is a particularly good source. The proportions of the individual tocopherols vary widely in different seed oils, e.g. principally β- in wheat oil, γ- in corn oil, α- in safflower oil, and γ- and δ- in soybean oil. Vitamin E deficiency is virtually unknown, with most of the dietary intake coming from food oils and margarine, though much can be lost during processing and cooking. Rats deprived of the vitamin display reproductive abnormalities. α-Tocopherol has the highest activity (100%), with the relative activities of β-, γ-, and δ-tocopherols being 50%, 10%, and 3% respectively. **α-Tocopheryl acetate** is the main commercial form used for food supplementation and

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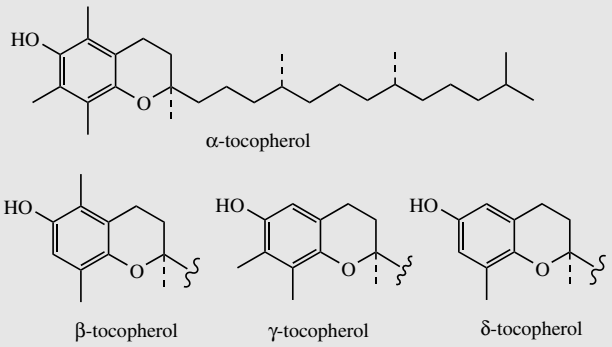

Figure 4.53

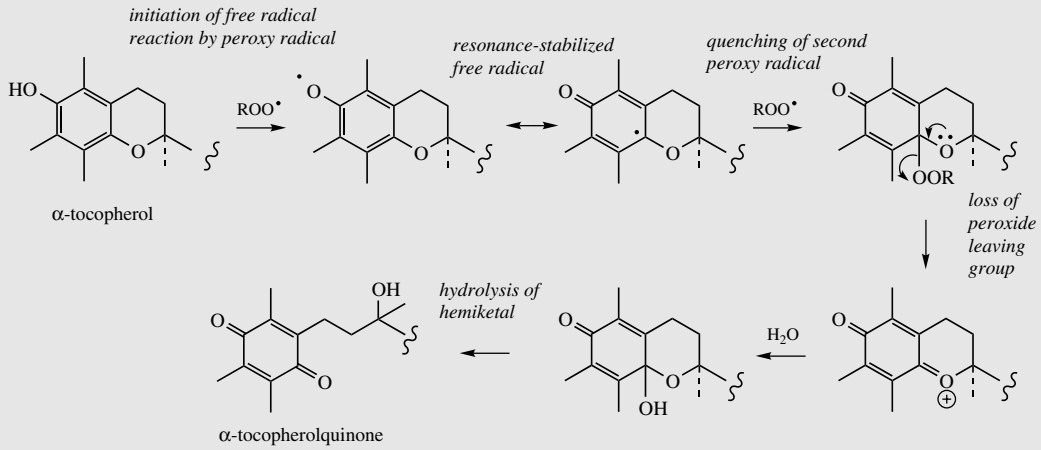

Figure 4.54

for medicinal purposes. The vitamin is known to provide valuable antioxidant properties, probably preventing the destruction by free radical reactions of vitamin A and unsaturated fatty acids in biological membranes. It is used commercially to retard rancidity in fatty materials in food manufacturing, and there are also claims that it can reduce the effects of ageing and help to prevent heart disease. Its antioxidant effect is likely to arise by reacting with peroxyl radicals, generating by one-electron phenolic oxidation a resonance-stabilized free radical that does not propagate the free radical reaction, but instead mops up further peroxyl radicals (Figure 4.54). In due course, the tocopheryl peroxide is hydrolysed to the tocopherolquinone.

more favoured aromatic tautomer from the hydrolysis of the coenzyme A ester. This compound is now the substrate for alkylation and methylation as seen with ubiquinones and plastoquinones. However, the terpenoid fragment is found to replace the carboxyl group, and the decarboxylated analogue is not involved. The transformation of **1,4-dihydroxynaphthoic acid** to the isoprenylated naphthoquinone appears to be catalysed by a single enzyme, and can be rationalized by the mechanism in Figure 4.56. This involves alkylation (shown in Figure 4.56 using the diketo tautomer), decarboxylation of the resultant β-keto acid, and finally an oxidation to the *p*-quinone.

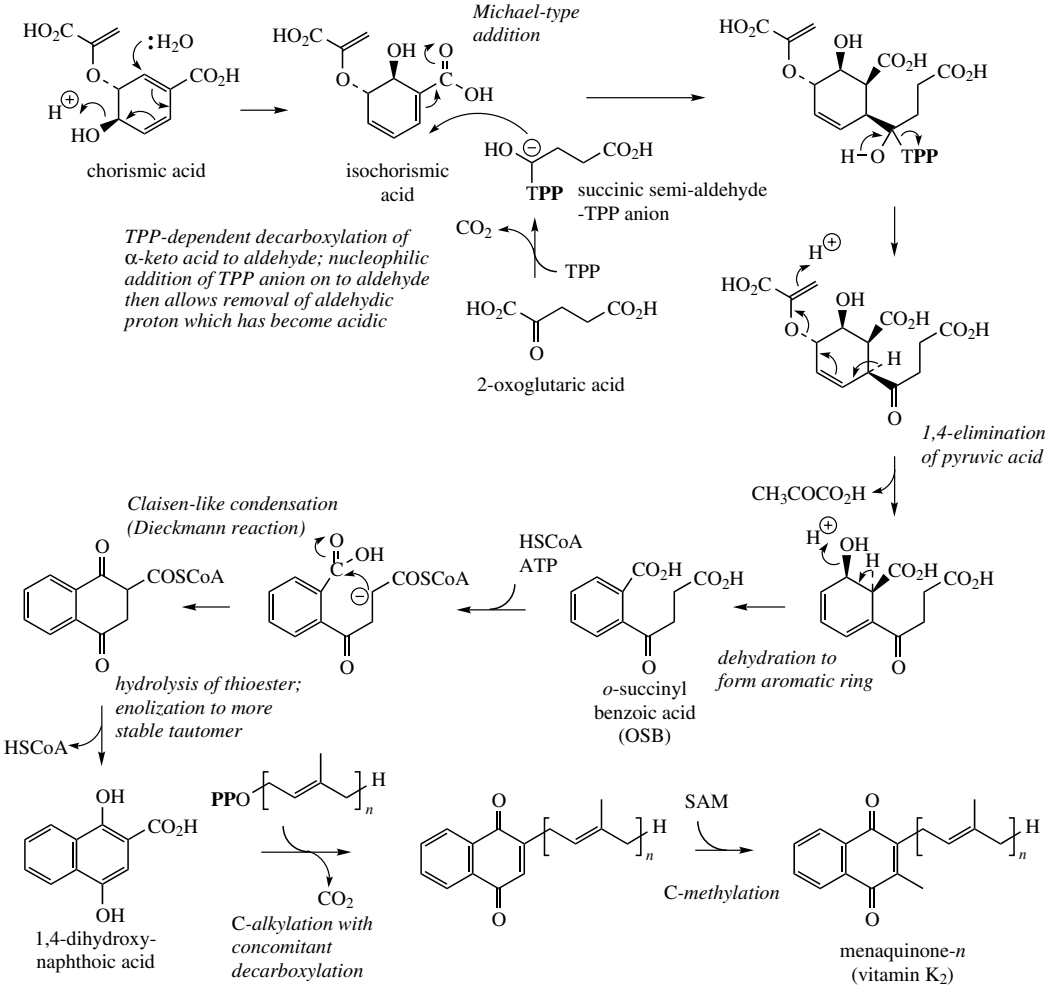

Figure 4.55

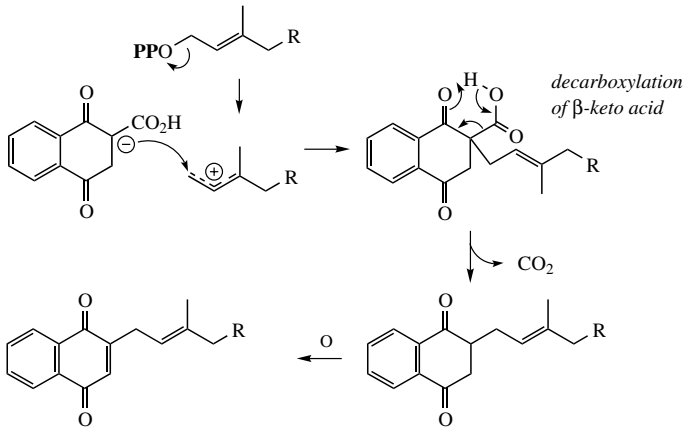

Figure 4.56

Vitamin K

Vitamin K comprises a number of fat-soluble naphthoquinone derivatives, with vitamin K_1 (phylloquinone) (Figure 4.50) being of plant origin whilst the vitamins K_2 (menaquinones) are produced by microorganisms. Dietary vitamin K_1 is obtained from almost any green vegetable, whilst a significant amount of vitamin K_2 is produced by the intestinal microflora. As a result, vitamin K deficiency is rare. Deficiencies are usually the result of malabsorption of the vitamin, which is lipid soluble. Vitamin K_1 (**phytomenadione**) or the water-soluble **menadiol phosphate** (Figure 4.57) may be employed as supplements. Menadiol is oxidized in the body to the quinone, which is then alkylated, e.g. with geranylgeranyl diphosphate, to yield a metabolically active product.

Vitamin K is involved in normal blood clotting processes, and a deficiency would lead to haemorrhage. Blood clotting requires the carboxylation of glutamate residues in the protein prothrombin, generating bidentate ligands that allow the protein to bind to other factors. This carboxylation requires carbon dioxide, molecular oxygen, and the reduced quinol form of vitamin K (Figure 4.57). During the carboxylation, the reduced vitamin K suffers epoxidation, and vitamin K is subsequently regenerated by reduction. Anticoagulants such as dicoumarol and warfarin (see page 144) inhibit this last reduction step. However, the polysaccharide anticoagulant heparin (see page 477) does not interfere with vitamin K metabolism, but acts by complexing with blood clotting enzymes.

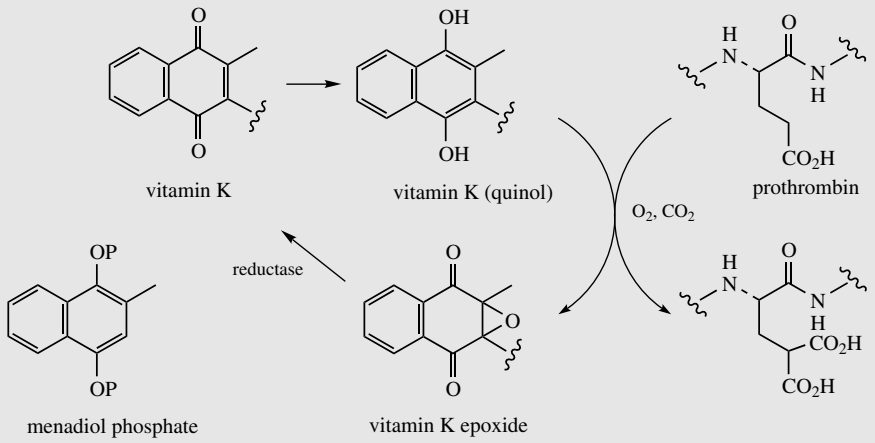

Figure 4.57

OSB, and 1,4-dihydroxynaphthoic acid, or its diketo tautomer, have been implicated in the biosynthesis of a wide range of plant naphthoquinones and anthraquinones. There are parallels with the later stages of the menaquinone sequence shown in Figure 4.55, or differences according to the plant species concerned. Some of these pathways are illustrated in Figure 4.58. Replacement of the carboxyl function by an isoprenyl substituent is found to proceed via a disubstituted intermediate in *Catalpa* (Bignoniaceae) and *Streptocarpus* (Gesneriaceae), e.g. **catalponone** (compare Figure 4.56), and this can be transformed to **deoxylapachol** and then **menaquinone-1** (Figure 4.58). **Lawsone** is formed by an oxidative sequence in which hydroxyl replaces the carboxyl. A further interesting elaboration is the synthesis of an anthraquinone skeleton by effectively cyclizing a dimethylallyl substituent on to the naphthaquinone system. Rather little is known about how this process is achieved but many examples are known from the results of labelling studies.

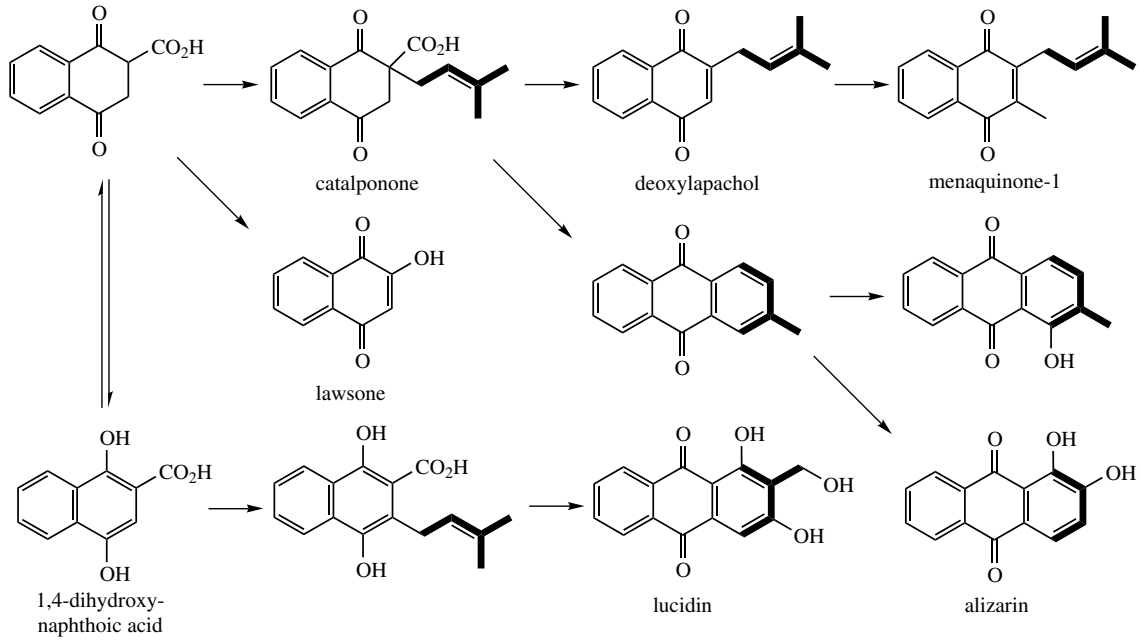

Figure 4.58

Some of these structures retain the methyl from the isoprenyl substituent, whilst in others this has been removed, e.g. **alizarin** from madder (*Rubia tinctorum*; Rubiaceae), presumably via an oxidation–decarboxylation sequence. Hydroxylation, particularly in the terpenoid-derived ring, is also a frequent feature.

Some other quinone derivatives, although formed from the same pathway, are produced by dimethylallylation of 1,4-dihydroxynaphthoic acid at the non-carboxylated carbon. Obviously, this is also a nucleophilic site and alkylation here is mechanistically sound. Again, cyclization of the dimethylallyl to produce an anthraquinone can occur, and the potently mutagenic **lucidin** from *Galium* species (Rubiaceae) is a typical example. The hydroxylation patterns seen in the anthraquinones in Figure 4.58 should be compared with those noted earlier in acetate/malonate-derived structures (see page 63). Remnants of the alternate oxygenation pattern are usually very evident in acetate-derived anthraquinones (Figure 4.59), whereas such a pattern cannot easily be incorporated into typical shikimate/2-oxoglutarate/isoprenoid structures. Oxygen substituents are not usually present in positions fitting the polyketide hypothesis.

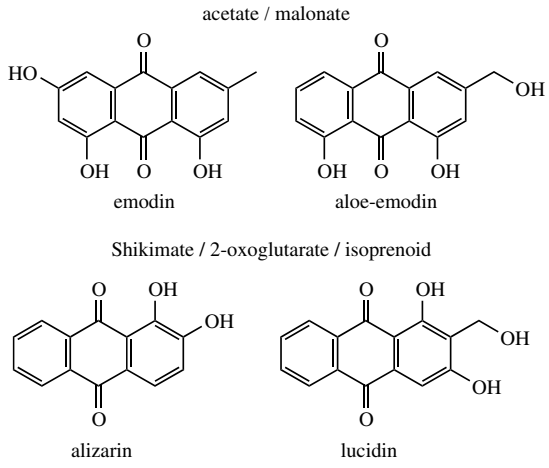

Figure 4.59

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