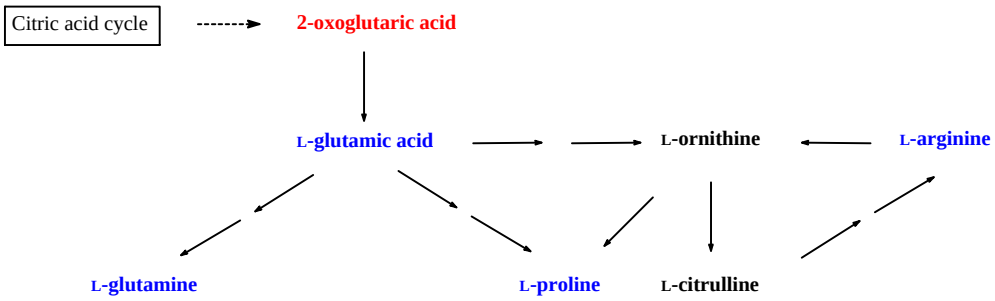
1 AMINO ACIDS

Commonly, 21 L-amino acids encoded by DNA represent the building blocks of animal, plant, and microbial proteins. The basic amino acids encountered in proteins are called proteinogenic amino acids [1.1)]. Biosynthesis of some of these amino acids proceeds by ribosomal processes only in microorganisms and plants and the ability to synthesize them is lacking in animals, including human beings. These amino acids have to be obtained in the diet (or produced by hydrolysis of body proteins) since they are required for normal good health and are referred to as essential amino acids. The essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The rest of encoded amino acids are referred to as non-essential amino acids (alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine). Arginine and histidine are classified as essential, sometimes as semi-essential amino acids, as their amount synthesized in the body is not sufficient for normal growth of children. Although it is itself non-essential, cysteine (classified as conditionally essential amino acid) can partly replace methionine, which is an essential amino acid. Similarly, tyrosine can partly replace phenylalanine.

1.1 The glutamic acid group

1.1.1 Glutamic acid and glutamine

Free ammonium ions are toxic to living cells and are rapidly incorporated into organic compounds. One of such transformations is the reaction of ammonia with 2-oxoglutaric acid from the citric acid cycle to produce L-glutamic acid. This reaction is known as reductive amination. Glutamic acid is accordingly the amino acid generated first as both constituent of proteins and a biosynthetic precursor. Many pathways and reactions involving glutamic acid are known. Glutamic acid itself can bind a further ammonium ion to form L-glutamine, a second amino acid. Glutamic acid then serves as the precursor of L-proline, L-ornithine, L-citrulline, and L-arginine. Biosynthetic pathway of the glutamic acid amino acid family is schematically shown in Figure 1.1.



[1.1)] Selenocysteine that is cotranslationally inserted into protein in eukaryota, archaea, and eubacteria has been recognized as the 21st amino acid in the genetic code.

Figure 1.1

In microorganisms, NAD^+-dependent glutamate dehydrogenase (EC 1.4.1.2), $NAD(P)^+$-dependent glutamate dehydrogenase (EC 1.4.1.3), $NADP^+$-dependent glutamate dehydrogenase (EC 1.4.1.4), glutamate synthase (EC 1.4.1.14), and glutamine synthetase (EC 6.3.1.2) are used for ammonia assimilation. Some of these organisms may use only glutamate dehydrogenase, or only glutamate synthase/glutamine synthetase, while others use either route, depending upon growth conditions. Most of the prokaryotic enzymes can use either NAD^+ (EC 1.4.1.2) or $NADP^+$ (EC 1.4.1.4). Plants have multiple forms of glutamate dehydrogenase enzymes and their role in glutamate biosynthesis is unclear [1.2)]. The NAD^+-specific enzymes are localized in the mitochondria, while the $NADP^+$-specific enzymes are associated with the chloroplasts. Glutamate synthase/glutamine synthetase functions in ammonia assimilation. Mammals use glutamate dehydrogenases located in the mitochondrial matrix that can use either NAD^+ or $NADP^+$ as cofactors (EC 1.4.1.3). Their glutamine synthetase acts in nitrogen metabolism. The formation of glutamic acid (and the reverse reaction) catalyzed by glutamate dehydrogenase (EC 1.4.1.3) is shown in Figure 1.2.

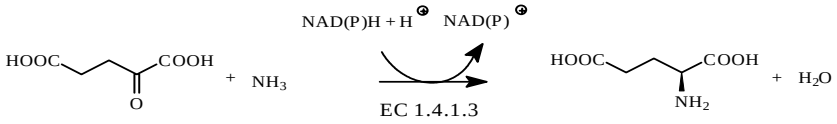
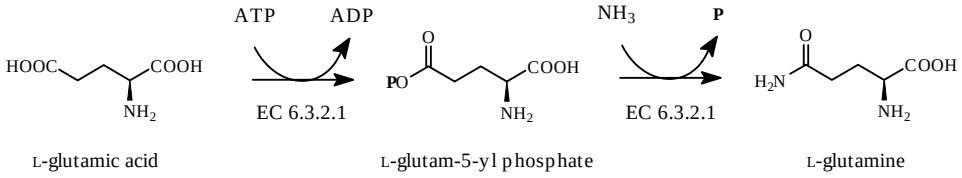


Figure 1.2

L-Glutamine is a constituent of proteins and a nitrogen donor for many biosynthetic reactions, including the biosynthesis of amino acids, purines, pyrimidines, glucosamine, and carbamoyl phosphate. The biosynthesis of glutamine is catalyzed by glutamine synthetase [1.3)] (EC 6.3.1.2), a key enzyme of nitrogen metabolism found in all domains of life. Glutamine arises as a product of amidation of glutamic acid that binds a further ammonium ion. This amidation proceeds through the intermediate L-glutam-5-yl phosphate-ATP, which splits off ADP and phosphoric acid yielding glutamine (Figure 1.3).



[1.2)] Plants take up nitrogen as nitrates and in smaller amounts also as ammonium ions. A few species, mainly leguminose plants, live in symbiosis with nitrogen-fixing bacteria that are able to reduce atmospheric nitrogen. Microorganisms mainly require ammonium ions as the nitrogen source, some bacteria and moulds are able to use nitrates similarly to plants. Plants reduce nitrates in two steps. In the first step, nitrate is reduced to nitrite by nitrate reductase (NADH) (EC 1.7.1.1). In the second step, nitrite is reduced to ammonia by ferredoxin-nitrite reductase (EC 1.7.7.1).

[1.3)] There are three types of glutamine synthetases, differing in number of subunits. Glutamine synthetase type I is found mostly in bacteria and archaea, glutamine synthetase type II in eukaryotes and some soil bacteria, and the type III glutamine synthetase has been described in anaerobic bacteria and cyanobacteria.

Figure 1.3

Very important for many amino acid syntheses is the group of enzymes called transaminases. They are able to transfer an amino group (mostly that of glutamine) to a 2-oxo acid according to the following equation:

$$(\text{amino acid})_1 + (\text{2-oxo acid})_2 = (\text{2-oxo acid})_1 + (\text{amino acid})_2$$

Thus, the glutamine amino group is transferred to 2-oxoglutaric acid (Figure 1.4) producing glutamic acid. The reaction is catalyzed by glutamate synthases having either NADPH (EC 1.4.1.13) or NADH (EC 1.4.1.14) as cofactors.

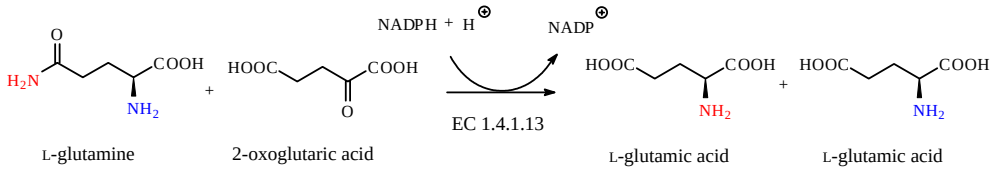


Figure 1.4

The transamination reaction catalyzed by transaminases is dependent on the coenzyme pyridoxal 5´-phosphate (PLP). The reaction starts with addition of the unprotonized amino group of an amino acid to the electron deficient carbon of the polarized carbonyl group of PLP under the formation of a monotopic carbinolamine. The carbinolamine dehydration then leads to an aldimine (Schiff base). The α-hydrogen atom of the original amino acid in the aldimine is now much more acidic and can be readily eliminated. Isomerization of the resulting intermediate leads to a ketimine (an imine of PLP with a 2-oxo acid). Hydrolysis of this ketimine generates pyridoxamine 5´-phosphate and the 2-oxo acid (Figure 1.5). The amino group of pyridoxamine 5´-phosphate is then transferred to another 2-oxo acid molecule and PLP is regenerated.

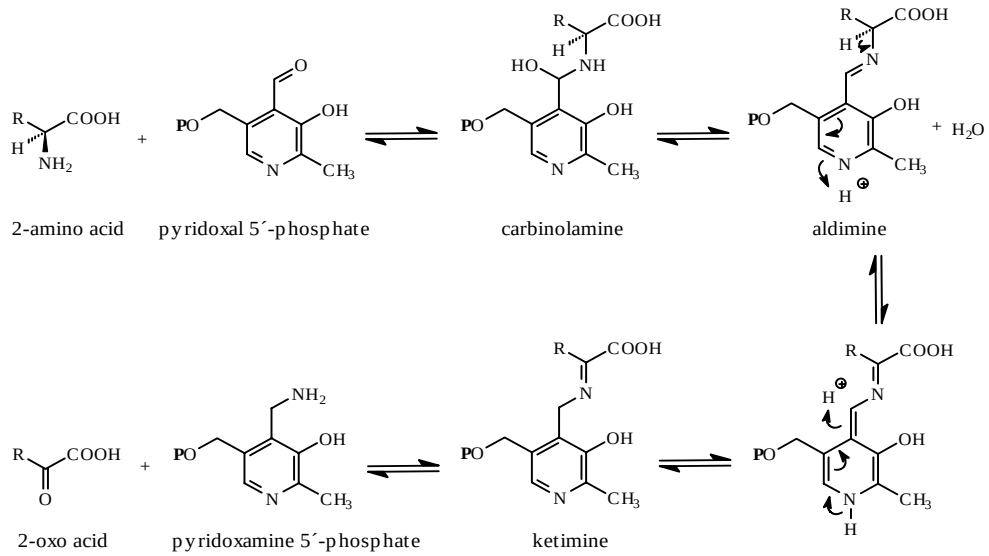


Figure 1.5

1.1.2 Proline

In addition to be a major constituent of proteins, L-proline also acts as an osmotic protectant in bacteria, plants and animals that are under osmotic stress. It is generated from L-glutamic acid by a ring formation. The reaction is facilitated by a preliminary phosphorylation of glutamic acid by γ-glutamylkinase (EC 2.7.2.11) that leads to an unstable intermediate L-glutam-5-yl phosphate under consumption of one molecule of ATP. Reduction of the phosphorylated γ-carboxyl to a carbonyl group is catalyzed by the NAD(P)H dependent glutamate semialdehyde dehydrogenase (EC 1.2.1.41). The formed glutamic acid 5-semialdehyde spontaneously yields a cyclic Schiff base (*S*)-1-pyrroline-5-carboxylic acid, i.e. (*S*)-3,4-dihydro-2*H*-pyrrole-2-carboxylic acid. Its reduction to proline is achieved by pyrroline-5-carboxylate reductase (EC 1.5.1.2) and the needed hydrogen is supplied by NAD(P)H. An alternative pathway of proline biosynthesis is a cyclization of L-ornithine catalyzed by ornithine cyclodeamidase (EC 4.3.1.12) (Figure 1.6).

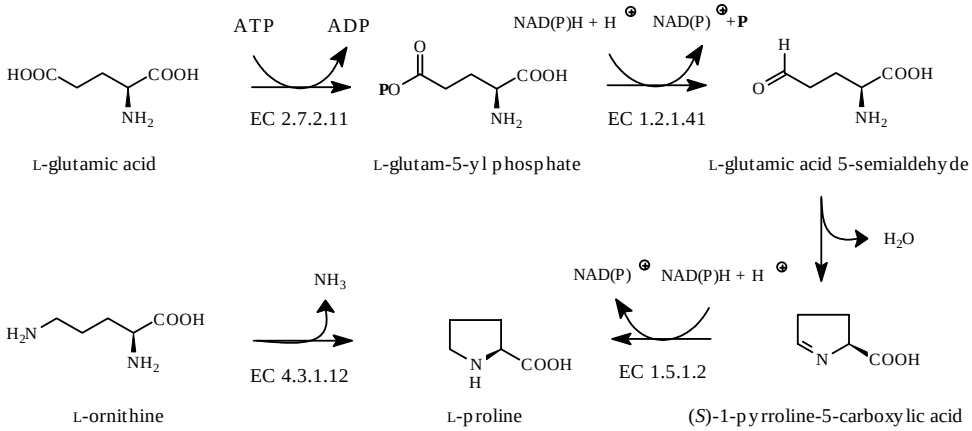


Figure 1.6

1.1.3 Arginine

The biosynthesis of the essential amino acid L-arginine in microorganisms and plants starts from L-glutamic acid. It proceeds via L-ornithine in several intermediate steps and needs reduction of the γ-carboxyl group to carbonyl group under the participation of ATP similarly to proline biosynthesis. To prevent the spontaneous cyclization of the semialdehyde, the first step is acetylation of glutamic acid with acetyl-CoA catalyzed by amino-acid *N*-acetyltransferase (EC 2.3.1.1). The carboxyl group of thus formed intermediate *N*-acetyl-L-glutam-5-yl phosphate is then reduced to a carbonyl group by NADPH-dependent *N*-acetyl-γ-glutamyl-phosphate reductase (EC 1.2.1.38) and the next intermediate *N*-acetyl-L-glutamic acid 5-semialdehyde is transformed to *N*-acetyl-L-ornithine in a transamination reaction catalyzed by a PLP protein N^2-acetylornithine 5-transaminase (EC 2.6.1.11). Finally, hydrolysis of *N*-acetyl-L-ornithine to L-ornithine is catalyzed by acetylornithine deacetylase (EC 3.5.1.16) (Figure 1.7).

In the second pathway (Figure 1.30), *O*-acetylhomoserine is synthesized from L-homoserine, an essential intermediate in methionine degradation, by homoserine *O*-acetyltransferase (EC 2.3.1.31). *O*-Acetylserine sulfhydrylase (EC 2.5.1.47) catalyzes the condensation of sulfide with *O*-acetylhomoserine to form L-homocysteine. Under normal metabolic conditions, essentially all the sulfur atoms derived from methionine degradation are transferred to cysteine before the sulfur atom is oxidized and excreted. Then homocysteine is transformed into cysteine by *trans*-sulfuration, i.e. L-homocysteine condenses with L-serine to form L,L-cystathionine by action of cystathionine β-synthase (EC 4.3.1.22). Cystathionine in turn dissociates into cysteine, 2-oxobutanoic acid, and ammonia by cystathionine γ-lyase (EC 4.4.1.1).

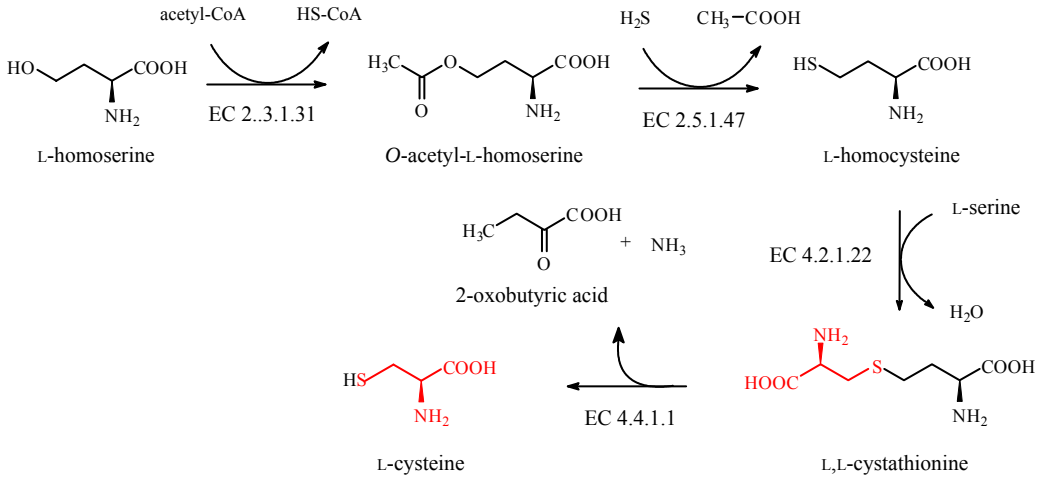


Figure 1.30

Cysteine may also be transformed into homocysteine by reverse *trans*-sulfuration catalyzed by cystathionine γ-synthase (EC 2.5.1.48) and cystathionine β-lyase (EC 4.4.1.8). The serine acetyltransferase pathway is used by plants and by enteric bacteria. Fungi use different cysteine biosynthetic pathways depending on the species.

1.4.4 Selenocysteine

L-Selenocysteine is a selenium-containing amino acid that is cotranslationally inserted into protein in all three lines of descent, eukaryota, archaea, and eubacteria. It is recognized as the 21st amino acid in the genetic code. The biosynthesis of selenocysteine is unique among amino acid biosyntheses as it occurs on its transfer RNA (tRNA), designated $tRNA^{[Ser]Sec}$. Initially, $tRNA^{[Ser]Sec}$ is aminoacylated with serine by seryl-tRNA synthetase (EC 6.1.1.11) and the seryl moiety provides the backbone for selenocysteine synthesis by selenocysteine synthase (EC 2.9.1.1). Selenocysteine synthase is a pyridoxal phosphate-dependent protein that converts the serine attached to $tRNA^{[Ser]Sec}$ to seleno-cysteine by initially removing the hydroxyl group from serine to form an 2-aminoacrylyl-t-RNA intermediate bound to the enzyme as an imine with the pyridoxal phosphate. This intermediate serves as the acceptor for activated selenium, and when selenium is donated,

selenocysteyl-tRNA$^{[Ser]Sec}$ is formed. This pathway is possibly also active in other eukaryotes and archaea that synthesize selenoproteins. The selenium donor in bacteria, selenophosphate, is synthesized from hydrogen selenide and ATP by selenidephosphate synthetase (selenide, water dikinase; EC 2.7.9.3) (Figure 1.31). Hydrogen selenide is synthesized from selenate via selenite by the same enzymes that reduce sulfate, via sulfite, to sulfide.

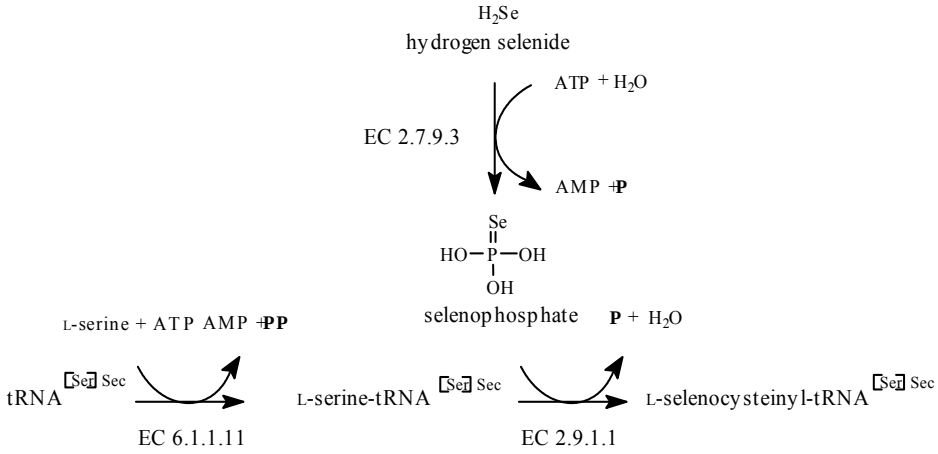


Figure 1.31

O-acetylserine sulfhydrylase (EC 2.5.1.47), acting in the biosynthesis of cysteine, also catalyzes the biosynthesis of free L-selenocysteine from *O*-acetyl-L-serine and hydrogen selenide (see Chapter 1.4.3). Selenocysteine can be then oxidized to L-selenocystine, methylated to *Se*-methyl-L-selenocysteine (thiol *S*-methyltransferase, EC 2.1.1.9), which can be transformed to γ-glutamyl-*Se*-methyl-L-selenocysteine using γ-glutamyltransferase (EC 2.3.2.2.). Reaction with L-cysteine and *O*-succinyl-L-homoserine yields L,L-selenocystathionine (cystathionine γ-synthase, EC 2.5.1.48) that can be transformed to L-selenohomocysteine (cystathionine β-lyase) and, finally, to L-selenomethionine (Figure 1.32). In plants growing in areas with high selenium content in the soil, *Se*-methylselenocysteine, γ-glutamyl-*Se*-methylselenocysteine, selenocystathionine, and selenomethionine are the main selenium-containing compounds. In many foods of plant or animal origin, the main selenium-containing compound is selenocysteine bound in proteins.

1.5 The aromatic and heterocyclic amino acids group

1.5.1 Phenylalanine and tyrosine

Biosynthetic pathways of the aromatic and heterocyclic amino acids family is schematically shown in Figure 1.33. The shikimic acid [1.9)] pathway provides a route to the

[1.9)] Shikimic acid has been isolated from plants of *Illicium anisatum* (syn. *I. religiosum*), Japanese shikimi.

aromatic amino acids L-phenylalanine and L-tyrosine and to the heterocyclic amino acid L-tryptophan that are not only constituents of proteins, but also precursors of many biologically active secondary metabolites found in microorganisms, plants, and animals. Shikimic acid, a central intermediate in this pathway, is formed by a sequence of reactions from the glycolytic product 3-phosphoenolpyruvic acid and D-erythrose 4-phosphate (formed in the pentose phosphate and the Calvin cycles). Both shikimic acid and the subsequent product chorismic acid are important intermediates. The latter is the starting compound for three different pathways that lead to the end products phenylalanine, tyrosine, and tryptophan. An activated form of D-ribose 5-phosphate (5-phospho-α-D-ribose 1-diphosphate), is another intermediate of tryptophan biosynthesis. It has also a key position in the biosynthesis of L-histidine. The biosynthetic pathway of histidine is unusual in that histidine is produced from a purine. Animals employ none of these pathways and, accordingly, these amino acids feature among those essential amino acids that have to be obtained in their diet.

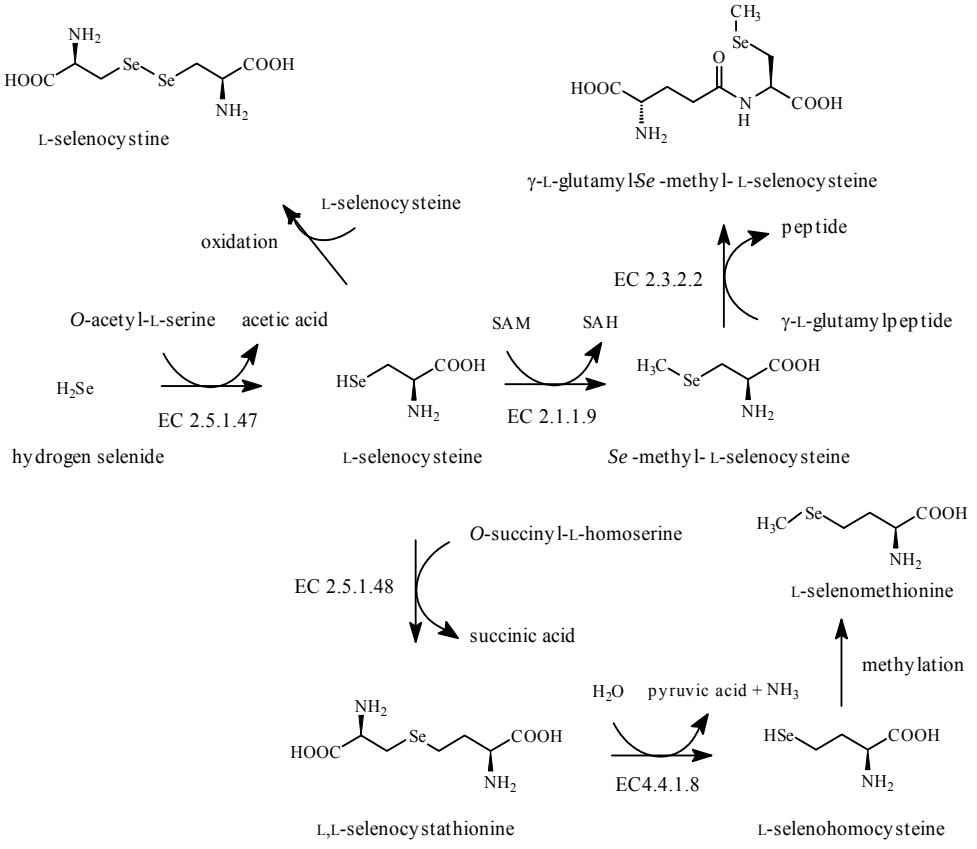


Figure 1.32

The shikimic acid pathway begins with an aldol-type condensation of phosphoenolpyruvic acid with D-erythrose 4-phosphate to give 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (3-deoxy-7-phospho-D-*arabino*-heptulosonic acid, Figure 1.34).

1.7.3 Alicyclic amino acids

1.7.3.1 Aminocyclopropane-1-carboxylic acid

Bacteria, fungi, and plants produce ethylene. In plants, ethylene is a signalling molecule (hormone) that regulates many processes of seed germination, plant growth, development, flowering, fruit ripening, abscission, and senescence. Plant cells can also synthesize ethylene when they encounter various stress factors, such as physical wounding, pathogen attack, flooding, chilling injury, or the presence of heavy metals. The induction of ethylene synthesis also plays a crucial role in a certain herbicide mode of action.

In plants, methionine is the only known precursor of ethylene biosynthesis that proceeds in several steps. The immediate ethylene precursor is 1-aminocyclopropane-1-carboxylic acid that is synthesized from methionine via SAM (see Chapter 1.2.3). The formation of 1-aminocyclopropane-1-carboxylic acid is catalyzed by the pyridoxal 5´-phosphate protein 1-aminocyclopropane-1-carboxylate synthase (EC 4.4.1.14). The enzymatic conversion of this acid into ethylene proceeds via unstable *N*-hydroxy-1-aminocyclopropane-1-carboxylic acid, which breaks down into ethylene (derived from the C-2 and C-3 carbons of 1-aminocyclopropane-1-carboxylic acid) and cyanoformic acid. The latter compound is further decomposed into hydrogen cyanide and CO_2 as shown in Figure 1.60.

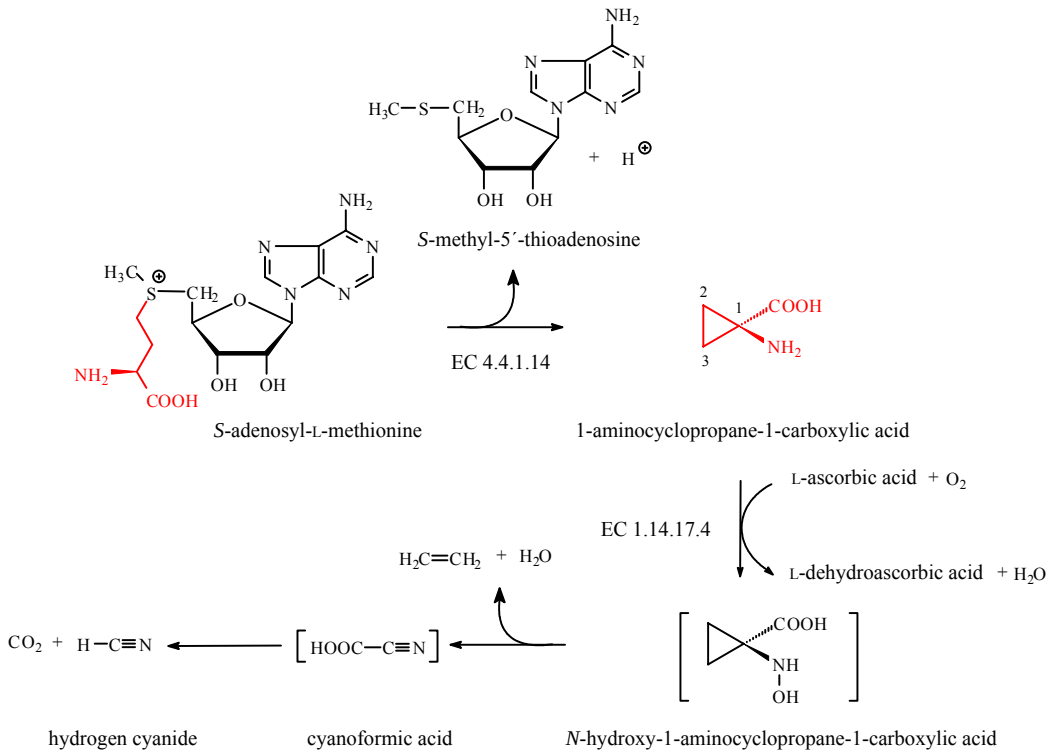
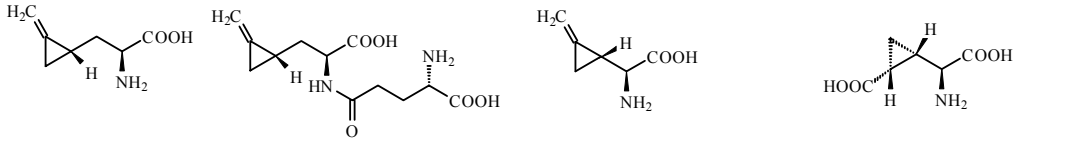


Figure 1.60

The enzyme involved in this process is 1-aminocyclopropane-1-carboxylic acid oxidase (ACC oxidase, EC 1.14.17.4), a member of the ferrous-dependent family of non-heme oxygenases. Like other members of this family, it requires ferrous ions and utilizes triplet oxygen and ascorbic acid. It also requires bicarbonate ions or CO_2 as an activator.

1.7.3.2 Hypoglycin and related compounds

The unripe fruits of the Jamaican ackee tree (*Blighia sapida*; Sapindaceae) contain an unusual free amino acid called hypoglycin, i.e. (2*S*,4*S*)-3-(methylenecyclopropyl)alanine, also known as L-β-(methylenecyclopropyl)alanine, L-3-(methylenecyclopropyl)alanine, or 2-amino-4,5-methanohex-5-enoic acid. The free amino acid (hypoglycin A) is found in the arils and seeds of the fruit. Its content significantly decreases in the arils with ripeness (from 1000-1110 mg/kg to less than 100 mg/kg). γ-Glutamylhypoglycin (hypoglycin B) is only found in the ackee seeds. Traces of the lower homologue of hypoglycin A with hypoglycemic activity, i.e. 2-(methylenecyclopropyl)glycine, its γ-glutamyldipeptide, and (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (Figure 1.61) are present in the immature seeds [1.16)].



hypoglycin A | hypoglycin B | 2-(methylenecyclopropyl)glycine | 2-(2′-carboxycyclopropyl)glycine

Figure 1.61

Significant amounts of hypoglycin A and hypoglycin B occur also in the seeds of *Billia hippocastanum* (Hippocastanaceae) from Costa Rica and *Acer pseudoplatanus* (Aceraceae), a common sycamore of temperate zones. The lower homologue of hypoglycin, L-α-(methylenecyclopropyl)glycine, was found in the seeds of lychee (*Litchi sinensis*; Sapindaceae).

Symptoms of ackee poisoning (commonly known as Jamaican vomiting sickness or more accurately the toxic hypoglycemic syndrome) occur 6-48 hours after the ingestion of unripe arils and include nausea, vomiting, drowsiness, muscular and mental exhaustion, and hypoglycemia. The mechanism of action is that the toxin follows a similar metabolic pathway as branched-chained amino acids, thus producing the active metabolite methylenecyclopropylacetyl-CoA. It is known that this metabolite then irreversibly binds to FAD and thereby inactivates medium-chain acyldehydrogenases that are essential for the complete β-oxidation of fatty acids.

There have been only a few reports on the biosynthesis of hypoglycin, which links to that of the lower homologue, L-α-(methylenecyclopropyl)glycine. It is inferred that the first step is analogous to isoleucine biosynthesis as it starts with the dehydration and deamination of threonine to yield 2-oxobutanoic (2-oxobutyric) acid. The addition of the C_1 unit from methionine (probably from SAM) to 2-oxobutanoic acid is supposed to give

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[1.16)] The Food and Drug Administration and Health Canada set the limit of 100 mg/kg.

5-hydroxy-2-oxopentanoic acid, which eliminates water to yield 2-oxopent-4-enoic acid. 2-Oxopent-4-enoic acid is oxidized to allenic (2-oxopent-3,4-dienoic) acid to which methionine adds the second C_1 unit giving rise to 2-oxo-3,4-methanopent-4-enoic acid. The formation of α-(methylenecyclopropyl)glycine is then accomplished by trans-amination (Figure 1.62).

Acetyl-CoA is subsequently added to 2-oxo-3,4-methanopent-4-enoic acid by the same mechanism by which (*S*)-2-isopropylmalic acid is formed in the leucine biosynthetic pathway as the relevant enzymes of this pathway appear not to be absolutely specific. The product, 2-hydroxy-2-(2-methylcycloprop-1-en-1-yl)butane-1,4-dioic acid (3-hydroxy-3-carboxy-4,5-methanohex-4-enoic acid), then exactly follows the remaining steps of that pathway. This sequence includes isomerization to 2-hydroxy-3-(2-methylcycloprop-1-en-1-yl)butane-1,4-dioic acid (2-hydroxy-3-carboxy-4,5-methanohex-4-enoic acid) and de-hydrogenation with decarboxylation yielding 2-oxo-4,5-methanohex-5-enoic acid. Finally, transamination of the latter compound, β-(methylenecyclopropyl)pyruvic acid, leads to hypoglycin A. The enzyme γ-glutamyltranspeptidase (EC 2.3.2.2) catalyzes the reaction in which glutathione (GSH), acting as the donor, forms γ-glutamylpeptide hypoglycin B with the acceptor hypoglycin A (Figure 1.62).

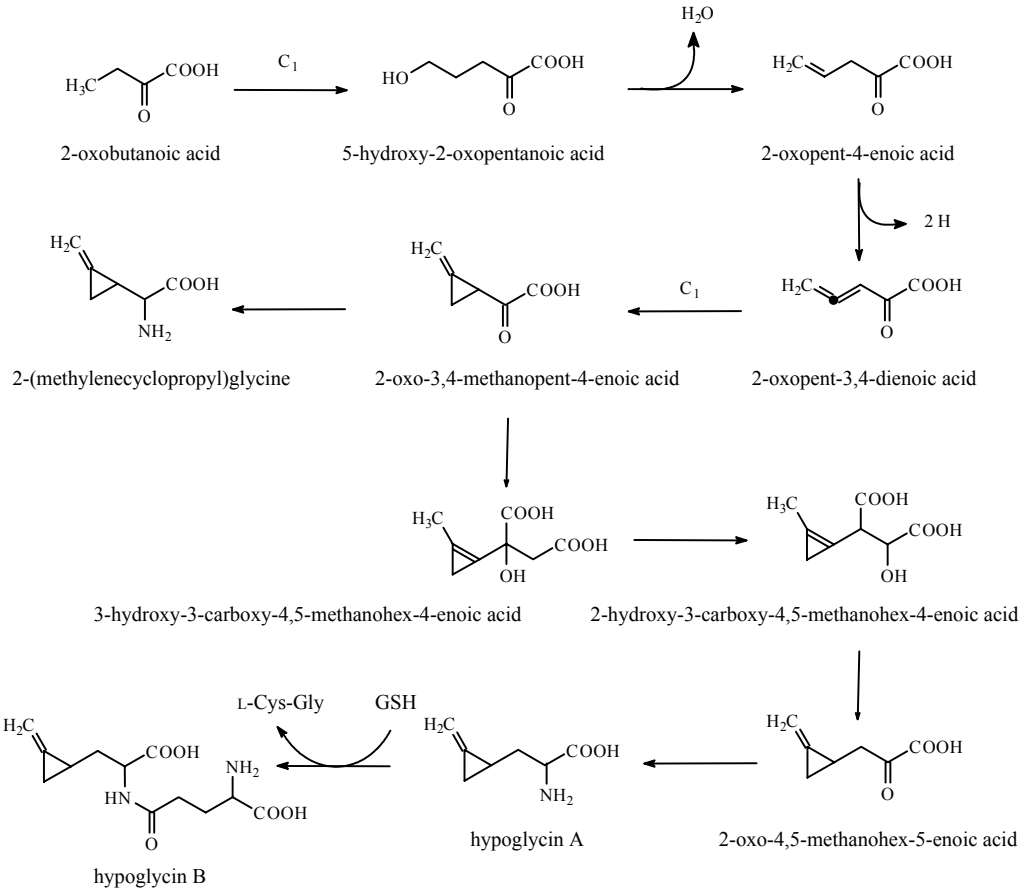


Figure 1.62

1.7.3.3 Cyclopentenylglycine

Cyclopentenylglycine has been demonstrated in several tropical plant species of Flacourtiaceae, Passifloraceae, and Turneraceae as a mixture of two stereoisomers, i.e. (2*S*,1′*R*)-2-(cyclopent-2′-en-1′-yl)glycine and (2*S*,1′*S*)-2-(cyclopent-2′-en-1′-yl)glycine (Figure 1.63), of which (2*S*,1′*R*)-2-(cyclopent-2′-en-1′-yl)glycine prevails. Cyclopentenylglycine is synthesized by two C_1-chain elongations of 2-oxoglutaric acid (formed by L-glutamic acid transamination) via 2-oxopimelic acid (Figure 1.64).).

(2*S*,1′*R*)-2-(cyclopent-2′-en-1′-yl)glycine (2*S*,1′*S*)-2-(cyclopent-2′-en-1′-yl)glycine

Figure 1.63

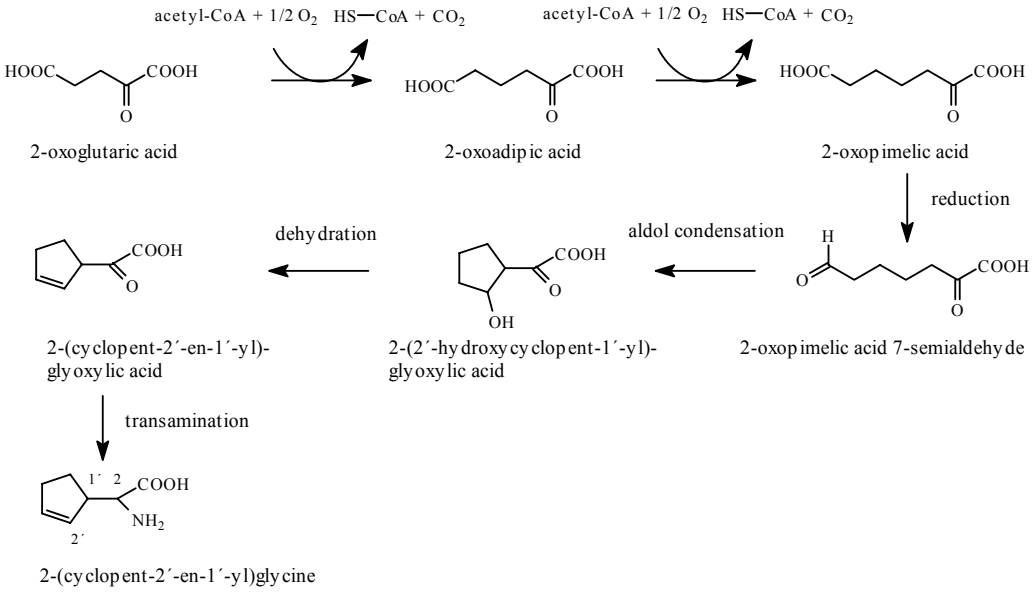


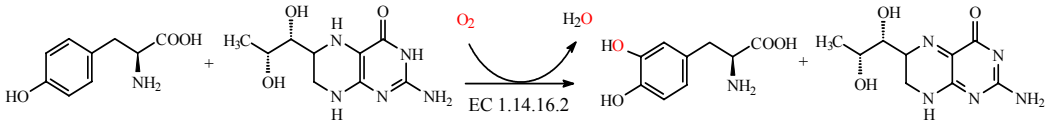
Figure 1.64

Reduction of 2-oxopimelic acid to the semialdehyde (2-oxopimelic acid 7-semialdehyde), yields the appropriate intermediate, 2-(2′-hydroxycyclopent-1′-yl)glyoxylic acid, which is used for the biogenesis of the five-membered carbon ring via intramolecular aldol condensation. This step creates the C-1′ chiral center, whose configuration depends on the site of nucleophilic attack of the β-carbon on the aldehyde carbon either above or below the plane of the functional group. The intermediate is dehydrated to cyclopentenyl-glyoxylic acid, which is finally transaminated yielding cyclopentenylglycine. Cyclopentenylglycine is the precursor of cyclopentenoic fatty acids (see Chapter 3.1.4.4) and cyclopentenoic cyanogenic glycosides (see Chapter 9.3).

1.7.4 Hydroxyamino acids

1.7.4.1 Dihydroxyphenylalanine

A relatively small number of tyrosine molecules are directly hydroxylated to give 3,4-dihydroxy-L-phenylalanine (L-DOPA, L-dopa, levodopa) [1.17]. This reaction is catalyzed by (6*R*)-5,6,7,8-tetrahydro-L-biopterin (L-*erythro*-5,6,7,8-tetrahydrobiopterin or BH_4)-dependent monooxygenase enzyme (tyrosine hydroxylase, EC 1.14.16.2) (Figure 1.65).



L-tyrosine 5,6,7,8-tetrahydrobiopterin 3,4-dihydroxy-L-phenylalanine 7,8-dihydrobiopterin

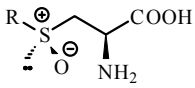
Figure 1.65

1.7.5 Sulfur-containing amino acids

1.7.5.1 *S*-Alk(en)ylcysteines and their sulfoxides

S-Alk(en)ylcysteines and their sulfoxides belong to the most common non-protein amino acids. Although their occurrence is associated almost exclusively with *Allium* species, their distribution in the plant kingdom appears to be much broader. They commonly occur in many other plants (e.g. genera *Brassica*, *Vigna*, *Petiveria*, *Tulbaghia*, *Scorodocarpus,* and *Acacia*, among many others) as well as in several mushrooms (genera *Marasmius*, *Collybia*, and *Lentinus*) and marine algae (genera *Chondria* and *Undaria*).

Garlic (*Allium sativum*), onion (*A. cepa*), and other members of the genus *Allium* (Liliaceae) typically contain 1-5% dry weight of *S*-alk(en)ylcysteines sulfoxides. The pool generally consists of varying proportions of the four major derivatives, (R_C,S_S)-*S*-allyl-, (R_C,S_S)-*S*-methyl-, (R_C,S_S)-*S*-propyl-, and (R_C,R_S)-(*E*)-*S*-(prop-1-en-1-yl)cysteine sulfoxides (alliin, methiin, propiin, and isoalliin, respectively) (Figure 1.66).



S-alk(en)yl-L-cysteine sulfoxides

Figure 1.66

[1.17] Dopa can undergo various biochemical fates in all living organisms. In animals, it is involved in melanogenesis and neurotoxicity as oxidation reactions catalyzed by tyrosinase (EC 1.14.18.1) convert Dopa into a heterogenous polymer melanin (see Chapter 8.2), the main pigment in the mammalian skin, hair, and eyes; other reactions can lead to catecholamines, the neurotransmitter norepinephrine (noradrenaline), and the hormone epinephrine (adrenaline). Its metabolism in plants is of particular importance as it leads to formation of some food components, such as alkaloids (e.g. salsalinol in bananas is a product from dopamine and acetaldehyde combining via the Pictet-Spengler reaction), certain pigments (betalains), and undesirable bitter substances (oleuropein in olives).

2 PEPTIDES

Peptides display a wide variety of biological functions and many of them have remarkable physiological properties. Some are widely distributed in nature and found in many different organisms, whereas others are only of a restricted occurrence. For example, peptides function as neurotransmitters and hormones and thus control many physiological processes. Furthermore, the toxic principles of some plants and mushrooms, insect, snake, and spider venoms are usually peptides.

Peptides are biosynthesized by ribosomal and nonribosomal processes (using multifunctional enzymes) from a wide range of amino acids. Ribosomal peptide biosynthesis leads to peptide enzymes, hormones, and many other physiologically active substances. The mechanisms of these processes are, however, beyond the scope of this publication.

Many structures biosynthesized by ribosomal processes are additionally modified. Glycopeptides are produced by adding sugar residues via *O*-glycoside linkages to the hydroxyls of serine and threonine residues or via *N*-glycoside linkages to the amino group of asparagine. Phosphopeptides and phosphoproteins have the hydroxyl group of serine or threonine esterified with phosphoric acid. Many peptides contain a pyroglutamic (glutiminic) acid residue at the *N*-terminus, which is a consequence of glutamine intramolecular cyclization between the γ-carboxyl and the α-amino group. The *C*-terminal carboxylic acids may also frequently be converted to an amide.

Nonribosomal processes synthesize many natural peptides occurring in foods. They are responsible for the formation of glutathione, histidine-containing dipeptides of skeletal muscles, peptide toxins, and peptide antibiotics. These peptides are formed by a sequence of enzyme-controlled reactions. During these processes, each amino acid (often not encoded by DNA) is added (after activation by conversion into AMP esters) as a result of the specificity of the enzyme involved. Unusual amino acids are often formed as a result of an elimination reaction from proteinogenic amino acids. For example, 2-aminobutanoic (α-aminobutyric) acid is formed by decarboxylation of glutamic acid, dehydroalanine (2-aminoprop-2-enoic acid, 2-aminoacrylic acid, anhydroserine) is generated by elimination of H_2S from cysteine or by elimination of water from serine, and threonine yields by dehydration (*Z*)-aminobut-2-enoic acid, also known as (*Z*)-dehydrobutyrine, 2-aminocrotonic acid, or anhydrothreonine. Except of activating the amino acid and catalyzing the peptide formation, these multifunctional enzymes may possess activities responsible e.g. for racemization of L-amino acids into D-amino acids and cyclization of the amino acids at both termini of a linear peptide chain.

2.1 γ-Glutamylpeptides

2.1.1 Glutathione

Glutathione in its reduced form (γ-L-glutamyl-L-cysteinylglycine, GSH) is present in high concentrations in most living cells, being the major reservoir of non-protein sulfur. In animal cells, its amounts range from 300 to 1500 mg/kg and lower quantities occur in

plants and microorganisms. For example, the amount of glutathione in wheat flour ranges from 10 to 15 mg/kg.

GSH biosynthesis is similar in all living organisms. GSH is synthesized from its constituent amino acids by two ATP-dependent reactions catalyzed by γ-glutamylcysteine synthetase (EC 6.3.2.2) and glutathione synthetase (EC 6.3.2.3). In the first step, γ-L-glutamyl-L-cysteine is formed from L-glutamic acid and L-cysteine. During the second step, glycine is added to the *C*-terminal of γ-glutamylcysteine yielding GSH (Figure 2.1).

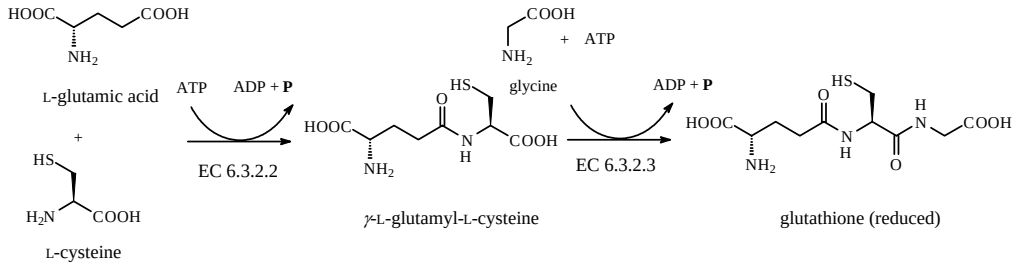


Figure 2.1

Because of its unique redox and nucleophilic properties, GSH serves in reductive reactions (Figure 2.2) and as an important line of defense against reactive oxygen species, heavy metals, xenobiotics, as well as pharmaceuticals (glutathione conjugates, such as mercapturic acids resulting from detoxification processes). In wheat flour, L-ascorbic acid is oxidized to L-dehydroascorbic acid (via L-monodehydroascorbic acid), which becomes a co-substrate of glutathione dehydrogenase (EC 1.8.5.1). It reduces dehydroascorbic acid back to ascorbic acid using GSH. In fruits, L-ascorbate peroxidase (EC 1.11.1.11) catalyzes oxidation of ascorbic acid by H_2O_2 to monodehydroascorbic acid and further to dehydroascorbic acid. Dehydroascorbic acid can be reduced back to ascorbic acid by GSH. Glutathione peroxidase (EC 1.11.1.9), a protein containing a selenocysteine residue, decomposes H_2O_2 (as well as steroid and lipid hydroperoxides) in biological membranes. This enzyme uses GSH (glutathione reduced), which is oxidized to GSSG (glutathione oxidized). GSH is then regenerated e.g. by the action of NADPH-dependent glutathione reductase (EC 1.8.1.7).

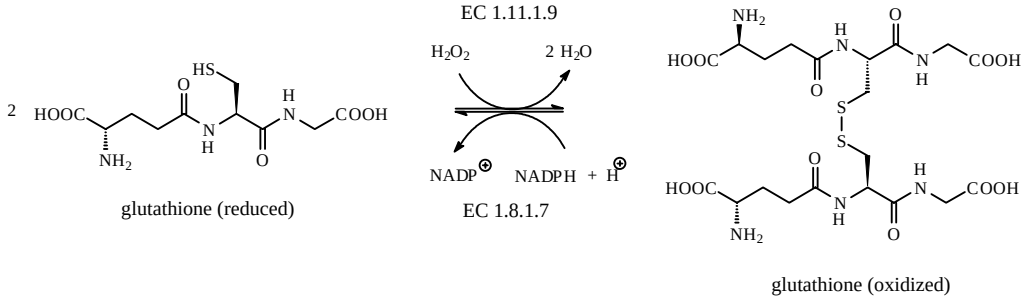
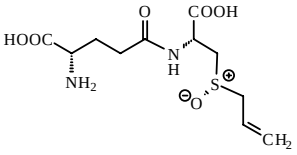


Figure 2.2

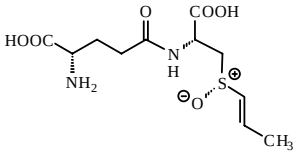
2.1.2 Other γ-glutamylpeptides

Although γ-glutamylpeptides are well represented in many organisms, their roles and properties are often not well understood yet. In some cases, plant γ-glutamylpeptides play a role in the transport of amino acids across the membranes, protect plant cells against phytotoxic heavy metals (they act as phytochelatins), or they may function as storage compounds of nitrogen and sulfur (in *Allium* and *Brassica* species), and may also represent a significant pool of bioactive organoselenium compounds.

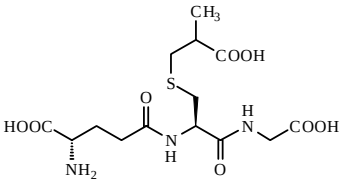
In *Allium* species, more than 20 γ-glutamylpeptides have been isolated, such as γ-glutamyl-*S*-alk(en)ylglutathiones, γ-glutamyl-*S*-alk(en)ylcysteines, and γ-glutamyl-*S*-alk(en)ylcysteine sulfoxides. Nine of these peptides occur as intermediates in the biosynthesis of *S*-alk(en)ylcysteine sulfoxides, including γ-glutamyl-*S*-allylcysteine sulfoxide in garlic. (*E*)-γ-Glutamyl-*S*-(prop-1-en-1-yl)cysteine sulfoxide and *S*-(2-carboxypropyl)glutathione occur in onion (Figure 2.3). In the prebulbing onions, these two peptides were found below 50 mg/kg fresh weight and at the bulbing onions they accumulated at the amount of 2100 and 400 mg/kg fresh weight, respectively. These amounts were maintained throughout storage and decreased by 50% during sprouting.



γ-glutamyl-*S*-allyl-L-cysteine sulfoxide



(*E*)-γ-glutamyl-*S*-(prop-1-en-1-yl)-L-cysteine sulfoxide



S-(2-carboxypropyl)glutathione

Figure 2.3

γ-Glutamylpeptides are formed in reactions catalyzed by the enzyme L-γ-glutamyl-transferase (EC 2.3.2.2). This enzyme catalyzes the splitting of the γ-glutamyl linkages in γ-glutamylpeptides (e.g. GSH) and the transfer of the glutamate moiety to amino acids, peptide acceptors, or water. For example, this enzyme catalyzes the reaction in which glutathione (GSH, acting as a donor) reacts with an acceptor amino acid yielding the corresponding dipeptide (γ-glutamylamino acid) (Figure 2.4). Such γ-glutamylpeptides may be formed by the reaction of GSH with the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (see Chapter 1.7.3.1) in tomato and hypoglycin A (see Chapter 1.7.3.2) in ackee fruit, respectively.

Most eukaryotic organisms posses Δ^9-desaturase that introduces a *cis*-double bond (by abstraction of the *pro-R* hydrogens from C-9 and C-10) into a saturated fatty acid at C-9 (Figure 3.9, the names are given for the appropriate fatty acid, the structures shown represent the thioesters involved in the conversions).

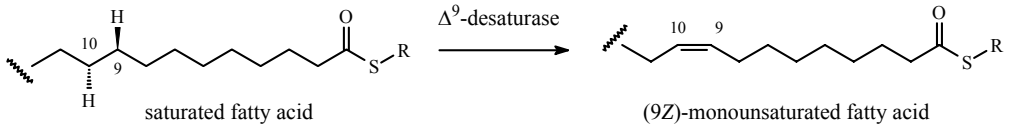


Figure 3.9

A palmitoyl and stearoyl thioesters are the preferred substrates for the mammalian stearoyl-CoA desaturase [3.6] (EC 1.14.19.1) and plant stearoyl-ACP desaturase (EC 1.14.19.2) that generate (9Z)-hexadec-9-enoic (palmitoleic) and (9Z)-octadec-9-enoic (oleic) acids [3.7], respectively (Figure 3.10). Both palmitoleic and oleic acid then become the dominant storage form of fatty acids in animal adipose tissue and are used for the synthesis of a variety of other long-chain unsaturated acids.

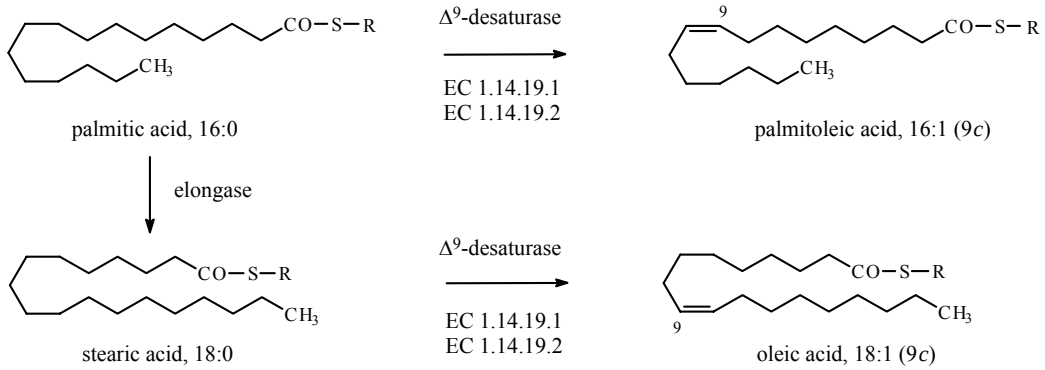


Figure 3.10

A number of plant species produce unusual fatty acid positional isomers. Chain elongation of palmitoleic acid towards carboxyl terminus yields (11Z)-octadec-11-enoic (vaccenic or asclepic) acid, the minor fatty acids of many seed oils and animal fats as the constituents of their triacylglycerols (Figure 3.11). Seeds of the Apiaceae family plants, commonly used as spices, such as parsley (*Petroselinum crispum*), fennel (*Foeniculum vulgare*), and coriander (*Coriandrum sativum*), are known to contain high levels (about

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3.6) The mammalian Δ^9-desaturase is a part of electron transport, which involves a non-heme iron enzyme (Fe^{2+}/Fe^{3+}), cytochrome b_5 and NADH-dependent cytochrome-b_5 reductase (EC 1.6.2.2) that reduces the formed ferricytochrome b_5. The plant Δ^9-desaturase requires ferredoxin.

3.7) A conventional shorthand representation for palmitoleic and oleic acids is C16:1 (9*c*) and C18:1 (9*c*), respectively. A less systematic numbering starting from the methyl (the ω end) may be also encountered. Major groups of fatty acids are then designated ω-3, ω-6, ω-9, etc. (or more correctly n-3, n-6, n-9), if there is a double bond that number of carbons from the methyl terminus.

50%) of a C-6 positional isomer of oleic acid, petroselinic acid, (6*Z*)-octadec-6-enoic acid, which represents an important oleochemical material for the food, cosmetic, chemical, and pharmaceutical industries. Its formation from palmitic acid is catalyzed by a Δ^4-palmitoyl-ACP desaturase and proceeds via (4*Z*)-hexadec-4-enoic acid. Chain elongation towards carboxyl terminus then yields petroselinoyl thioester, which is hydrolyzed to petroselinic acid. The seed oil of black-eyed Susan vine (*Thunbergia alata*; Acanthaceae) has an unusual fatty acid composition, which consists of more than 80% of the unsaturated fatty acid (6*Z*)-hexadec-6-enoic acid. This fatty acid forms from palmitic acid by the action of Δ^6-palmitoyl-ACP desaturase in the plastid.

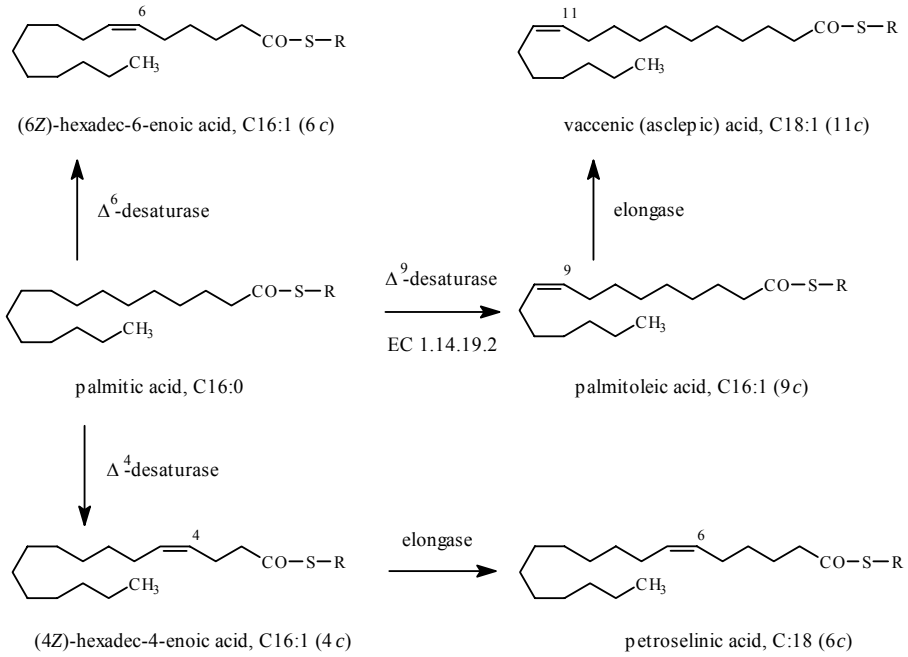

Figure 3.11

Seed oils of some plants are rich in triacylglycerols containing very-long-chain and ultra-long-chain monounsaturated fatty acids belonging to the ω-9 group. They are derived from oleic acid (the most common fatty acid from nearly all seed oils formed by *de novo* synthesis) by a series of chain elongation reactions towards carboxyl terminus (Figure 3.12). The first product of this elongation is (11*Z*)-eicos-11-enoic (gondoic) acid, which is further elongated to (13*Z*)-docos-13-enoic (erucic) acid, (15*Z*)-tetracos-15-enoic (nervonic or selacholeic) acid, and (17*Z*)-hexacos-17-enoic (ximenic) acid. Some of these fatty acids are of considerable interest as renewable raw materials for oleochemicals. Developing seeds of some plants belonging to the Brassicaceae family have been shown to synthesize high amounts of erucic and nervonic acids. For example, erucic acid is found in high amounts (up to 50%) in seed oils of crambe (*Crambe abyssinica*) and white mustard (*Brassica alba*). Nervonic acid (together with erucic acid) is present in high amounts (22-25%) in honesty (*Lunaria annua*) seed oils used e.g. for production of high-temperature lubricants and engineering polyamide fibers, similar to nylon.

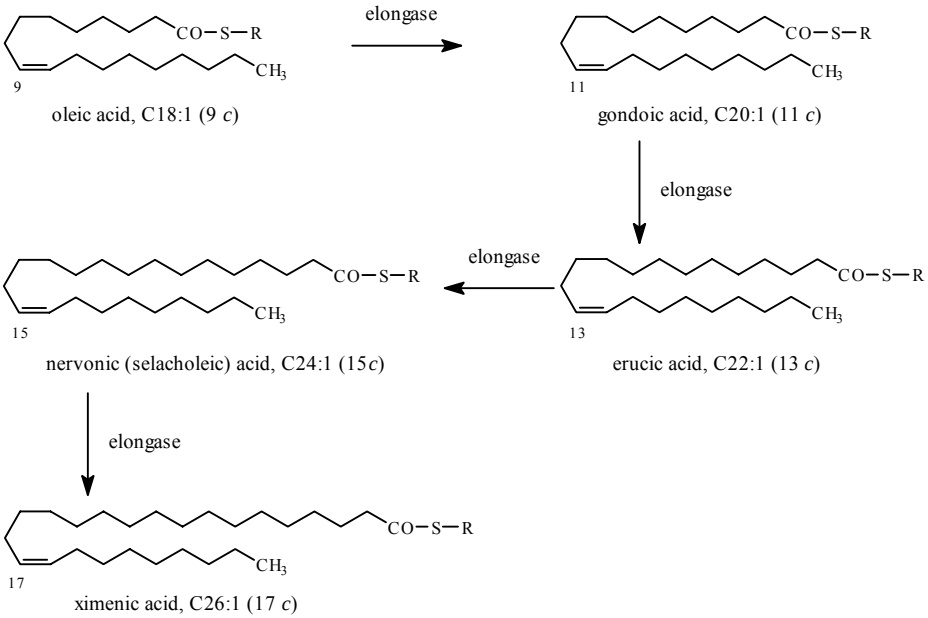

Figure 3.12

3.1.3.3 Long-chain polyunsaturated fatty acids

Polyunsaturated fatty acids are usually fatty acids of 18 carbon atoms or more that contain two or more *cis*-double bonds in a non-conjugated array (isolated double bonds, isolene-type fatty acids) as a repeating unit -(CH=CH-CH_2)$_n$- (i.e. they have a methylene group inserted between the two *cis*-double bonds). They are formed by sequential desaturation and fatty acyl elongation reactions. The position of further desaturation then depends very much on the organism. Animal enzymes introduce new *cis*-double bonds towards the carboxyl group (mammalian systems dispose Δ^9-, Δ^6-, Δ^5-, and Δ^4-desaturases, a minimum chain length of 16-18 carbons is required) [3.8)], but never beyond C-9, with *cis*-configuration. Besides, plant and fungal enzymes tend to introduce additional *cis*-double bonds between the existing double bond and the methyl terminus (Δ^{12}- and Δ^{15}-desaturases).

Accordingly, oleic acid is further desaturated to (6*Z*,9*Z*)-octadeca-6,9-dienoic acid (Δ^6-desaturase) in mammals, but to (9*Z*,12*Z*)-octadeca-9,12-dienoic (linoleic) acid (Δ^{12}-desaturase, plastidal oleate desaturase, EC 1.14.99.-) and further to linolenic (α-linolenic) acid, (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid (Δ^{15}-desaturase, plastidal linoleate desaturase, EC 1.14.99.-), in plants and fungi [3.9)]. The inability of animal systems to desaturate closer to the methyl terminus than C-9 renders them unable to convert palmitic

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[3.8)] Invertebrates (e.g. insect) produce a large number of compounds, derived from unusual fatty acid, as sex pheromones using different desaturases (e.g. Δ^{11Z}- and Δ^{11E}-desaturase).

[3.9)] In so called 16:3 plants (e.g. cocoa bean tissue, *Theobroma cacao*, Sterculiaceae), α-linolenic acid is also biosynthesized by desaturation of dodecanoic (lauric) acid to (3*Z*,6*Z*,9*Z*)-dodeca-3,6,9-trienoic acid followed by chain elongation.

acid to linoleic acid or α-linolenic acid. Accordingly, linoleic and α-linolenic acid are referred to as essential fatty acids since they cannot be synthesized *de novo* and must be obtained from plant material in the diet (Figure 3.13).

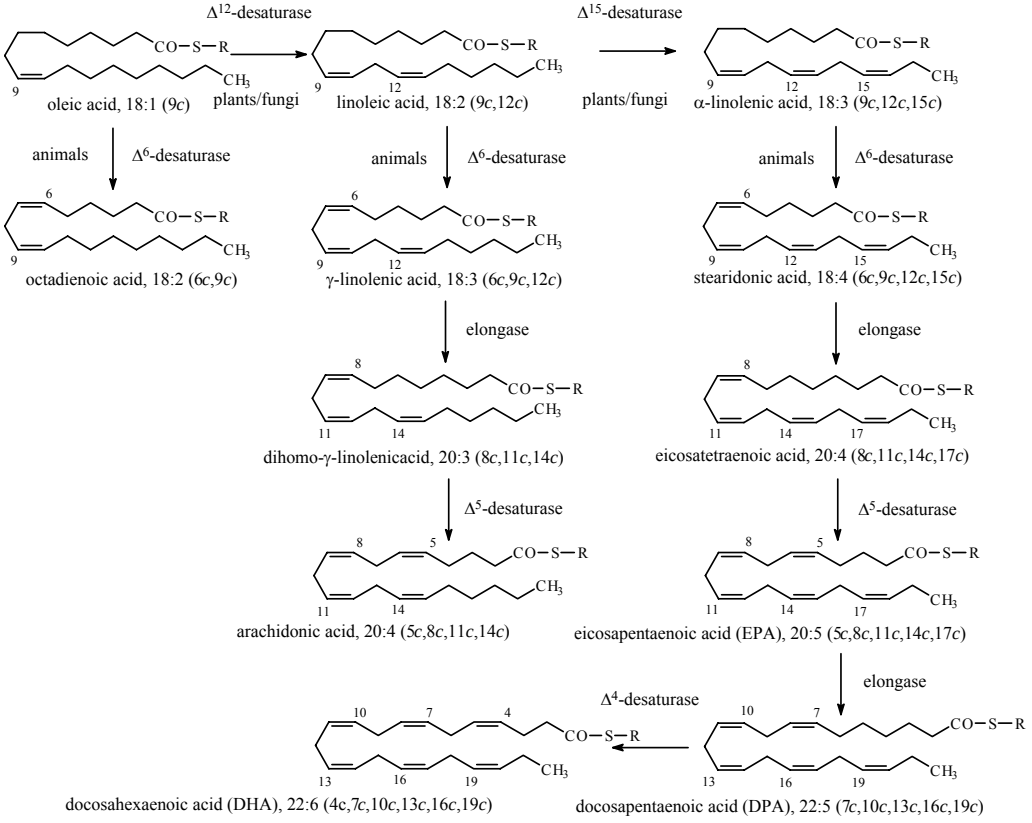


Figure 3.13

Animals need linoleic acid for the biosynthesis of two other fatty acids of the so called ω-6 (or n-6) group, i.e. (8*Z*,11*Z*,14*Z*)-eicosa-8,11,14-trienoic (dihomo-γ-linolenic) acid and (5*Z*,8*Z*,11*Z*,14*Z*)-eicosa-5,8,11,14-tetraenoic (arachidonic) acid that are synthesized via γ-linoleic acid by the action of Δ^6-desaturase (linoleoyl-CoA desaturase, EC 1.14.19.3). These polyunsaturated C_{20} fatty acids become the precursors of prostaglandins of the 1- and 2-series and other biologically active compounds (see Chapter 3.1.3.3). Similarly, α-linolenic acid, belonging to the so called ω-3 (or n-3) group of poly-unsaturated fatty acids, is the starting compound on the way to (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapenta-5,8,11,14,17-enoic acid (EPA), the precursor of prostaglandins of the 3-series [3.10)].

3.10) Marine algae also contain some homoallylic polyunsaturated compounds as their major fatty acids. For example, arachidonic acid occurs in brown and red algae, EPA in diatoms and some brown and red algae, stearidonic acid in a cryptomonas and some green and brown algae. This high level of unsaturation is

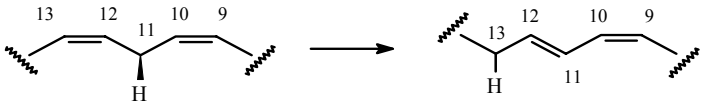
3.1.4 Less common fatty acids

Under appropriate conditions (during biohydrogenation as well as under heating and metal-catalyzed hydrogenation), *cis*-double bonds in natural unsaturated fatty acids tend to isomerize to thermodynamically more stable *trans*-double bonds. These processes also produce unusual positional fatty acid isomers. Some fats contain one or more acetylenic bonds. Branched-chain fatty acids form by using methylmalonyl-CoA or 2-oxoacids, from the amino acid syntheses, instead of malonyl-CoA as the chain-extending unit. Fats also contain branched-chain fatty acid originating by the breakdown of terpenoids. Some plants produce seed oils of unusual structure, which includes variation of chain length or acetylenic, cycloprop(en)yl, cyclopent(en)yl, epoxy, hydroxy, and oxo functional groups. Many of these fatty acids have a wide range of pharmacological and industrial uses.

3.1.4.1 Geometrical and positional isomers of unsaturated fatty acids

Unsaturated fatty acids with unusual structures (geometrical and positional isomers of common unsaturated fatty acids) are those with one *trans*-double bond and/or conjugated (non-methylene-interrupted) double bonds. They are formed in low concentrations on microbial hydrogenation (biohydrogenation) in the stomach of ruminants from dietary unsaturated fatty acids [3.11)] and are consequently found in milk and meat. Biohydrogenation of unsaturated fatty acids requires a free acid to proceed and the product is then absorbed and incorporated into ruminant fat.

The isomerization starts by elimination of the *pro-S* hydrogen from the C-11 carbon, the (12*Z*)-double bond then moves into conjugation with the (9*Z*)-double bond via an allylic isomerization giving more stable (*E*)-configuration at C-11 (Figure 3.14).



(9*Z*,12*Z*)-9,12-dienoic fatty acid (9*Z*,11*E*)-9,11-dienoic fatty acid

Figure 3.14

The major substrates are linoleic and α-linolenic acids, which are hydrogenated in the rumen [3.12)] to the extent of 70-95% and 85-100%, respectively. The major biochemical pathways for the biohydrogenation of these acids are illustrated in Figure 3.15. The initial

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presumably to assist maintaining the membrane fluidity in cold seawater. Arachidonic acid is also widespread in ferns and mosses.

3.11) When dietary lipids enter the rumen, the initial step of metabolism is the hydrolysis of the ester linkages found in triacylglycerols, phospholipids, and glycolipids. Hydrolysis of dietary lipids is predominantly due to rumen bacteria with little evidence for a significant role by rumen protozoa and fungi or salivary and plant lipases.

3.12) The rumen bacteria involved in biohydrogenation have been classified into two groups based on their metabolic pathways. To obtain complete biohydrogenation of polyunsaturated fatty acids, bacteria from both groups are generally required.

step typically involves an isomerization of the (12*Z*)-double bond to a (11*E*)-double bond resulting in conjugated di- and trienoic fatty acids. Next is a reduction of the (9*Z*)-double bond resulting in a (11*E*)-fatty acid. The final step is a further hydrogenation of the (11*E*)-double bond producing stearic acid (linoleic and α-linolenic acid pathways) or (15*E*)-octadec-15-enoic acid (α-linolenic acid pathway). The extent to which the various pathways of biohydrogenation are associated with specific enzymes and species of bacteria is unknown.

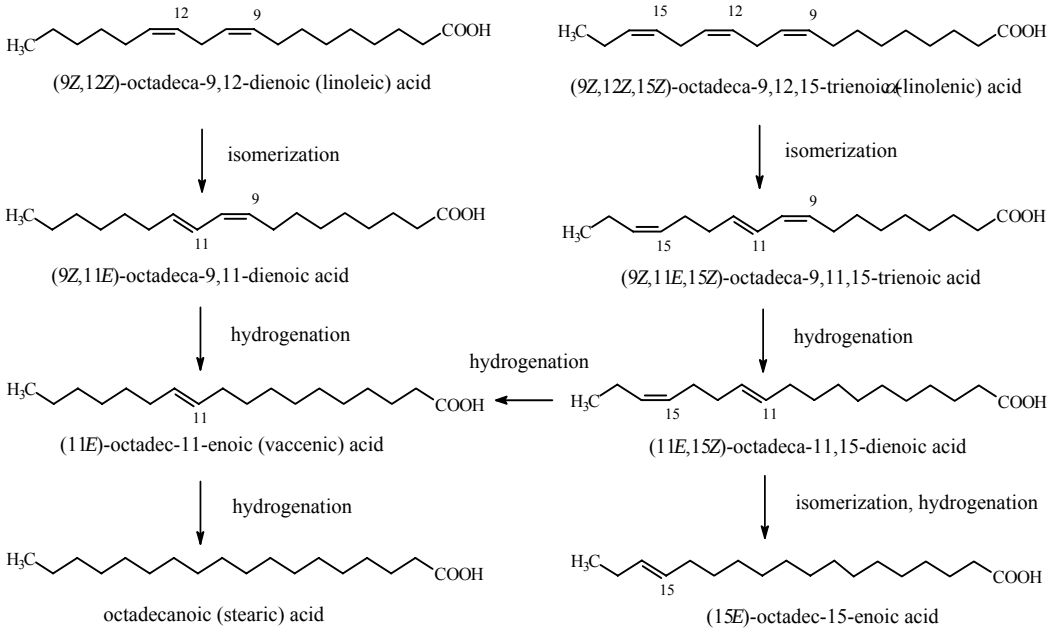


Figure 3.15

The key biohydrogenation intermediates are (11*E*)-octadec-11-enoic (*trans*-vaccenic) acid, which is formed from linoleic and α-linolenic acids, and (9*Z*,11*E*)-octadeca-9,11-dienoic acid, so called conjugated linoleic acid (CLA) [3.13)], formed in the biohydrogenation of α-linolenic acid. These intermediates are present in appreciable quantities in ruminant fat at a ratio of about 3:1. (9*Z*,11*E*)-Octadeca-9,11-dienoic acid is also formed by desaturation of vaccenic (asclepic) acid in the mammary gland via Δ^9-desaturase.

Conjugated linoleic acids have been reported to have a wide range of beneficial effects (anticarcinogenic, antiatherogenic, antidiabetic, and immune stimulatory). Studies have established that the (9*Z*,11*E*)-octadeca-9,11-dienoic acid typically represents more than 90% of total conjugated linoleic acid present in milk fat and over 75% of that present in beef fat.

[3.13)] This collective term is used to describe positional and geometric isomers of linoleic acid with conjugated double bonds. The major conjugated fatty acids arising from linoleic acid are (9*Z*,11*E*)-octadeca-9,11-dienoic acid and (10*E*,12*Z*)-octadeca-10,12-dienoic acid. Minor products are (8*E*,10*Z*)-, (9*Z*,11*Z*)-, (9*E*,11*E*)-, (10*Z*,12*Z*)-, (10*E*,12*E*)-octadeca-10,12-dienoic acid and some other isomers.

Many other *trans*-unsaturated fatty acids, including (9*E*)-octadec-9-enoic (elaidic) acid, its positional isomers, and isomers of conjugated linoleic acid, have been found in the ruminal outflow. A portion of *trans*-octadecenoic acids found in ruminant fat may be derived from (9*Z*)-octadec-9-enoic (oleic) acid or may originate in the mammary gland and adipose tissue from endogenous synthesis involving Δ^9-desaturase with rumen-derived *trans*-vaccenic acid as the substrate [3.14)].

Seed oils of a few plant species are also rich in conjugated fatty aids derived from oleic and linoleic acids. Dimorphecolic acid, (10*E*,12*E*)-9-hydroxyoctadeca-10,12-dienoic acid, is the major fatty acid in seeds of *Dimorphotheca* species (one of eight genera of the Calenduleae tribe; Asteraceae). Dimorphecolic acid is derived from oleic acid by the concerted activity of *trans*-Δ12-oleic acid desaturase and Δ9-fatty acid conjugase via (9*Z*,12*E*)-octadeca-9,12-dienoic acid. Calendic acid, (8*E*,10*E*,12*Z*)-octadeca-9,11,13-trienoic acid, is formed by (8,11)-linoleoyl desaturase as the major fatty acid (50-60%) in the seed oil of pot marigold, also known as English marigold (*Calendula officinalis*; Asteraceae). Punicic acid, (9Z,11E,13Z)-octadeca-9,11,13-trienoic acid, forms by the action of (11,14)-fatty acid desaturase and accounts for 40% of the total seed oil of the herb *Trichosanthes kirilowii* (Cucurbitaceae) used in traditional Chinese medicine. It also occurs in the pomegranate (*Punica granatum*; Lythraceae) seeds at the amount of 60%. α-Eleostearic acid, (9*Z*,11*E*,13*E*)-octadeca-9,11,13-trienoic acid, occurs in a large quantity (80%) in tung oil (also called China wood oil or nut oil) that is made from the nuts of the tung tree (*Vernicia fordii*, syn. *Aleurites fordii*; Euphorbiaceae). Its formation is catalyzed by fatty acid conjugase. Tung oil has traditionally been used in lamps in China. In modern times, it is used as an ingredient in paint, varnish, and caulk (Figure 3.16).

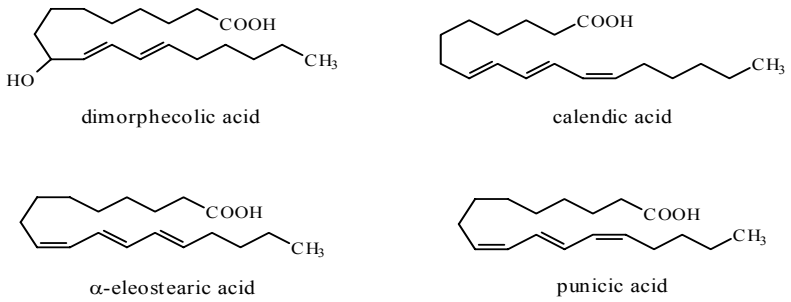


Figure 3.16

3.1.4.2 Acetylenic fatty acids

Many unsaturated fatty acids found in nature contain one or more acetylenic bonds. Acetylenic (alkynoic or ethynoic) fatty acids are widespread in mosses and liverworts and they appear to be common in tropical plants particularly in the families Santalaceae and Olacaceae. They also occur in a number of plants that belong to the Asteraceae, Caesalpiniaceae, and other families.

[3.14)] The positional isomers of octadecenoic acid (having their *trans*-double bond at carbons from C-4 to C-16) and the positional isomers of octadecadienoic acid (conjugated linoleic acid), i.e. (7*E*,9*Z*)-, (7*E*,9*E*)-, (8*E*,10*Z*), (8*E*,10*E*)-, (9*Z*,11*Z*)-, (9*Z*,11*E*)-, (9*E*,11*E*)-, (10*E*,12*Z*)-, (10*E*,12*E*)-, (11*Z*,13*E*)-, (11*E*,13*E*)-, (12*Z*,14*E*)-, and (12*E*,14*E*)-.

Acetylenic fatty acids are predominantly formed by further desaturation of olefinic systems in unsaturated fatty acid molecules. For example, desaturation of oleic acid at C-9 thus yields octadec-9-ynoic (stearolic) acid (18:1, 9*A*), which is present in seeds of Santalaceae (*Exocarpus*, *Santalum, Ximenia*) plants (Figure 3.17). Desaturation of linoleic acid at C-9 by crepenynate synthase (EC 1.14.99.33) produces (9*Z*)-octadec-9-en-12-ynoic (crepeninyc) acid (18:2, 9*Z*,12*A*) that is found in high concentration in the seed oil of *Ixiolaena brevocompta* (Asteraceae). Toxic effect of this plant seeds has been reported in Australian sheep. Crepeninyc acid is then desaturated at C-14 to yield (9*Z*,*14Z*)-octadec-9,14-dien-12-ynoic (dehydrocrepeninyc) acid (18:3, 9*Z*,12*A*,14*Z*). Both these fatty acids occur e.g. in triacylglycerols of the aril and cotyledon oils of *Afzelia cuanzensis* (Caesalpiniaceae) (65% of total fatty acids). Further desaturation to (9*Z*)-octadec-9-en-12,14-diynoic acid and its chain shortening by β-oxidation then yields short-chain acetylenic acids. Isomerization of crepeninyc acid gives (11*E*)-octadec-11-en-9-ynoic (santalbic) acid (18:2, 9*A*,11*E*), also known as ximeninic acid. This acid occurs in the seed oil from several species of the genera *Exocarpus*, *Santalum*, and *Ximenia* (Santalaceae), of which some seeds are eaten as Australian bush foods. For example, ximeninic acid is the major fatty acid (40%) in the seed oil of *Santalum spicatum* seeds.

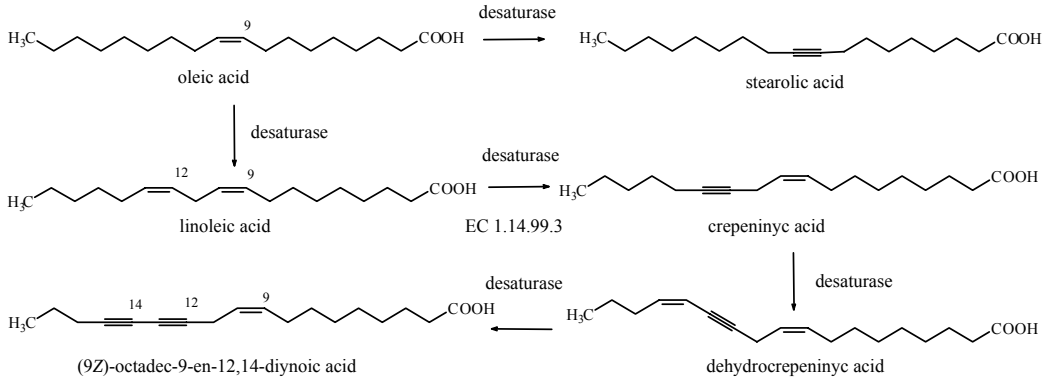


Figure 3.17

The metabolic effect of these unusual fatty acids on animals or humans is not well known. It was proposed that they interfere with the metabolism of lipids and fatty acids by inhibiting cyclooxygenase and lipoxygenase enzymes.

3.1.4.3 Branched-chain fatty acids

Branched-chain fatty acids predominantly occur in mammalian fats. They are also characteristic constituents of the lipid part of cell walls in some pathogenic bacteria. Several mechanisms appear to operate in their formation. Methyl side-chains can be introduced when methylmalonyl-CoA replaces malonyl-CoA as the chain-extending unit. Methylmalonyl-CoA arises by biotin-dependent carboxylation of propionyl-CoA, catalyzed by propionyl-CoA carboxylase (EC 6.4.1.3), in exactly the same way as malonyl-CoA is formed. Thus, 2,4,6,8-tetramethyldecanoic acid is produced from an acetyl-CoA starter and four methylmalonyl-CoA chain extender units (Figure 3.18). For example, it occurs in the preen gland wax of the goose (*Anser anser*). It has been shown

that some branched- and medium straight-chain fatty acids of tomato (*Solanum lycopersicum*; Solanaceae) arise from acetyl-CoA elongation of 2-oxoacids acting as the precursors of amino acids, without the involvement of fatty acid synthase mediated reactions, suggesting integration of amino acid and fatty acid metabolism (Figure 3.19). The enzymes involved include 2-isopropylmalate synthase (EC 2.3.3.13), 3-isopropylmalate dehydratase (EC 4.2.1.33), 3-isopropylmalate dehydrogenase (EC 1.1.1.85), and enzymes acting in the transformation of acyl-CoA thioesters.

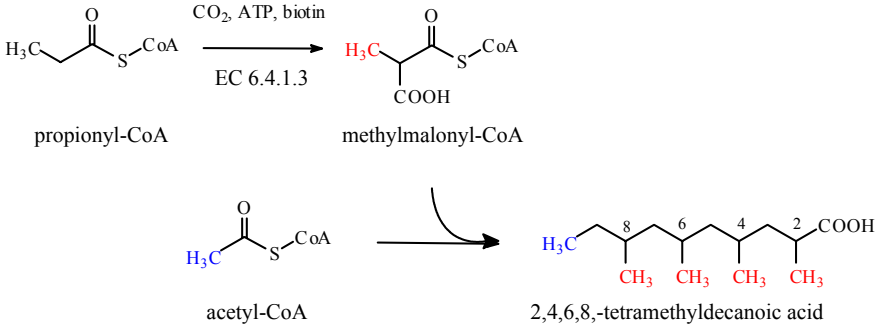


Figure 3.18

(3*RS*,7*R*,11*R*,15)-3,7,11,15-Tetramethylhexadecanoic (phytanic) acid in the mammalian diet is derived via microbial cleavage of chlorophyll followed by reduction/oxidation of the resultant phytol, i.e. (2*E*,3,7*R*,11*R*,15)-3,7,11,15-tetramethylhexadec-2-ene-1-ol (see Chapter 6.5.2), side-chain (Figure 3.20). Phytanic acid occurs in dairy products (e.g. in butter fat) and other ruminant fats as a mixture of (3*RS*)-epimers.

Due to the presence of a methyl group at it's β-position, the β-oxidation pathway cannot degrade phytanic acid. Instead, its α-methylene group is oxidatively excised to give pristanic acid, which can be metabolized by the β-oxidation pathway [3.15]. Both epimers of phytanic acid are first converted to phytanoyl-CoA by the action of a non-specific phytanoyl-CoA ligase (EC 6.2.1.24), which is hydroxylated at C-2 (phytanoyl-CoA 2-hydroxylase, EC 1.14.11.18, requires Fe^{2+} and ascorbic acid) to two *threo*-stereoisomers. The (3*R*)-epimer is hydroxylated to (2*S*,3*R*)-product and the (3*S*)-epimer to (2*R*,3*S*)-product. The steps in the pathway subsequent to that catalyzed by phytanoyl-CoA hydroxylase are less well characterized. It is believed that 2-hydroxyphytanoyl-CoA is then converted to pristanic acid via two enzymatic steps. In the first step, both 2-hydroxyphytanoyl-CoA epimers undergo cleavage (by peroxisomal α-oxidation using thiamine diphosphate-dependent enzyme 2-hydroxyphytanoyl-CoA lyase) into the corresponding pristanal epimers and formyl-CoA, which rapidly hydrolyzes to formic acid and HS-CoA. Both pristanal epimers, (2*RS*)-pristanal, are subsequently oxidized to

[3.15] Phytanic acid is normally present in small amounts in human tissues. Many defects in the α-oxidation pathway, including Refsum´s disease, result in an accumulation of phytanic acid leading to neurological distress, deterioration of vision, deafness, loss of coordination, and eventually death.

(2*RS*,6*R*,10*R*)-tetramethylpentadecanoic (pristanic) acid via an aldehyde dehydrogenase (pristanal dehydrogenase, EC 1.2.1.3).

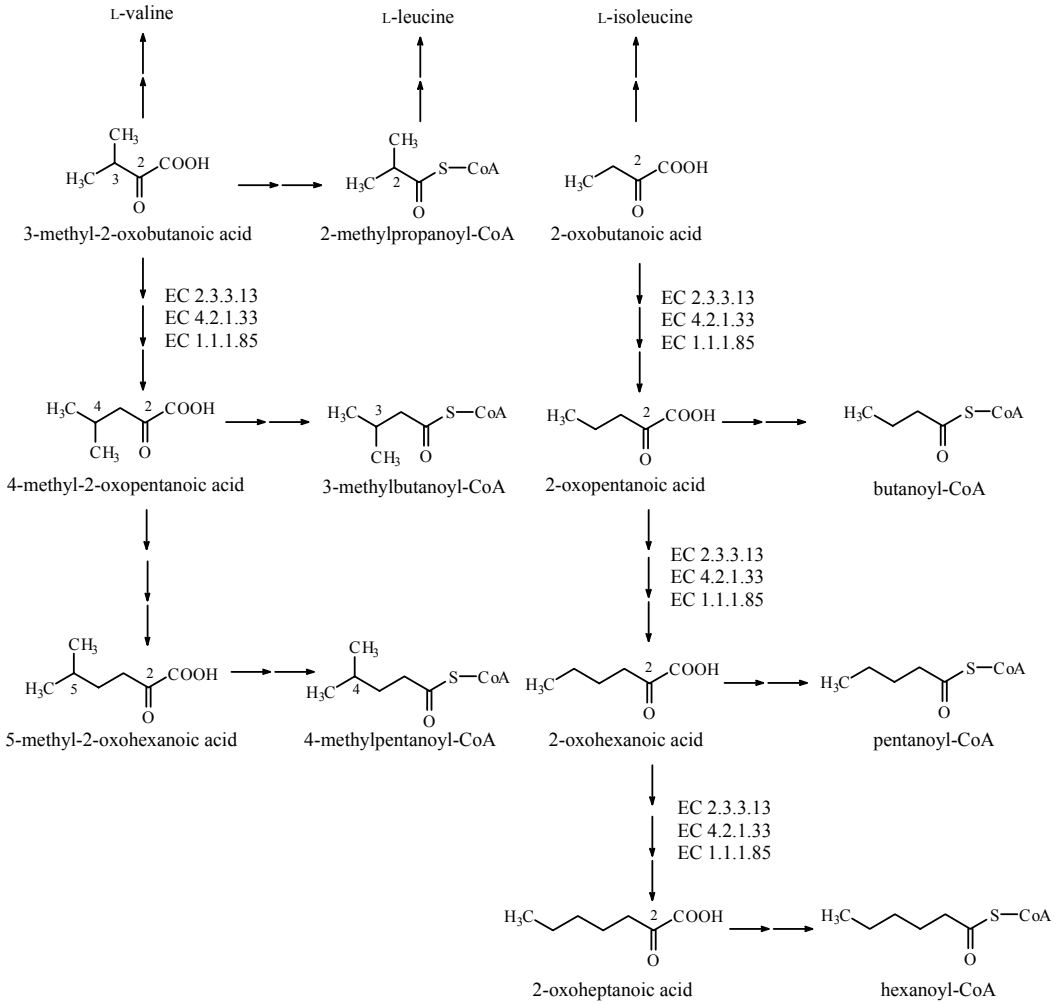


Figure 3.19

The activation of pristanic acid by acyl-CoA synthetase (long-chain fatty acyl-CoA synthetase, EC 6.2.1.3) gives a mixture of (2*R*,*S*)-pristanoyl-CoA epimers. Conversion of the (2*R*)-epimer of pristanoyl-CoA to the (2*S*)-epimer by α-methylacyl-CoA epimerase (also known as 2-methylacyl-CoA 2-racemase, EC 5.1.99.4) allows further oxidation of the (2*S*)-epimer via peroxisomal β-oxidation system [3.16)] to 2-methylpropionyl-CoA, propionyl-CoA, and acetyl-CoA.

3.16) Peroxisomes are not only able to metabolize the 2-hydroxyphytanic-CoA, but also the free acid itself via 2-oxophytanic acid (hydroxyphytanate oxidase, EC 1.1.3.27), which is further decarboxylated (e.g. by branched-chain-2-oxoacid decarboxylase, EC 4.1.1.72) to pristanic acid.

3.1.4.4 Alicyclic fatty acids

Cyclopropane and cyclopropene fatty acids are found in the phospholipids of many plants, bacteria, and parasitic protozoa. Cyclopropene fatty acids, such as sterculic and malvalic acids, are present in the seed oil (used for technical purposes) from *Sterculia foetida* (Sterculiaceae), malvalic acid also occurs in the edible cottonseed oil (*Gossypium* species; Malvaceae). Dihydrosterculic acid was found in the seed oil (35-48%) of lychee (*Litchi sinensis*) and longan fruits (*Dimocarpus longan*) from the Sapindaceae family.

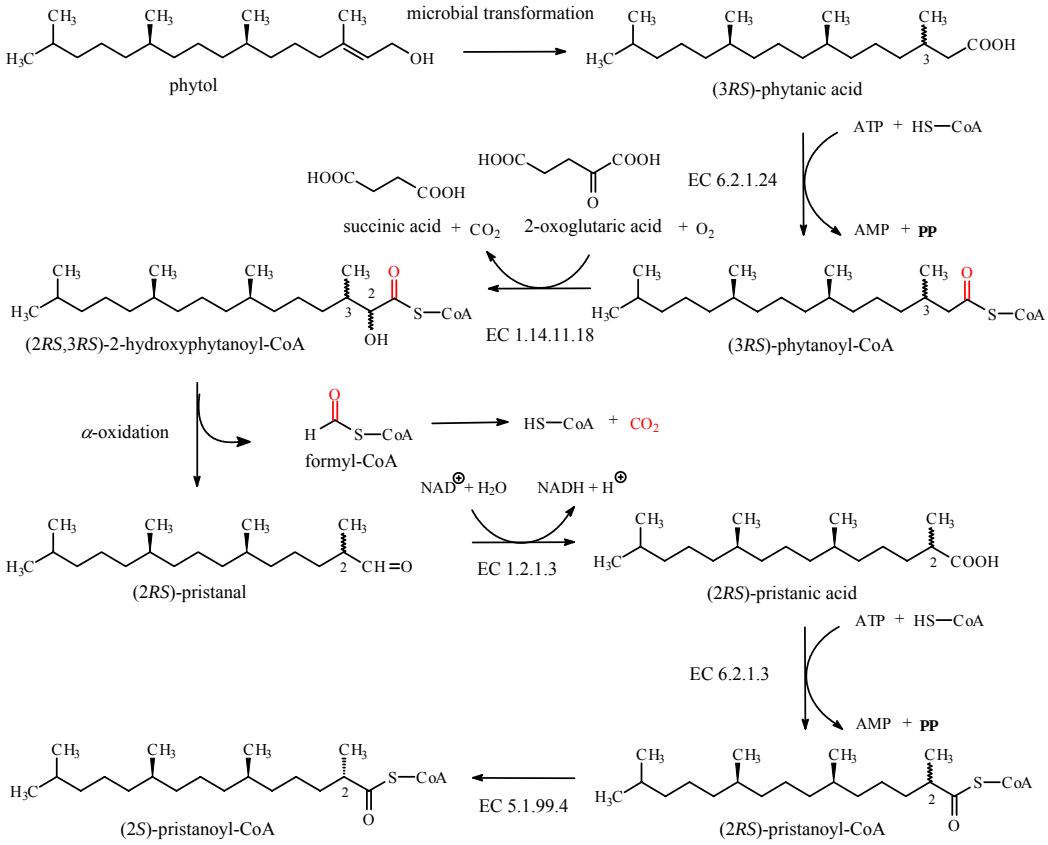


Figure 3.20

Cyclopropane and cyclopropene fatty acids are derived from oleic acid bound in glycerophospholipids, such as (3-*sn*-phosphatidyl)ethanolamine (and to lesser extent in phosphatidylglycerol or phosphatidylinositol), by *C*-methylation (electrophilic addition) with *S*-adenosylmethionine (SAM or AdoMet) as the alkylating agent, to the *cis* double bond) on C-10, initiated by the double bond electrons. The reaction is catalyzed by cyclopropane fatty acid synthetase (EC 2.1.1.79). Loss of a proton from the postulated carbocation intermediate, via cyclopropane ring formation, gives dihydrosterculic acid, (9*R*,10*S*)-8-(octacyclopropyl)octanoic acid, which is dehydrogenated by a desaturase to sterculic acid, (*Z*)-8-(octylcycloprop-2-ene-1-yl)octanoic acid. Malvalic acid, (*Z*)-7-

(octylcycloprop-2-ene-1-yl)heptanoic acid, is produced from sterculic acid by chain shortening from the carboxyl end by α-oxidation (Figure 3.21).

Plants lack an immune system in the sense that it exists in animals, but they possess mechanisms that recognize potential pathogens and initiate defense responses. It has become evident that various types of oxygenated fatty acids, collectively termed oxylipins or sometimes octadecanoids, are involved in responses to physical damage (by animals or insects), stress, and attack by pathogens. These compounds are similar in many ways to the eicosanoids derived from arachidonic acid in animals, which have so many varied functions, but especially in the inflammatory process. Compounds derived from cyclopentyl fatty acids, e.g. jasmonic acid and methyl jasmonate, collectively referred to as jasmonates, are discussed in Chapter 3.4.

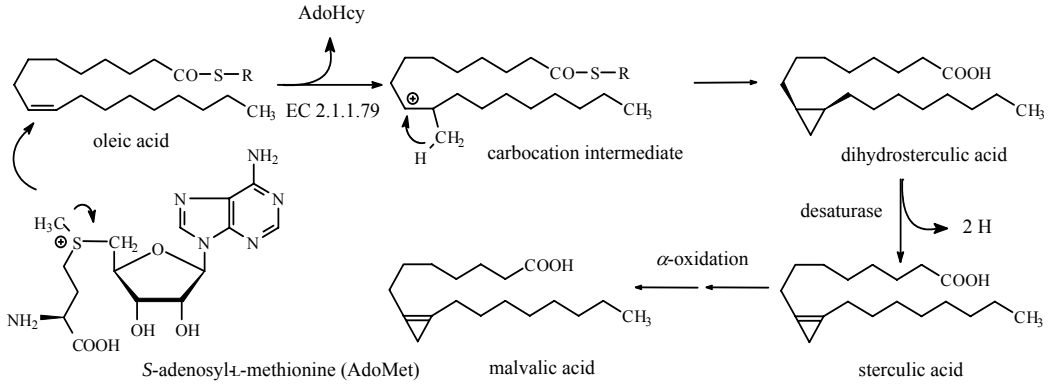


Figure 3.21

Fatty acids with a terminal cyclopent-2-en-1-yl ring are found in seed oils from many species of the plant family Flacourtiaceae. Chaulmoogra oil, expressed from the seeds of *Hydnocarpus wightiana*, provided for many years the only treatment for the relief of leprosy. The three most common fatty acids are hydnocarpic acid, (11*R*)-11-(cyclopent-2′-en-1′-yl)hendecanoic acid, its higher homologue chaulmoogric acid, (13*R*)-13-(cyclopent-2′-en-1′-yl)tridecanoic acid, and gorlic acid, (13*R*)-13-(cyclopent-2′-en-1′-yl)tridec-6-enoic acid. In addition, various cyclopentenyl unsaturated fatty acids and lower homologues of chaulmoogric acid are the minor components of most oils of this type, e.g. hormelic acid, (15*R*)-15-(cyclopent-2′-en-1′-yl)pentadec-9-enoic acid, alepric acid, (9*R*)-9-(cyclopent-2′-en-1′-yl)nonanoic acid, aleprylic acid (7*R*)-7-(cyclopent-2′-en-1′-yl)heptanoic acid, and aleprestic acid, (5*R*)-5-(cyclopent-2′-en-1′-yl)pentanoic acid, while (13*R*)-13-(cyclopent-2′-en-1′-yl)tridec-4-enoic acid is the main component of the seeds of *H. anthelmintica*.

Cyclopentenyl fatty acids (alternative starter units to acetyl-CoA) are known to arise by malonate chain extension of the coenzyme A ester of aleprolic acid, (1*R*)-cyclopent-2′-en-1′-ylcarboxylic acid (Figure 3.22). The precursor of aleprolic acid is the non-proteinogenic amino acid 2-(cyclopent-2′-en-1′-yl)glycine (see Chapter 1.7.3.3). The conversion of this amino acid to aleprolic acid may occur via transamination and oxidative decarboxylation.

In the endoplasmic reticulum and in the peroxisomes, glycerone phosphate may be directly reduced to glycerol 3-phosphate by the action of various dehydrogenases, such as glycerol 3-phosphate dehydrogenase (NAD^+) (EC 1.1.1.8) and glycerol 3-phosphate dehydrogenase $NAD(P)^+$ (EC 1.1.1.94), or esterified to 1-acylglycerone 3-phosphate by glycerone phosphate *O*-acyltransferase (EC 2.3.1.42). 1-Acylglycerone 3-phosphate is then reduced to 1-acylglycerol 3-phosphate (3-*sn*-lysophosphatidic acid) by acylglycerone phosphate reductase (EC 1.1.1.101) or used for the biosynthesis of glycerophospholipids.

Alternatively, 2-acylglycerols produced by hydrolysis of dietary fats by lipases [3.21)], can also serve as substrates for the synthesis of 1,2-diacylglycerols [3.22)]. This transformation of 2-acylglycerols to 1,2-diacylglycerols is catalyzed by the action of 2-acylglycerol *O*-acyltransferase (EC 2.3.1.22) (Figure 3.32).

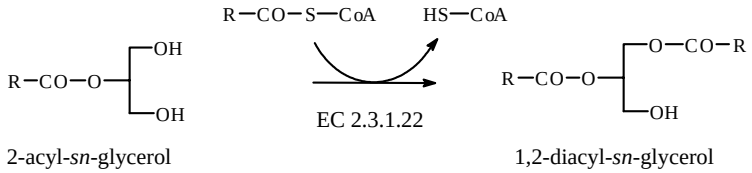


Figure 3.32

3.3.2 Glycerophospholipids

Glycerophospholipids constitute three groups of fatty acid esters. The first two group representatives are derived from either 3-*sn*-phosphatidic acid (phosphatides) or 3-*sn*-lysophosphatidic acid (lysophosphatides) (Figure 3.33).

The third group of glycerophospholipids is glycerol ether phospholipids or plasmalogens [3.23)]. There are two types of plasmalogens, alk-2-en-1-yl ethers derived from 3-*sn*-plasmenic acid and 1-alkyl ethers derived from 3-*sn*-plasmanic acid (Figure 3.34).

In animals and plants, glycerophospholipids are mostly synthesized in the endoplasmic reticulum and in the mitochondria by esterification of an alcohol to the phosphate of phosphatidic acid (1,2-diacyl-*sn*-glycerol 3-phosphate). The most commonly encountered alcohols in phosphatides and lysophosphatides are choline (*N*,*N*,*N*,-trimethylethanolamine), ethanolamine (2-aminoethanol), L-serine, *myo*-inositol, and glycerol. Plasmalogens are derived from choline, ethanolamine, and serine.

[3.21)] The human fat-digestive enzymes include triacylglycerol- and phospholipases. Triacylglycerol lipase catalyzes the hydrolysis of triacylglycerol to free fatty acid, mono- and diacylglycerol. The human lipases include the pre-duodenal lingual and gastric lipases (EC 3.1.1.3), extra-duodenal pancreatic and hepatic lipases, endothelial lipase (EC 3.1.1.3), and lipoprotein lipase (EC 3.1.1.34).

[3.22)] Various 2-acyl-*sn*-glycerols can act as acceptor. Palmitoyl-CoA and other long-chain acyl-CoAs can act as donors. The *sn*-1 position and the *sn*-3 position are both acylated, at about the same rate.

[3.23)] The term plasmalogen may be used as a generic term for glycerophospholipids in which the glycerol moiety bears an alk-1-en-1-yl ether group. The term plasmenic acid signifies a derivative of *sn*-glycerol 3-phosphate in which C-1 bears an *O*-(alk-1-en-1-yl) residue, and C-2 is esterified with a fatty acid. The terms plasmanic acid and plasmanyl may also be applied to ethers with an alkyl group bearing a double bond within the chain. In such cases, the proper term alkenyl, if used without the ene locant(s), would be misleading.

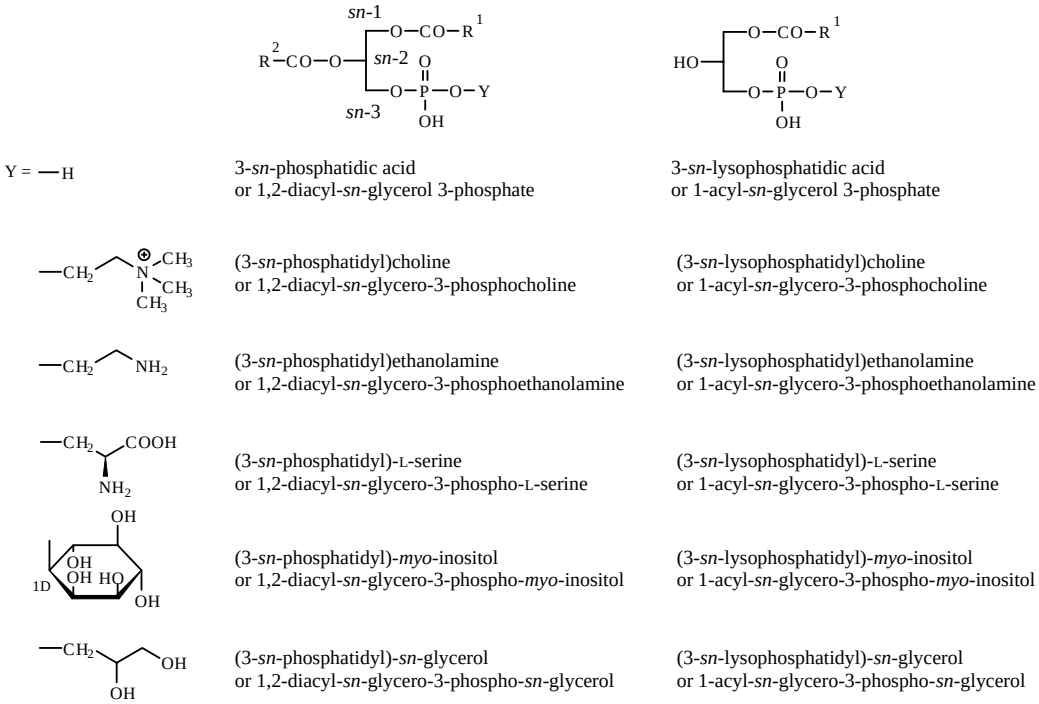


Figure 3.33

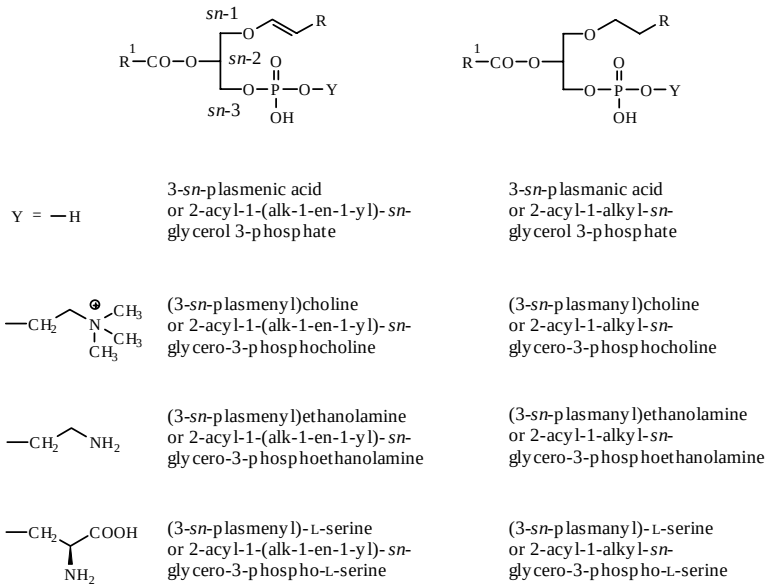


Figure 3.34

For example, non-starch phospholipids of wheat flour are composed of phosphatidylcholines (4.9%), *N*-acylphosphatidylethanolamines (4.9%), phosphatidylinositols (0.5%), phosphatidylglycerols (1.0%), lysophosphatidylcholines (1.5%), *N*-acyllyso-

phosphatidylethanolamines (1.7%), and lysophosphatidylglycerols (0.3%). Starch phospholipids of wheat flour comprise lysophosphatidylcholines (74.8%), lysophosphatidylethanolamines (9.9%), lysophosphatidylserines / lysophosphatidylinositols (2.5%), and lysophosphatidylglycerols (2.2%).

3.3.2.1 Phosphatides

Phosphatides can be synthesized by two mechanisms. One utilizes a CDP-activated alcohol (choline or ethanolamine) for the attachment to the phosphate moiety of phosphatidic acid. The other utilizes CDP-activated 1,2-diacylglycerol and an inactivated alcohol.

The alcohols are transferred to the CDP-activated alcohols in two steps (Figure 3.35). The first step, formation of the corresponding *O*-phosphoalcohol, is catalyzed by choline kinase (EC 2.7.1.32) and ethanolamine kinase (EC 2.7.1.82), respectively. In the second step, the *O*-phosphoalcohol is transformed to the corresponding CDP-alcohol by the action of CTP-phosphocholine cytidyltransferase (CDP-choline synthetase, EC 2.7.7.15) and CTP-phosphoethanolamine cytidyltransferase (CDP-ethanolamine synthetase, EC 2.7.7.14), respectively.

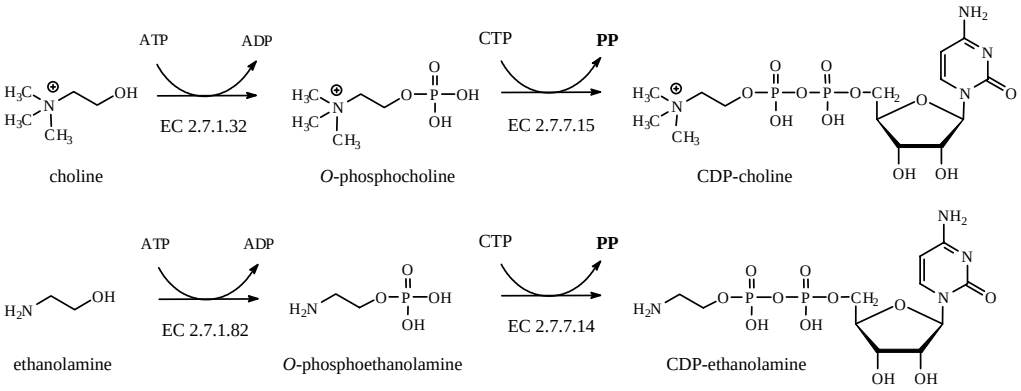


Figure 3.35

The CDP-activated 1,2-diacylglycerol is formed from phosphatidic acid in the reaction that is catalyzed by phosphatidate cytidyltransferase (CDP-diacylglycerol synthetase, EC 2.7.7.41) (Figure 3.36).

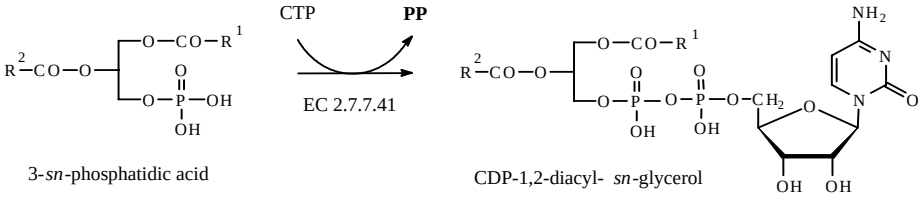


Figure 3.36

Synthesis of phosphatidylcholine (sometimes incorrectly called lecithin) can occur by three pathways. In the first pathway (Figure 3.37), CDP-choline is attached to diacylglycerol by diacylglycerol cholinephosphotransferase (EC 2.7.8.2). In the second pathway, phosphatidylcholine is synthesized by the addition of choline to CDP-activated 1,2-diacylglycerol by phosphatidylcholine synthase (EC 2.7.8.24). The third pathway involves the conversion of either phosphatidylserine or phosphatidylethanolamine to phosphatidylcholine (Figure 3.38). The conversion of phosphatidylserine to phosphatidylcholine first requires decarboxylation of phosphatidylserine by the pyridoxal phosphate protein phosphatidylserine decarboxylase (EC 4.1.1.65) to yield phosphatidylethanolamine; this then undergoes a series of three methylation reactions utilizing SAM as the methyl group donor. In the first methylation reaction catalyzed by phosphatidylethanol amine *N*-methyltransferase (EC 2.1.1.17), phosphatidylethanolamine yields phosphatidyl-*N*-methylethanolamine, which is then methylated by phosphatidyl-*N*-methylethanolamine *N*-methyltransferase (EC 2.1.1.71) to phosphatidyl-*N*,*N*-dimethylethanolamine. The enzyme also catalyzes the transfer of the third methyl group, producing phosphatidylcholine.

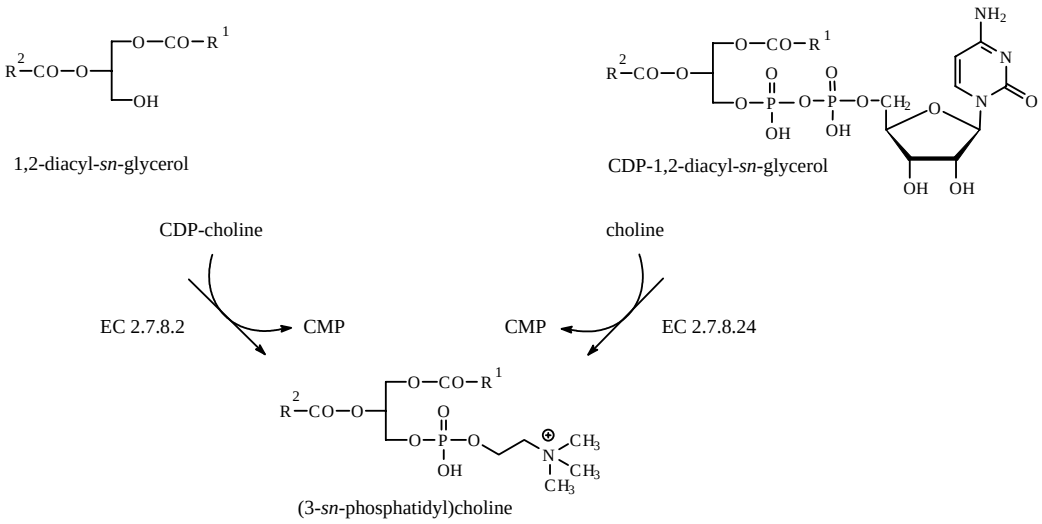


Figure 3.37

For example, phosphatidylcholine containing palmitic acid at both C-1 and C-2 positions is the major glycophospholipid in the extracellular lipid layer lining the pulmonary alveoli. Phosphatidylcholines are also the major glycophospholipids of milk, egg, and oil seed phosphatides.

Synthesis of phosphatidylethanolamine can occur by two pathways. The first pathway requires that ethanolamine be activated by phosphorylation and then by coupling to CDP. The ethanolamine is then transferred from CDP-ethanolamine to diacylglycerol by diacylglycerol ethanolaminephosphotransferase (EC 2.7.8.1) to yield phosphatidylethanolamine (Figure 3.38). The second pathway involves decarboxylation of phosphatidylserine (Figure 3.39).

Animal phosphatidylethanolamines contain primarily palmitic or stearic acid at C-1 carbon and a long-chain unsaturated fatty acid (linoleic, arachidonic acids, DHA) at carbon C-2. In milk, egg, and oil seeds, phosphatidylethanolamines (together with phosphatidylcholines) represent the major constituents of the phosphatide fraction.

The pathway for phosphatidylserine synthesis involves a reaction of serine with CDP-diacylglycerol catalyzed by phosphatidylserine synthase (EC 2.7.8.8) (Figure 3.40). Phosphatidylserines are composed of fatty acids similar to phosphatidylethanolamines. In milk, eggs, and oil seeds, phosphatidylserines are minor components of phosphatide fraction.

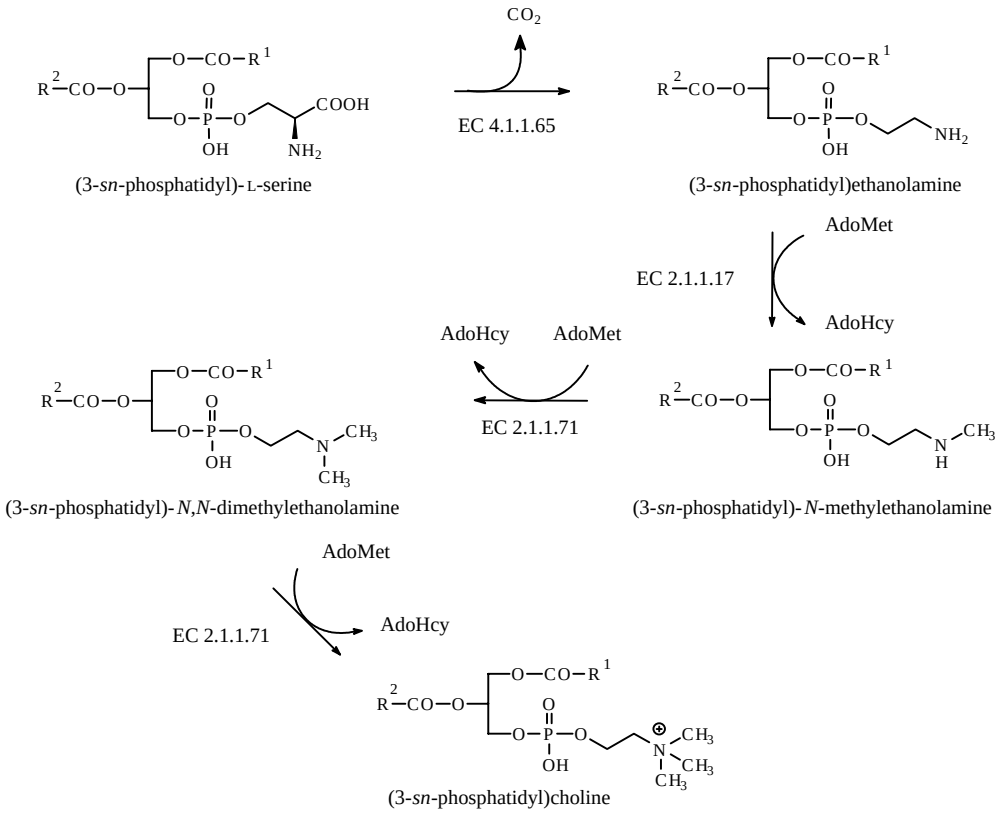


Figure 3.38

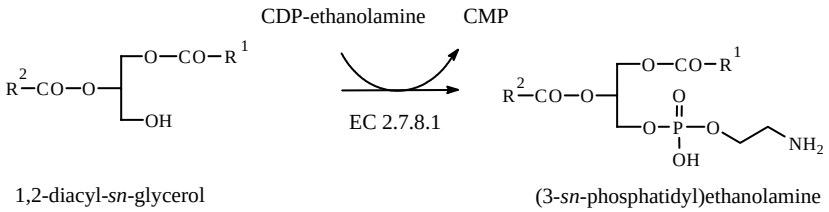


Figure 3.39

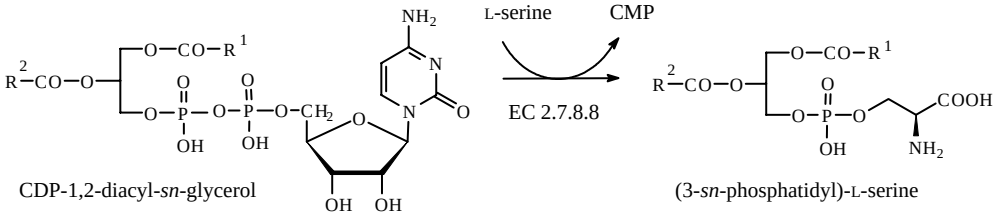


Figure 3.40

The synthesis of phosphatidylinositol involves CDP-activated diacylglycerol condensation with *myo*-inositol, which is catalyzed by phosphatidylinositol synthase (EC 2.7.8.11) (Figure 3.41). For example, the mammalian phosphatidylinositols contain almost exclusively stearic acid at carbon C-1 and arachidonic acid at carbon C-2. The released arachidonic acid is then a substrate for the synthesis of eicosanoids. Although *myo*-inositol (bound at 1D position) is the predominant form, the presence of *scyllo*-inositol-containing phosphatides has been found in plant cells and *chiro*-inositol-containing phosphatides in animal cells. The biosynthetic conversion of D-glucose 6-phosphate to *myo*-inositol is described in Chapter 4.1.6.1.

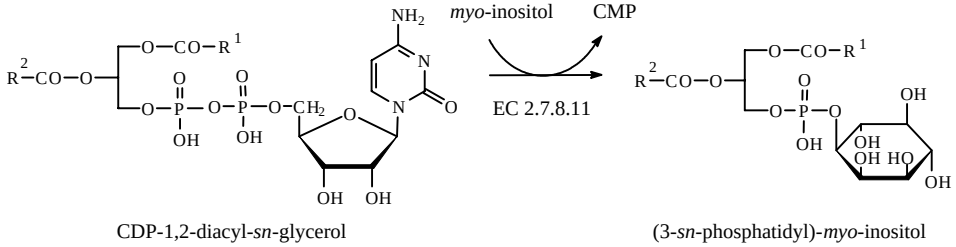


Figure 3.41

Phosphatidylinositols are further phosphorylated by specific kinases to yield phosphorylated phosphatidylinositol derivatives (phosphoinositides). Phosphoinositides exist in animal and plant membranes with various levels of phosphate esterified to the hydroxyls of the *myo*-inositol moiety. Thus, phosphatidylinositol 4-phosphate (1-phosphatidyl-1D-*myo*-inositol 4-phosphate) forms by the action of 1-phosphatidylinositol 4-kinase (EC 2.7.1.67) and phosphatidylinositol 4,5-bisphosphate (1-phosphatidyl-1D-*myo*-inositol 4,5-bisphosphate) by the action of 1-phosphatidylinositol 4-phosphate kinase (EC 2.7.1.68). They represent extremely important membrane phospholipids involved in the transduction of signals for cell growth and differentiation. Their level in oil seeds is much higher than that in milk or eggs.

Phosphatidylglycerol is synthesized from CDP-diacylglycerol and glycerol 3-phosphate by the action of phosphatidylglycerol phosphate synthase (EC 2.7.8.5) (Figure 3.42).

Phosphatidylglycerols are found in high levels in mitochondrial membranes and as components of pulmonary surfactant. The vital role of phosphatidylglycerols is to serve as the precursors of diphosphatidylglycerols derived from 1´,3´-di-*O*-(*sn*-3-phosphatidyl)-*sn*-glycerol (diphosphatidic acid) (Figure 3.43).

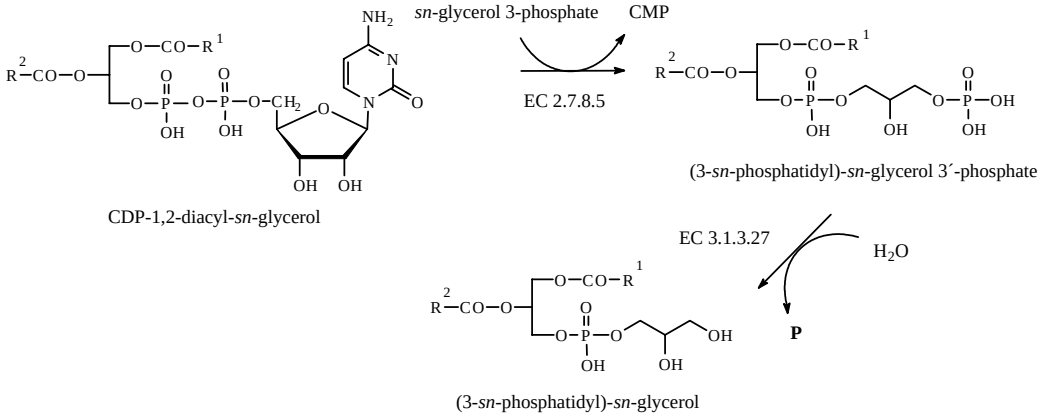


Figure 3.42

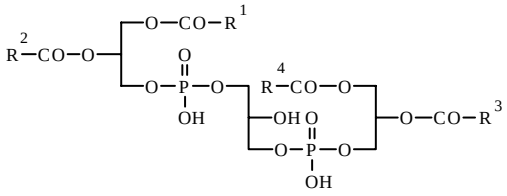


Figure 3.43

3.3.2.1.1 Ethanolamine and choline

Choline is a fundamental metabolite in plants because of its contribution to the synthesis of the membrane phospholipid phosphatidylcholine, which accounts for 40 to 60% of lipids in non-plastid plant membranes. Although choline biosynthesis accommodates different pathways in plants, most of them use L-serine as the starting compound (Figure 3.44).

The first step in choline biosynthesis is the direct decarboxylation of serine to ethanolamine, which is provided by serine decarboxylase (EC 4.1.1.-) unique to plants. Ethanolamine is widely recognized as the entrance compound to choline biosynthesis. The synthesis of choline from ethanolamine may take place at two parallel pathways, where three consecutive *N*-methylation steps are carried out either on free-bases or their phosphates. The enzymes involved are ethanolamine kinase (EC 2.7.1.82), phosphoethanolamine *N*-methyltransferase (EC 2.1.1.103), and ethanolamine *N*-methyltransferase (EC 2.1.1.-). The release of choline from the different pathway levels is species-specific and can be e.g. found in spinach. Phosphocholine can either be directly dephosphorylated by phosphocholine dephosphorylase (EC 3.1.3.75) to release choline or incorporated into phosphatidylcholine via CDP-choline (CDP-choline synthetase, EC 2.7.7.15) with the subsequent release of choline from phosphatidylcholine by phospholipase D (EC 3.1.4.4). Analogously, ethanolamine phosphate can be incorporated to phosphatidylethanolamine by CDP-ethanolamine synthetase (EC 2.7.7.14).

GDP-L-galactose, on the other hand, is formed by GDP-D-mannose 3,5-epimerase (EC 5.1.3.18), which also generates GDP-L-gulose (GDP-L-Gul). This conversion represents a 3,5-epimerization via enol intermediates. L-Galactose is incorporated into xyloglucans and *N*-linked glycans.

4.1.4 Aminosugars

Aminosugars, such as D-glucosamine (2-amino-2-deoxy-D-glucose), D-galactosamine (2-amino-2-deoxy-D-galactose), and D-mannosamine (2-amino-2-deoxy-D-mannose), and their *N*-acetyl derivatives occur as principal components of various biologically active oligosaccharides and biopolymers. For instance, *N*-acetyl-D-glucosamine (GlcNAc) is a building unit of milk oligosaccharides, chitin, peptidoglycans of bacterial cell walls (mureins), and glycosaminoglycans (mucopolysaccharides) that function in various biological systems as components of proteoglycans (hyaluronic acid and dermatan sulfate). *N*-Acetyl-D-galactosamine (GalNAc) is a building unit of proteoglycans (chondroitin sulfate and dermatan sulfate).

The starting compound for the synthesis of *N*-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose, UDP-GlcNAc) is D-Fru6P. It is first converted to D-glucosamine 6-phosphate in a reaction with L-glutamine catalyzed by glucosamine 6-phosphate synthase (EC 2.6.1.16) [4.3)]. D-Glucosamine 6-phosphate is acetylated and forms *N*-acetyl-D-glucosamine 6-phosphate using acetyl-CoA as the acetyl group donor (glucosamine 6-phosphate *N*-acetyltransferase, EC 2.3.1.4); *N*-acetyl-D-glucosamine 6-phosphate is converted to *N*-acetyl-D-glucosamine 1-phosphate by phosphoacetylglucosamine mutase (EC 5.4.2.3).

α-D-Glucosamine 1,6-phosphomutase (EC 2.4.2.10) catalyzes the isomerization of D-glucosamine 6-phosphate to D-glucosamine 1-phosphate in the pathway leading to bacterial cell wall peptidoglycan and lipopolysaccharide biosyntheses (Figure 4.13). D-Glucosamine 1-phosphate is then acetylated to *N*-acetyl-D-glucosamine 1-phosphate by D-glucosamine 1-phosphate *N*-acetyltransferase (EC 2.3.1.157). *N*-acetyl-D-glucosamine 1-phosphate reacts with UTP yielding UDP-*N*-acetyl-D-glucosamine (UDP-GlcNAc pyrophosphorylase, EC 2.7.7.23). This activated form of *N*-acetyl-D-glucosamine is further converted to UDP-*N*-acetyl-D-glucuronic acid (UDP-*N*-acetylglucosamine 6-dehydrogenase, EC 1.1.1.136), UDP-*N*-acetyl-D-galactosamine, also known as UDP-2-acetamido-2-deoxy-D-galactose (UDP-*N*-acetylglucosamine 4-epimerase, EC 5.1.3.7), and UDP-*N*-acetyl-D-mannosamine, i.e. UDP-2-acetamido-2-deoxy-D-mannose (UDP-*N*-acetylglucosamine 2-epimerase, EC 5.1.3.14). Finally, various transferases are involved in the biosynthesis of biopolymers containing aminosugars.

[4.3)] The reverse reaction, i.e. hydrolysis of *N*-acetyl-D-glucosamine to D-fructose 6-phosphate and ammonia, is catalyzed by glucosamine 6-phosphate deaminase (EC 3.5.99.6); hydrolysis of *N*-acetyl-D-glucosamine 6-phosphate to D-glucosamine 6-phosphate is provided by *N*-acetylglucosamine 6-phosphate deacetylase (EC 3.5.1.25).

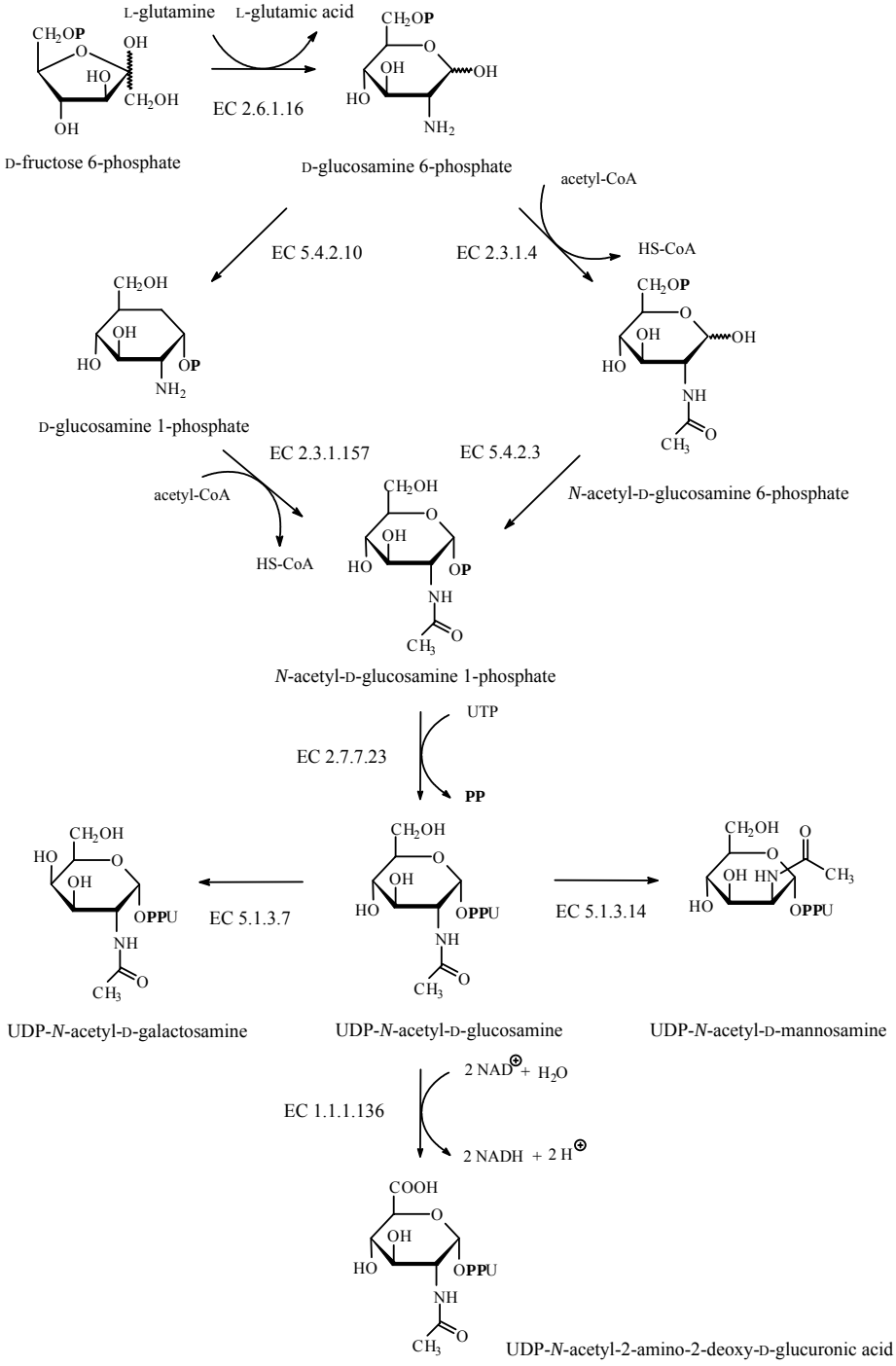

L-glutamine
L-glutamic acid
EC 2.6.1.16
D-fructose 6-phosphate
D-glucosamine 6-phosphate
acetyl-CoA
HS-CoA
EC 5.4.2.10
EC 2.3.1.4
D-glucosamine 1-phosphate
N-acetyl-D-glucosamine 6-phosphate
EC 2.3.1.157
EC 5.4.2.3
acetyl-CoA
HS-CoA
N-acetyl-D-glucosamine 1-phosphate
UTP
PP
EC 2.7.7.23
EC 5.1.3.7
EC 5.1.3.14
UDP-N-acetyl-D-galactosamine
UDP-N-acetyl-D-glucosamine
UDP-N-acetyl-D-mannosamine
2 NAD⊕ + H2O
2 NADH + 2 H⊕
EC 1.1.1.136
UDP-N-acetyl-2-amino-2-deoxy-D-glucuronic acid

Figure 4.13

4.1.4.1 Acetylmuramic acid

Bacterial cell walls contain an unusual saccharide, *N*-acetyl derivative of *N*-acetyl-β-muramic acid, (*R*)-2-acetamido-3-*O*-(1-carboxyethyl)-2-deoxy-β-D-glucopyranose (β-MurAc), bound in peptidoglycan structures called murein. The peptidoglycan chains are composed of alternating β-(1→4) linked *N*-acetyl-D-glucosamine and *N*-acetylmuramic acid residues that are cross-linked via peptide structures (through the lactyl group of the *N*-MurAc to link the peptide via and amide/peptide bond). The peptidoglycan building stone, UDP-*N*-MurAc, forms from UDP-*N*-acetyl-D-glucosamine and phosphoenolpyruvic acid via the intermediate UDP-*N*-acetyl-3-*O*-(1-carboxyvinyl)-D-glucosamine (UDP-*N*-acetyl-D-glucosamine 1-carboxyvinyl transferase, EC 2.5.1.7), which is reduced to UDP-*N*-MurAc by UDP-*N*-acetylmuramate dehydrogenase (EC 1.1.1.158) (Figure 4.14).

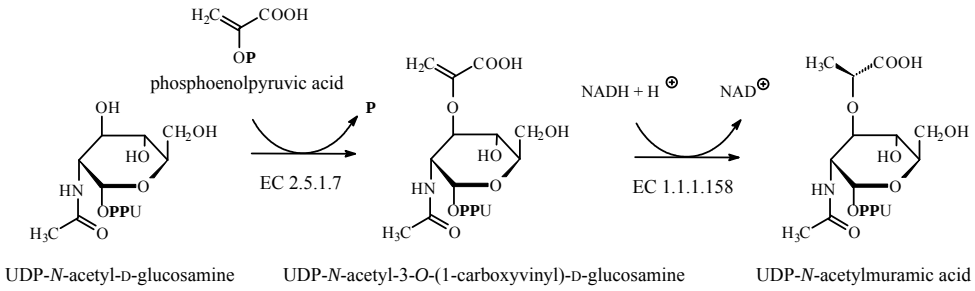


Figure 4.14

4.1.4.2 Acetylneuraminic acid

Sialic acids are a family of nine carbons α-oxo acids that play a wide variety of biological roles in higher animals and some microorganisms. Sialic acids comprise about 50 members, which are derivatives of neuraminic (5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic) acid (Neu). They carry various substituents at the amino or hydroxyl groups. The amino group of Neu is acetylated or glycolated, while at all non-glycosidic hydroxyl residues one or various acetyl groups may occur. In mammals, sialic acids are found at the distal ends of cell surface conjugates and thus are major determinants of specific biological functions, such as cellular adhesion, formation, masking of recognition determinants, and stabilization of glycoprotein structures. In certain strains of pathogenic bacteria, they occur as a homopolysaccharide (polysialic acid) with α-(2→8) and/or α-(2→9) ketosidic linkages in capsular polysaccharides that mask the organism from the immune system.

N-acetylneuraminic acid (5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic) acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Gc), and *N*-acetyl-9-*O*-acetylneuraminic acid (Neu5,9Ac_2) are the three most frequently occurring members of the sialic acids family. Only Neu5Ac is ubiquitous, while the others are not found in all species.

N-acetyl-α-neuraminic acid, a constituent of food glycoproteins (e.g. milk κ-caseins) and glycolipids, (e.g. gangliosides) forms by aldol-type reaction from *N*-acetyl-D-mannos-

amine and pyruvic acid (Figure 4.15). The reaction is more complex in the enzyme-catalyzed version. The two enzymes initiating the biosynthesis of Neu5Ac from *N*-acetyl-D-mannosamine in mammals, the hydrolyzing UDP-*N*-acetylglucosamine 2-epimerase (EC 5.1.3.14) and *N*-acetylmannosamine kinase (Mg^{2+}/K^{+}-dependent enzyme, EC 2.7.1.60), are parts of one bifunctional enzyme that catalyzes hydrolysis of UDP-*N*-acetyl-D-glucosamine, its isomerization to *N*-acetyl-D-mannosamine, and phosphorylation of *N*-acetyl-D-mannosamine to *N*-acetyl-D-mannosamine 6-phosphate. The next step, aldol-type condensation of *N*-acetyl-D-mannosamine 6-phosphate (open-chain form) with phosphorenolpyruvic acid to *N*-acetylneuraminic acid 9-phosphate, is catalyzed by *N*-acetylneuraminate 9-phosphate synthetase (EC 2.5.1.57). Hydrolysis of this phosphate by *N*-acetylneuraminate 9-phosphatase (EC 3.1.3.29) yields Neu5Ac. *N*-Acetylneuraminate synthase (EC 2.5.1.56) generates Neu5Ac from *N*-acetyl-D-mannosamine and phosphoenolpyruvic acid in bacteria (Figure 4.16).

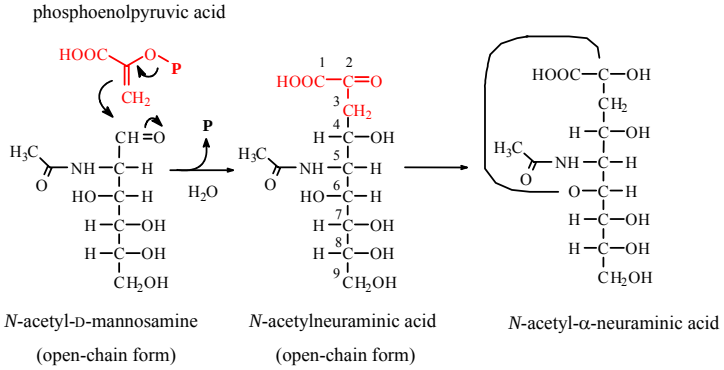


Figure 4.15

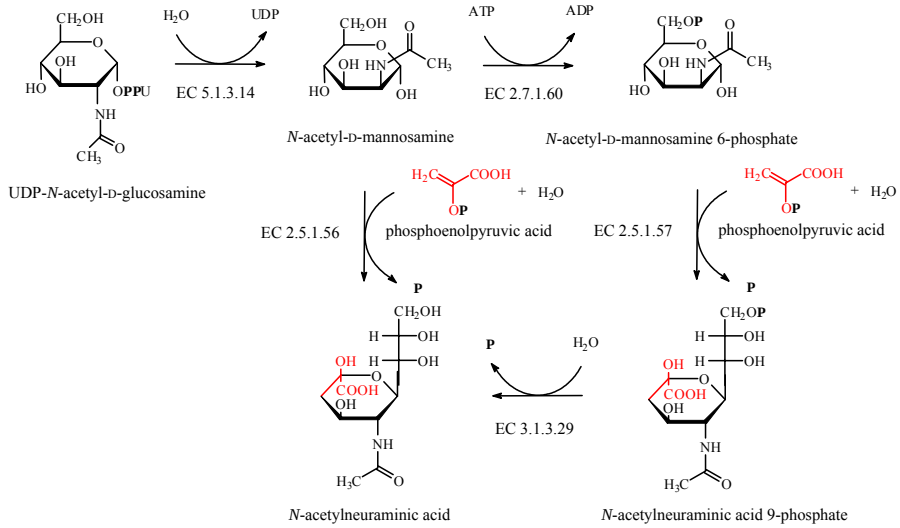


Figure 4.16

4.1.5 Sugar alcohols

It has been suggested that sugar alcohols, because of their water-like hydroxyl groups, may mimic the structure of water and maintain an artificial sphere of hydration around macromolecules. They have also been postulated as scavengers of activated oxygen species preventing peroxidation of lipids resulting in cell damage.

4.1.5.1 Glucitol

D-Glucitol (also incorrectly known as D-sorbitol) is a hexitol (2*R*,3*S*,4*S*,5*S*)-hexane-1,2,3,4,5,6-hexol commonly found in higher plants. In Rosaceae plants, glucitol is the main photoassimilate produced in the leaves from which it is translocated to sink tissues. The biosynthesis of glucitol shares a common hexose phosphate pool (D-Fru6P is the starting compound) with sucrose synthesis and it was recently shown in apple that these two pathways are not independent from one another. The biosynthesis of glucitol involves three enzymatic steps. In the first step, the glycolytic enzyme phosphoglucose isomerase (EC 5.3.1.9) catalyzes isomerization of D-Fru6P to D-Glc6P and *vice versa*. In the next step, D-Glc6P is reduced to D-glucitol 6-phosphate by sorbitol-6-phosphate dehydrogenase (EC 1.1.1.200), which is finally hydrolyzed to D-glucitol using sorbitol-6-phosphate phosphatase (EC 3.1.3.50) [4.4]. The translocated glucitol in fruit tissues is eventually converted to fructose via a NAD^+-dependent sorbitol dehydrogenase (EC 1.1.1.14), which is a key enzyme for the metabolic utilization of glucitol and plays an important role in supplying carbon during fruit development of plants that use glucitol as the main product of photosynthesis (Figure 4.17).

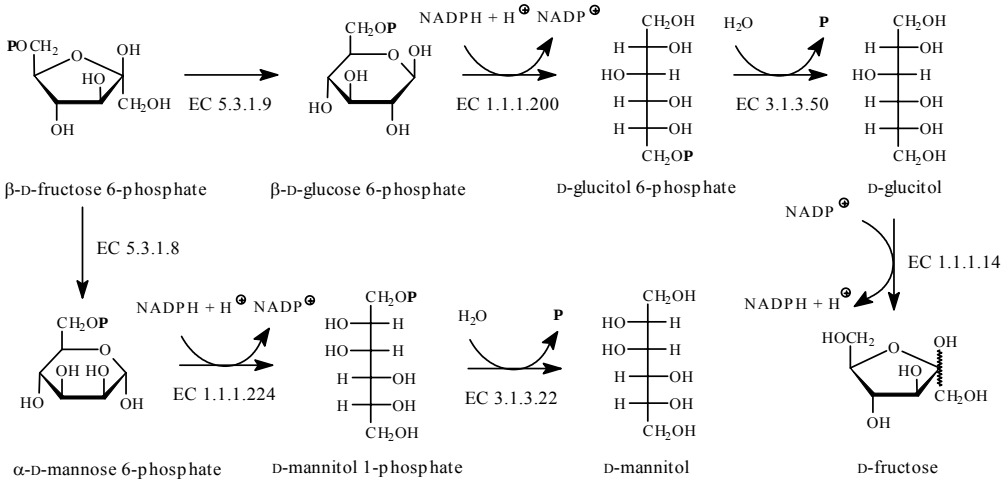


Figure 4.17

Glucitol occurs in variable amount in many stone fruits of the Rosaceae family. Its concentration in e.g. apples is 0.2-0.8% and in plums 0.6-13.9% (fresh weight). Glucitol

[4.4] The Gram-negative, fermentative, α-proteobacterium *Zymomonas mobilis* has been shown to biosynthesize sorbitol and gluconolactone from glucose and fructose in a one-step enzymatic reaction, which is catalyzed by a unique enzyme, glucose-fructose oxidoreductase (EC 1.1.99.28).

is also used commercially as a laxative, slowly metabolized sweetener (it provides dietary energy of 11 kJ/g versus the average 17 kJ/g of saccharose or starch, while retaining 60% of the saccharose sweetness), humectant, texturizer, softener, and is a precursor of other products of commercial interest including vitamin C.

4.1.5.2 Mannitol

D-Mannitol, (2*R*,3*R*,4*R*,5*R*)-hexane-1,2,3,4,5,6-hexol, is the most widely distributed sugar alcohol in nature and has been reported in more than 100 species of vascular plants. Its accumulation prevents loss of water and mannitol-producing plants exhibit a high degree of salt tolerance. The amount of mannitol in fruits and many vegetables is low being in the range of 0.01-0.02%. Very high levels of mannitol can be found in some fungi. The amount of mannitol in table mushroom (*Agaricus bisporus*; Agaricaceae) can reach 20% (dry matter). Higher amount of mannitol in wines is an indicator of grape infection caused by *Botrytis cinerea* or the so-called mannitol fermentation provided by *Bacterium mannitopoeum*, thus spoiling the wine.

Mannitol synthesis in higher plants (Figure 4.17) occurs simultaneously with either saccharose synthesis or raffinose synthesis via isomerization of Fru6P to Man6P, which is catalyzed by phosphomannose isomerase (EC 5.3.1.8). Man6P is then converted to D-mannitol 1-phosphate by NADPH-dependent mannose-6-phosphate reductase (EC 1.1.1.224). The final step of the irreversible conversion of mannitol 1-phosphate to D-mannitol is provided by mannitol-1-phosphatase (EC 3.1.3.22).

4.1.6 Cyclitols

Cyclitols (Figure 4.18) comprise a group of alcoholic sugars formally derived from cyclohexane (cyclohexitols), of which the most prominent naturally occurring form is *myo*-inositol, (1*Z*,2*Z*,3*Z*,5*Z*,4*E*,6*E*)-cyclohexane-1,2,3,4,5,6-hexol.

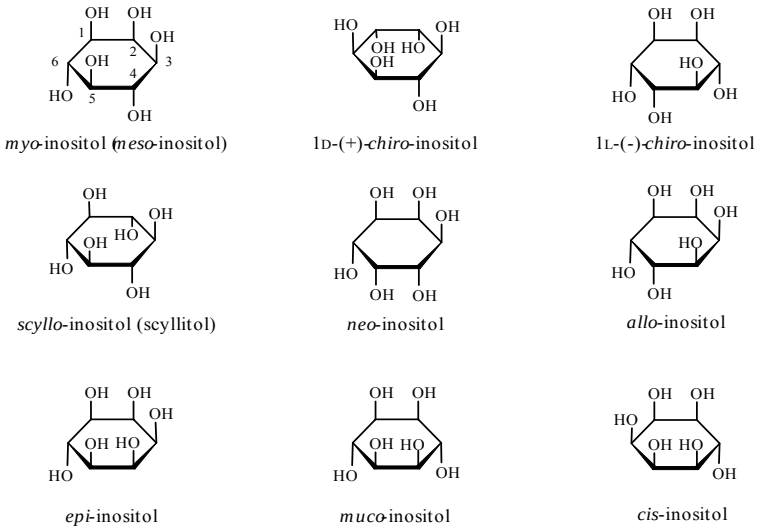


Figure 4.18

Myo-inositol (also known as *meso*-inositol) plays an important role as the structural basis for a number of secondary messengers in eukaryotic cells; including inositol phosphates and phosphatidylinositol in phospholipids (see Chapter 3.3.2.1). It is found in many foods, in particular, in cereals with high bran content, nuts, legumes (Table 4.2), and fruits. In cereals and seeds, it mainly occurs as phytic acid (phytate), i.e. *myo*-inositol-1,2,3,4,5,6-hexa*kis*dihydrogenphosphate, and its salts that are known as phytin. Formerly, *myo*-inositol has been considered a vitamin (referred to as vitamin B_8), but it is synthesized by the human body.

4.1.6.1 Inositols

Biosynthetic conversion of D-glucose 6-phosphate to free *myo*-inositol involves two enzymatic steps. The first step is an irreversible cyclization of D-Glc6P to 1L-*myo*-inositol 1-phosphate (1D-*myo*-inositol 3-phosphate). This reaction is catalyzed by inositol 1-phosphate synthetase (EC 5.5.1.4) [4.5)]. The second step, loss of phosphate catalyzed by inositol phosphatase (EC 3.1.3.25), releases free *myo*-inositol (Figure 4.19). Overall, this scheme constitutes the sole pathway of *myo*-inositol biosynthesis in cyanobacteria, algae, fungi, plants, and animals and occupies a central role in their cellular metabolism.

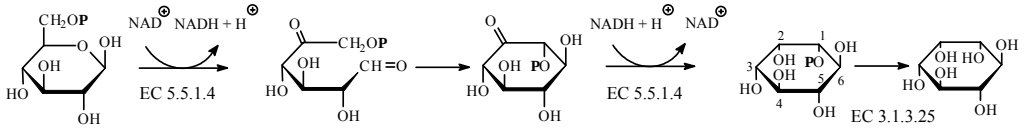


Figure 4.19

Functionally, the conversion of D-Glc6P to 1L-*myo*-inositol 1-phosphate involves three steps: NAD^+-coupled oxidation of D-Glc6P at C-5, aldol condensation between C-1 and C-6 of D-*xylo*-5-hexulose 6-phosphate (5-oxo-D-glucose 6-phosphate), and NADH catalyzed reduction of 1L-2-*myo*-inosose 1-phosphate (1D-2-*myo*-inosose 3-phosphate, also known as D-2,3,6/3,5-pentahydroxycyclohexane 2-phosphate) to yield 1L-*myo*-inositol 1-phosphate (1D-*myo*-inositol 3-phosphate).

Only *myo*-inositol is biosynthesized *de novo* from D-Glc6P. Metabolic processing of *myo*-inositol then produces many biologically important products. Oxidation of *myo*-inositol gives D-GlcA, conjugation with UDP-D-Gal forms galactinol, the galactosyl donor for biosynthesis of α-galactooligosaccharides raffinose, stachyose, verbascose, and ajugose (see Chapter 4.2.3.2). Isomerization of *myo*-inositol produces other stereoforms of inositols. 1D-*chiro*-inositol is a constituent of α-galactooligosaccharides fagopyritols (see Chapter 4.2.3.3). Methylation of *myo*-inositol and other isomeric (*scyllo*-, *chiro*-, *muco*-, and *neo*-) inositols leads to *O*-methyl inositols bornesitol, ononitol, sequoyitol, pinitol,

[4.5)] This enzyme requires NAD^+, which dehydrogenates the -CHOH- group to -CO- at C-5 of the glucose 6-phosphate, changing C-6 into an active methylene, able to condense with the –CH=O group at C-1. Finally, the enzyme-bound NADH reconverts C-5 into the -CHOH- form. The enzyme has a preference for the β-anomeric form of D-glucose 6-phosphate.

quebrachitol, etc. (Figure 4.20), which participate in stress-related responses, storage of seed products, and production of glycosides derived from inositol. The research on inositol-linked stress-related processes in plants is still in its pioneering stage. For example, the biochemical conversion of *myo*-inositol to D-pinitol is catalyzed by *O*-methyl transferase yielding D-ononitol. The enzyme catalyzing the next step, epimerization of C-l of ononitol, has yet to be examined. This reaction proceeds via 1D-4-*O*-methyl-1-*myo*-inosose as the intermediate and yields D-pinitol as the final product. Furthermore, *myo*-inositol is involved in biosynthesis of phytic acid, phosphatidylinositol, its polyphosphates, and other lipids.

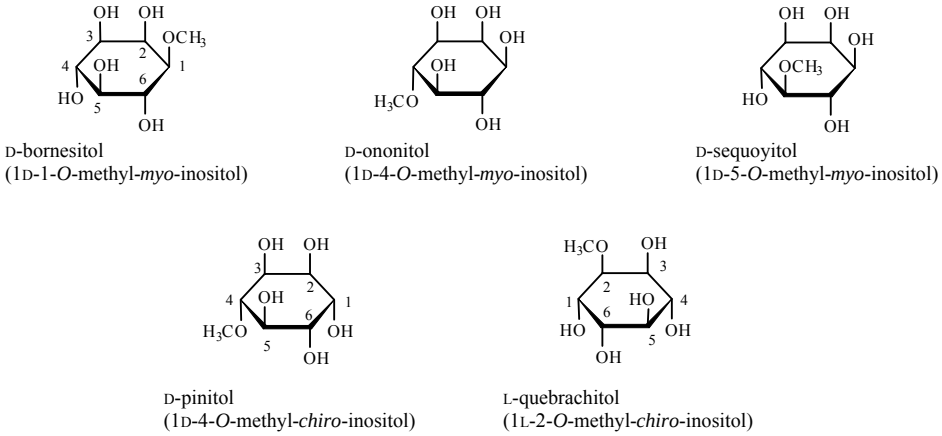


Figure 4.20

4.1.6.2 Phytic acid

myo-Inositol-1,2,3,4,5,6-hexa*kis*dihydrogenphosphate, known as phytic acid or phytate, is the most abundant *myo*-inositol phosphate in plant cells. It is usually present as mixed salts with K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, and Zn^{2+}, known as phytin. Phytin is the principal storage form of phosphorus and mineral nutrients in seeds for utilization during seed germination and seedling growth. It is also present in other plant tissues and organs, such as pollen, roots, and tubers.

The potential of phytin to form very stable salts with minerals confers upon this molecule its notorious antinutritional properties. Humans and monogastric animals lack the hydrolytic phytases (EC 3.1.3.-) in their digestive tract. Therefore, high concentrations of phytin in food grains and its ability to chelate mineral cations compromise mineral absorption. Wheat phytin content is approximately 10 mg/kg and about 70-80% of this amount is hydrolyzed to *myo*-inositol by the action of yeast (*Saccharomyces cerevisiae*) phytases in dough.

Currently, little is known of the biosynthesis of phytic acid. There appears to be several routes of its biosynthesis. The first route is a lipid-independent route relying on the phosphorylation of 1L-*myo*-inositol 1-phosphate (1D-*myo*-inositol 3-phosphate) (see Figure 4.19) or free *myo*-inositol that can be esterified by *myo*-inositol kinase

(EC 2.7.1.64). A step-wise phosphorylation of 1L-*myo*-inositol 1-phosphate (1D-*myo*-inositol 3-phosphate) by not yet characterized *myo*-inositol phosphate kinases (EC 2.7.1-) produces 1L-*myo*-inositol 1,6-bisphosphate (1D-*myo*-inositol 3,4-phosphate), 1L-*myo*-inositol 1,4,6-trisphosphate (1D-*myo*-inositol 3,4,6-trisphosphate), and 1L-*myo*-inositol 1,4,5,6-tetra*kis*phosphate (1D-*myo*-inositol 3,4,5,6-tetra*kis*phosphate), which is further phosphorylated (*myo*-inositol polyphosphate kinase, EC 2.7.1.134) yielding 1L-*myo*-inositol 1,3,4,5,6-penta*kis*phosphate (1D-*myo*-inositol 1,3,4,5,6-penta*kis*phosphate) and then the final product phytic acid (EC 2.7.1.-) (Figure 4.21).

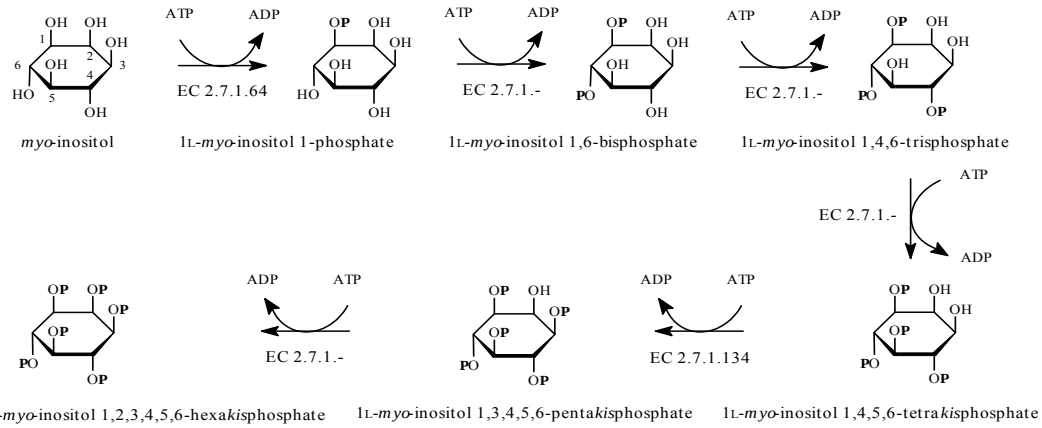


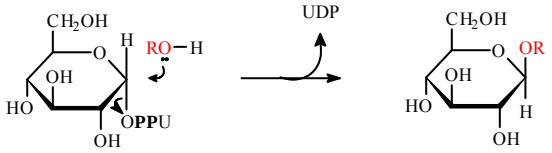
Figure 4.21

The second route is a lipid-dependent route, which uses 1L-*myo*-inositol 3,5,6-trisphosphate (1D-*myo*-inositol 1,4,5-trisphosphate) produced from 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate hydrolyzed by phospholipase C (EC 3.1.4.3). To date, the biosynthesis of phytic acid following this pathway has only been fully reported in several aquatic monocotyledonous plants.

4.2 Oligosaccharides

The formation of glycosides, oligosaccharides, and polysaccharides is dependent on the activation of sugar by its binding to a nucleoside diphosphate. Nucleophilic displacement of the respective nucleoside diphosphate-leaving group by a suitable nucleophile then generates the new sugar derivative [4.6]. The product will be a glycoside if the nucleophile is a suitable aglycone molecule or oligosaccharide if the nucleophile is another sugar molecule. This reaction, if mechanistically of S_N2 type, should give an inversion of configuration at C-1 in the electrophile, generating a product with the β-configuration and nucleoside diphosphate (Figure 4.22).

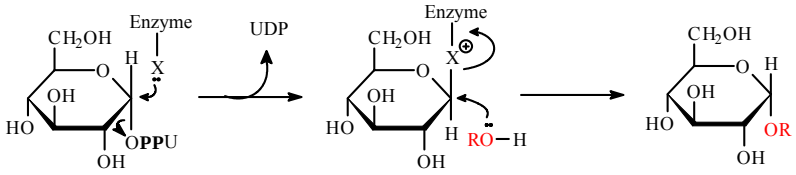
[4.6] Synthesis of nucleoside triphosphates from nucleoside diphosphates is realized by nucleoside diphosphate kinases. Many ribo- and deoxyribonucleoside triphosphates can act as donors; the most usual one is ATP. For example, UTP from UDP is formed by uridine diphosphate kinase (EC 2.7.4.6) that carries the phosphate residue from ATP to UDP.



UDP-α-D-glucose β-D-glucoside

Figure 4.22

Many of the linkages formed between sugar monomers actually have α-configuration. It is believed that a double S_N2 mechanism operates, which also involves a nucleophilic group of the enzyme (Figure 4.23).



UDP-α-D-glucose α-D-glucoside

Figure 4.23

4.2.1 Glucooligosaccharides

4.2.2.1 Trehalose

α,α-Trehalose is a non-reducing disaccharide that occurs in plants and a large range of organisms, such as bacteria, fungi, nematodes, and crustaceans. In addition to its function as a storage and transport sugar, α,α-trehalose plays an important role in stress protection, especially during heat stress and dehydration. It occurs in small amounts in fruits, vegetables, and wines (0-61 mg/l). Higher amounts (7-31 g/kg) can be found in table mushrooms (*Agaricus bisporus*; Agaricaceae).

Biosynthesis of α,α-trehalose is a two-step reaction [4.7)]. It starts with UDP-D-Glc that reacts with D-Glc6P and forms α,α-trehalose 6-phosphate (trehalose 6-phosphate

[4.7)] While UDP-D-glucose is the most common substrate for the biosynthesis of trehalose in plants and yeast cells, it is not the sole donor. ADP-glucose trehalose 6-phosphate synthase (EC 2.4.1.-) has been identified in a mutant yeast strain of *Saccharomyces cerevisiae.* GDP-glucose trehalose 6-phosphate synthase (EC 2.4.1.36) has been demonstrated in streptomycetes. Several organisms are capable of synthesizing trehalose directly from maltose (trehalose synthase, EC 5.4.99.16). In some of these organisms (*Thermus caldophilus*), this is the only trehalose biosynthesis pathway, while in others (such as mycobacteria and *Corynebacterium glutamicum*) several trehalose biosynthesis pathways coexist. *C. glutamicum* e.g. can synthesize trehalose also from maltodextrin. In this pathway, trehalose is formed by the action of several enzymes. Isoamylase (also known as glycogen- or starch-debranching enzyme, EC 3.2.1.68) hydrolyzes the α-1,6-glucosidic linkages in glycogen or the α-1,4-glucosidic linkages found in other polysaccharides, such as starch, to produce maltodextrins. Maltooligosyl-trehalose synthase (EC 5.4.99.15) then

synthase, EC 2.4.1.15). Trehalose 6-phosphate phosphatase (EC 3.1.3.12) catalyzes the phosphate hydrolysis to α,α-trehalose (Figure 4.24).

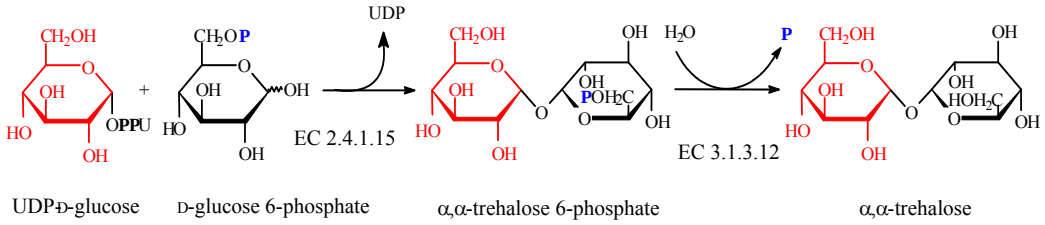


Figure 4.24

4.2.2 Fructooligosaccharides

4.2.2.1 Saccharose

The non-reducing disaccharide saccharose (often referred to as sucrose) is one of the most abundant products in nature. Most photosynthetic eukaryotes and some specific prokaryotes synthesize saccharose in the cytoplasm. In higher plants, saccharose occupies a unique position, comparable only to glucose in the animal world. It is a major product of photosynthesis with a central role as a primary transport and storage sugar. It has also roles in signal transduction and stress. Storage glycosides, including saccharose, starch, and fructans, are mobilized and utilized during seed germination and plant growth. The only known enzymatic paths of saccharose cleavage in plants are catalyzed by the degradative enzyme invertase (EC 3.2.1.26) and reversible sucrose synthase (EC 2.4.1.13). Invertase-catalyzed hydrolysis of saccharose into D-glucose and D-fructose has been associated with cell expansion, e.g. fruit growth and ripening (Table 4.1), whereas sucrose synthase-catalyzed decomposition into D-fructose and UDP-D-Glc has been linked with biosynthetic processes, such as starch biosynthesis.

Table 4.1 Content of glucose, fructose, and saccharose in common fruits and vegetables (% in edible portion)

Fruits	Glucose	Fructose	Saccharose	Vegetables	Glucose	Fructose	Saccharose
Apple	1.8	5.0	2.4	Beet	0.18	0.16	6.11
Banana	5.8	3.8	6.6	Broccoli	0.73	0.67	0.42
Date	32.0	23.7	8.2	Carrot	0.85	0.85	4.24
Grape	8.2	8.0	0.0	Cucumber	0.86	0.86	0.06
Orange	2.4	2.4	4.7	Endive	0.07	0.16	0.07
Peach	1.5	0.9	6.7	Onion	2.07	1.09	0.89
Pineapple	2.3	1.4	7.9	Spinach	0.09	0.04	0.06
Strawberry	2.6	2.3	1.3	Tomato	1.12	1.34	0.01

catalyzes the conversion of maltodextrin to maltooligosyl-trehalose by forming α,α-1,1-glucosidic linkage, using a mechanism of intermolecular transglucosylation. The third enzyme, maltooligosyl-trehalose trehalo-hydrolase (EC 3.2.1.141), hydrolyzes the product to form trehalose and maltodextrin, which is shorter by two glucosyl residues.

The biosynthesis of saccharose is provided using UDP-α-D-Glc as the starting compound. The biosynthetic enzymes sucrose-phosphate synthase (EC 2.4.1.14), sucrose-phosphate phosphohydrolase (EC 3.1.3.24), and sucrose synthase (EC 2.4.1.13) have been well characterized in plants and unicellular eukaryotes. The glucose donor UDP-D-Glc reacts with the sugar acceptor β-D-Fru6P and forms saccharose 6^F-phosphate and UDP. The saccharose 6^F-phosphate is then hydrolyzed to saccharose and phosphate. Alternatively, UDP-D-Glc can react with β-D-Fru yielding saccharose and UDP (Figure 4.25).

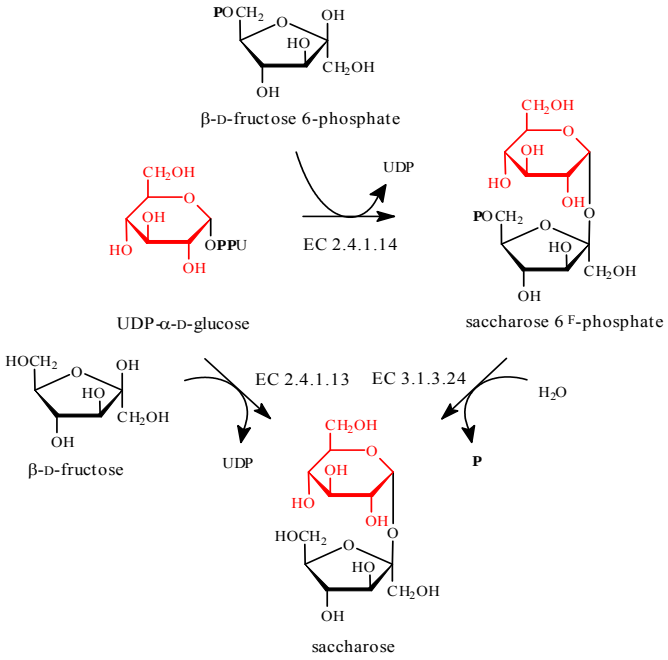


Figure 4.24

4.2.3 Galactooligosaccharides

4.2.3.1 Lactose

Lactose, the major reducing disaccharide in most mammalian milks, is synthesized from UDP-D-Gal and D-glucose exclusively in the lactating mammary glands. The content of lactose in cow milk ranges between 4 and 5%. Glucose and some oligosaccharides are present in small quantities. The biosynthesis of lactose is catalyzed by the enzyme lactose synthase (EC 2.4.1.22) [4.8] (Figure 4.26).

[4.8] Lactose synthase (EC 2.4.1.22) is a complex composed of two distinct protein components, a catalytic and a regulatory subunit. The catalytic subunit, UDP-galactose-glycoprotein galactosyltransferase (EC 2.4.1.38), does not catalyze the biosynthesis of lactose. It participates in the biosynthesis of oligosaccharide chains of secretory and membrane-bound glycoconjugates (e.g. glycoproteins and glycopeptides) by catalyzing the transfer of Gal from UDP-Gal to the terminal *N*-acetyl-β-D-glucosaminyl residue. The regulatory subunit (i.e. the modifier protein α-lactalbumin) in the lactose synthase complex, reversibly binds to UDP-galactose-

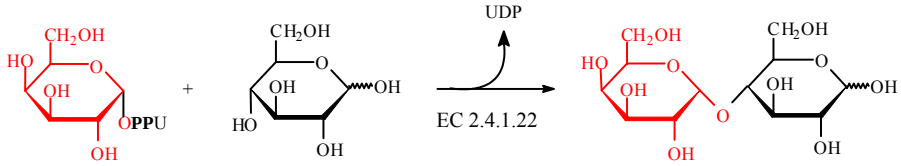


Figure 4.26

4.2.3.2 Raffinose and higher α-galactooligosaccharides

Raffinose is a member of the so-called raffinose family oligosaccharides that represent a large portion of oligosaccharides in plants. The sugars of this series consist of α-1,6-linked chains of D-galactose attached to the 6-glucosyl position of saccharose; therefore they are often referred to as α-galactooligosaccharides. They comprise raffinose, stachyose, verbascose, ajugose as well as several other compounds with a higher degree of polymerization (the highest is a nonasaccharide; none of these higher oligosaccharides in the series have not yet been named). α-Galactooligosaccharides occur at least in traces in each plant family. In higher amounts, they can be found in legumes. They are synthesized in leaves, roots and tubers. Raffinose is usually found in all parts of the plant, including seeds. Stachyose is often the main oligosaccharide in storage organs, although variations occur between species (Table 4.2). Functionally, these soluble carbohydrates are used for carbon transport and storage by the plant, although they have also been reported as protective agents acting during maturation of drying seeds. Sugars of this series in legumes have long been considered as undesirable non-digestible factors that promote flatulence.

Table 4.2 Content of *myo*-inositol, galactinol, and oligosaccharides in some common legumes (% dry matter)

Legume name	*Myo*-inositol	Galactinol	Saccharose	Raffinose	Stachyose	Verbascose
Common bean (*Phaseolus vulgaris*)	0.02-0.06	0.04-0.05	2.2-4.9	0.3-1.1	3.5-5.6	0.1-0.3
Pea (*Pisum sativum*)	0.10-0.17	0.07	2.3-3.5	0.6-1.0	1.9-2.7	2.5-3.1
Lentil (*Lens culinaris*)	0.07-0.11	0.10-0.12	1.3-2.0	0.3-0.5	1.9-3.1	1.2-1.4
Soybean (*Glycine max*)	0.03-0.10	0.0	2.8-7.7	0.2-1.8	0.02-4.8	0.1-1.8
Chickpea (*Cicer arietinum*)	0.10-0.30	0.08-0.20	2.0-3.5	0.7-0.9	1.5-2.4	0.0

Biosynthesis of trisaccharide raffinose starts with UDP-D-Gal that, under catalysis of galactinol synthase (EC 2.4.1.123), reacts with *myo*-inositol and forms pseudooligosaccharide galactinol (1-*O*-α-D-galactopyranosyl-1L-*myo*-inositol). The only known

glycoprotein galactosyltransferase and thus promotes, in the presence of Mn^{2+} ions, glucose binding and facilitates the biosynthesis of lactose.

function of galactinol is in the biosynthesis of α-galactooligosaccharides. The subsequent reaction of galactinol with saccharose, catalyzed by raffinose synthase (EC 2.4.1.82), yields raffinose and *myo*-inositol is released (Figure 4.27).

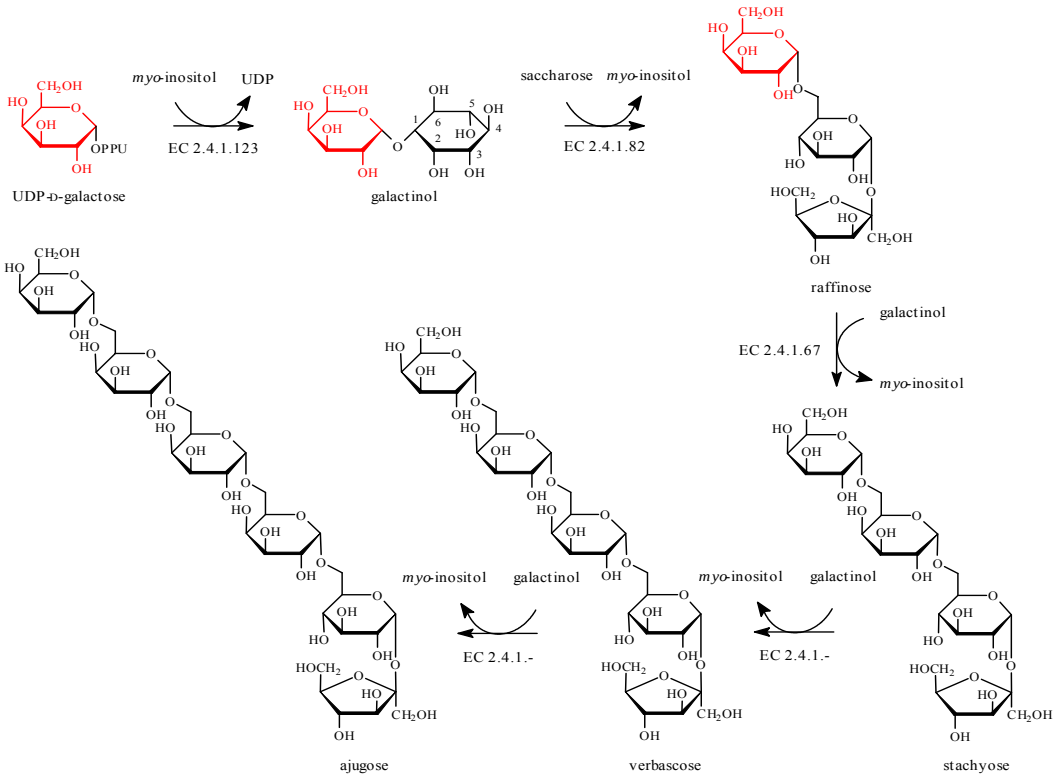


Figure 4.27

Another galactosyltransferase (stachyose synthase, EC 2.4.1.67) catalyzes galactosyl transfer from galactinol to raffinose resulting in the formation of tetrasaccharide stachyose and *myo*-inositol. The biosynthesis of higher α-galactooligosaccharides appears to occur via two routes. One pathway (shown in Figure 4.27) is dependent on galactinol and requires enzymes similar to that of the stachyose biosynthesis pathway (verbascose synthase, EC 2.4.1.-; ajugose synthase, EC 2.4.1.-). The other pathway does not appear to require galactinol. Instead, two raffinose molecules yield stachyose and saccharose is released (stachyose synthase, EC 2.4.1.-), two molecules of stachyose then yield raffinose and verbascose (verbascose synthase, EC 2.4.1.-), and two molecules of verbascose yield stachyose and ajugose (ajugose synthase, EC 2.4.1.-).

4.2.3.3 Fagopyritols

Maturing embryos of many plant seeds accumulate saccharose, raffinose, and derived higher homologues as the predominant soluble sugars, whereas maturing embryos of buckwheat (*Fagopyrum esculentum*; Polygonaceae) seeds accumulate α-D-galactosyl

cyclitols derived from 1D-*chiro*-inositol that are known as fagopyritols. These compounds are grouped into two main series: A and B, which differ from the positioning of the *O*-α-D-galactopyranosyl linkage to 1D-*chiro*-inositol. The first fagopyritol of the A series is *O*-α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol termed fagopyritol A1. The first fagopyritol of the B series is *O*-α-D-galactopyranosyl-(1→2)-1D-*chiro*-inositol termed fagopyritol B1. The subsequent di- and tri-galactopyranosylfagopyritols (A2, B2, A3, and B3) result from the addition of galactosyl units linked by (1→6)-*O*-α-D-galactopyranosyl unit to fagopyritol A1 and B1, respectively. Fagopyritols of the A series have only been detected so far in the seeds of buckwheat, whereas fagopyritols of the B series have been detected in several other species (mostly fagopyritol B1). Fagopyritols have attracted attention as they may be useful in the treatment of non-insulin-dependent *diabetes mellitus* and polycystic ovary syndrome.

The enzyme fagopyritol synthase (EC 2.4.1.-) catalyzes the formation of fagopyritol A1 and B1, but not the subsequent compounds in the series. To date, no enzyme has been identified that can catalyze the formation of fagopyritols A2, B2, A3 or B3 (Figure 4.28).

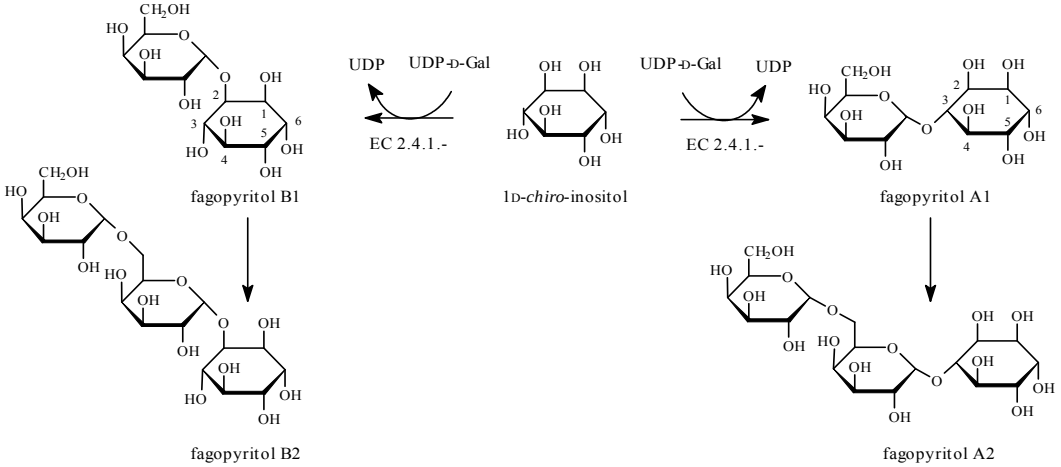


Figure 4.28

4.3 Polysaccharides

Polysaccharides fulfill two main functions in living organisms, as structural elements and food reserves. Most photosynthetically fixed carbon in plants is incorporated into cell wall polysaccharides. The central process of polysaccharide synthesis is the action of glycosyltransferases (also called glycosylsynthases). These enzymes form glycosidic bonds by attaching a sugar moiety of an appropriate donor substrate (mainly a nucleotide sugar) to a specific acceptor substrate, whereas amylose, callose, cellulose, and other linear polysaccharides are synthesized in a single-step reaction involving no intermediates. The assembly of the branched polysaccharides, such as xyloglucan, galactomannan, and pectin, requires a number of enzyme activities.

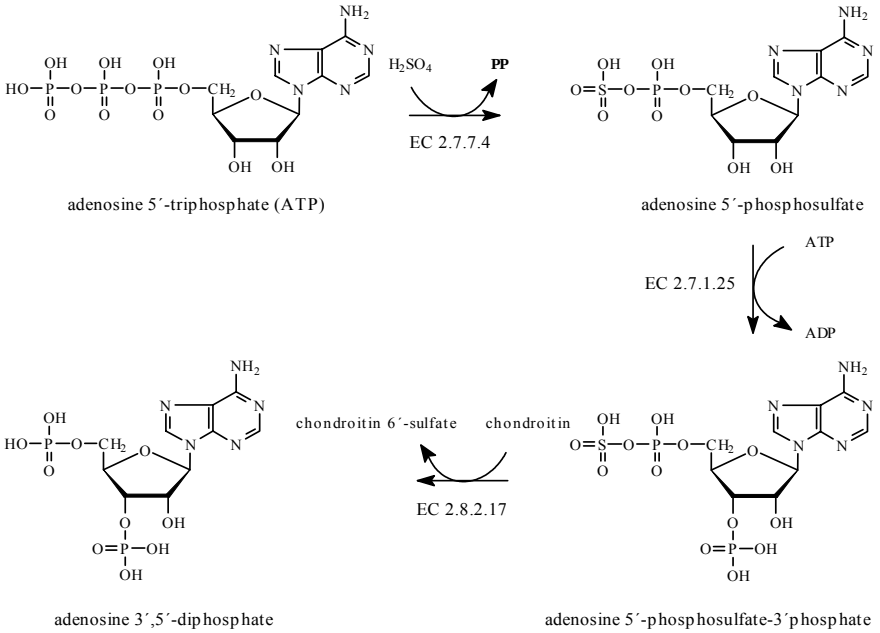


Figure 4.43

4.4 Transformation products of sugars

Glycolysis is the archetype of universal metabolic processes that converts glucose into pyruvic acid. It is known to occur, with variations, in many types of cells in nearly all organisms. Glycolysis, through anaerobic respiration, is the main energy source in many prokaryotes (e.g. fermenting yeast), eukaryotic cells devoid of mitochondria, eukaryotic cells under low-oxygen conditions (e.g. heavily-exercising muscle), and *post mortem*.

With few exceptions, all dietary sugars can serve as substrates for microbial fermentation performed by many types of microorganisms. The ability of a particular microorganism to ferment a particular sugar depends on the presence of a suitable enzyme system to convert that sugar to glucose. The actual biochemical pathway varies depending on the sugars involved, but the most common involves a part of the glycolysis pathway, which is shared with the early stages of aerobic respiration in most organisms. The later stages of the pathway vary considerably depending on the final product. The glycolysis pathway produces two molecules of ATP and creates two molecules of pyruvic acid that is used to regenerate two molecules of NAD^+ from NADH. Pyruvic acid, the product of glycolysis, is converted to lactic acid in the absence of oxygen, e.g. in the *post mortem* muscle or by lactic acid fermentation. Lactic acid fermentation is used for production of e.g. yoghurt, some cheeses, pickled cucumbers, olives, and sauerkraut. Propionic acid fermentation typically occurs in the late stage of Emmental cheese production. Other types of fermentation (mixed acid fermentation, butyric acid fermentation, capronic acid fermentation, glyoxylic acid fermentation, butanol fermentation, acetone fermentation, butanediol fermentation, etc.) have been widely employed in the production of a range of products.

Under anaerobic conditions, yeasts convert pyruvic acid to ethanol and carbon dioxide in the so-called alcohol or ethanol fermentation (e.g. in cider, beer, and wine production). Carbon dioxide is responsible for the rising of bread dough. Bacteria and some fungi typically produce carboxylic acids under aerobic conditions. Acetic acid fermentation (in the production of vinegar) refers to oxidation of ethanol to acetic acid.

4.4.1 Lactic acid fermentation

The lactic acid bacteria produce lactic acid as the major end product of the fermentation of sugars. These bacteria are divided into two main groups depending on their fermentation products. During homolactic acid fermentation, one molecule of glucose is ultimately converted via the Embden-Meyerhof glycolytic pathway to two molecules of pyruvic acid, which is then converted to lactic acid. In the heterolactic acid fermentation, sometimes referred to as the phosphoketolase pathway, the products of fermentation include one molecule of lactic acid, one molecule of ethanol, and one molecule of carbon dioxide.

4.4.1.1 Homolactic acid fermentation

Sugars can enter the cell in two different mechanisms. In most homofermentative strains, such as *Lactococcus lactis* and *Streptococcus lactis*, sugars are transported by a sugar phosphotransferase system (protein-N^{π}-phosphohistidine-sugar phosphotransferase, EC 2.7.1.69). During this transport, the sugar is phosphorylated. α-Lactose is phosphorylated to α-lactose 6′-phosphate, and hydrolyzed to α-D-galactose 6-phosphate and β-D-glucose (Figure 4.44). α-D-Galactose 6-phosphate is then degraded (via D-tagatofuranose 6-phosphate and D-tagatofuranose 1,6-bisphosphate) to D-glyceraldehyde 3-phosphate. β-D-Glucose is phosphorylated to β-D-glucose 6-phosphate, while D-fructose is usually phosphorylated to D-fructose-1-phosphate (although some phosphotransferase systems generate D-fructose 6-phosphate). The protein-N^{π}-phosphohistidine-sugar phosphotransferase is then regenerated by phosphopyruvate-protein phosphotransferase, EC 2.7.3.9). The second mechanism involves the transport of free sugars using permease (EC 2.7.1.69) and their subsequent phosphorylation.

All sugar phosphates are then activated to D-fructose 1,6-bisphosphate through different routes, depending on the nature of the sugar and on the way it enters the cell. The common product, fructose 1,6-bisphosphate, is processed via the Embden-Meyerhof pathway via D-glyceraldehyde 3-phosphate and other metabolites to pyruvic acid, which is then converted to L-(+)-lactic acid, the sole end-product of homolactic fermentation.

The enzymes involved are phosphoglycerate kinase (EC 2.7.2.3), phosphoglyceromutase (EC 5.4.2.1), enolase (EC 4.2.1.11), pyruvate kinase (2.7.1.40), L-lactate dehydrogenase (EC 1.1.1.27), galactokinase (EC 2.7.1.6), UDP-galactose pyrophosphorylase (EC 2.7.7.10), UDP-galactose 4-epimerase (EC 5.1.3.2), UDP-glucose pyrophosphorylase (EC 2.7.7.9), phosphoglucomutase (EC 5.4.2.2), glucokinase (EC 2.7.1.2), fructokinase (EC 2.7.1.4), phosphoglucose isomerase (EC 5.3.1.9), fructose bisphosphate aldolase (EC 4.1.2.13), 1-phosphofructokinase (EC 2.7.1.56), phospho-β-galactosidase (EC 3.2.1.85), D-galactose 6-phosphate isomerase (EC 5.3.1.26), D-tagatose 6-phosphate kinase (EC 2.7.1.144), and tagatose 1,6-bisphosphate aldolase (EC 4.1.2.40).

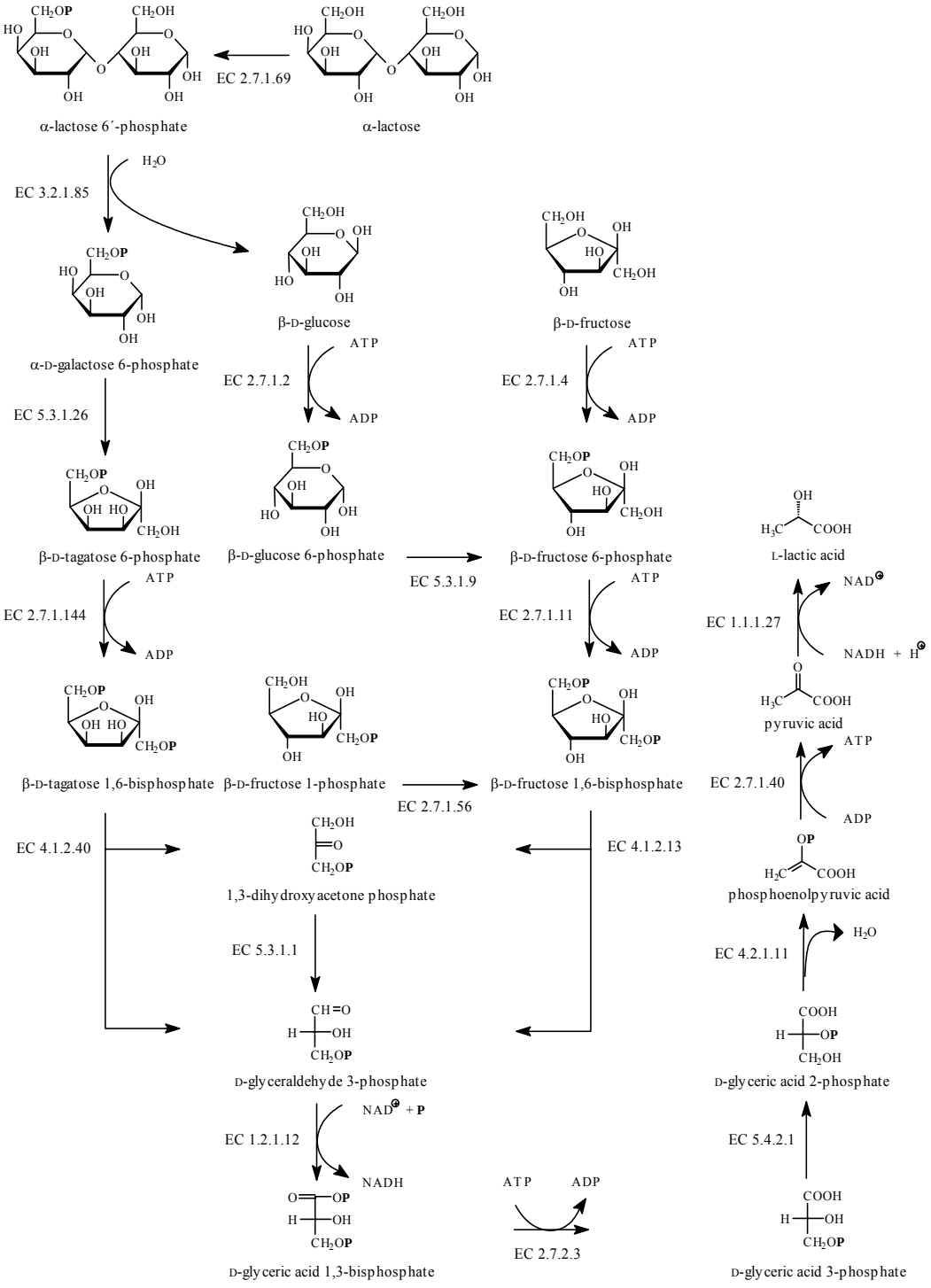

α-lactose 6´-phosphate
EC 2.7.1.69
α-lactose
EC 3.2.1.85
H2O
α-D-galactose 6-phosphate
β-D-glucose
β-D-fructose
EC 2.7.1.2
EC 2.7.1.4
ATP
ADP
EC 5.3.1.26
β-D-tagatose 6-phosphate
β-D-glucose 6-phosphate
β-D-fructose 6-phosphate
EC 5.3.1.9
L-lactic acid
EC 2.7.1.144
EC 2.7.1.11
EC 1.1.1.27
NAD⊕
NADH + H⊕
β-D-tagatose 1,6-bisphosphate
β-D-fructose 1-phosphate
β-D-fructose 1,6-bisphosphate
EC 2.7.1.56
pyruvic acid
EC 2.7.1.40
EC 4.1.2.40
EC 4.1.2.13
1,3-dihydroxyacetone phosphate
phosphoenolpyruvic acid
EC 4.2.1.11
H2O
EC 5.3.1.1
D-glyceraldehyde 3-phosphate
D-glyceric acid 2-phosphate
EC 1.2.1.12
NAD⊕ + P
NADH
EC 5.4.2.1
ATP
ADP
EC 2.7.2.3
D-glyceric acid 1,3-bisphosphate
D-glyceric acid 3-phosphate

Figure 4.44

5 VITAMINS

5.1 Fat - soluble vitamins

Fat-soluble vitamins include biologically active members of vitamin A (diterpenes), vitamin D (modified triterpenes), vitamin E (tocochromanols), vitamin K (naphthoquinones) families and related compounds, of which some act as their precursors. Vitamin A, vitamin D and the corresponding provitamins (provitamins A and provitamins D) are biosynthesized as products of the mevalonate and deoxyxylulose phosphate pathways leading to terpenoids and steroids. The biologically active compounds derived from quinones with phytyl or isoprenoid side-chains include members of the vitamins E and K families as well as structurally related plastoquinones and ubiquinones. Vitamin E, vitamin K, plastoquinones, and ubiquinones principally arise as products of the shikimate pathway that further produces aromatic amino acids and a number of phenolic compounds, whereas their side-chains form by the non-mevalonate pathways.

5.1.1 Vitamin A

Carotenoid cleavage products, known as apocarotenoids, are widespread in living organisms as they exert key biological functions. In animals, apocarotenoids function as vitamins, visual pigments, important regulatory signalling molecules in cell division, growth, and differentiation of tissues as well as in controls of reproduction. In plants, apocarotenoids play roles of hormones, pigments, flavors, and defense compounds.

The A group of fat-soluble vitamins historically includes vitamin A_1 (all-*trans*-retinol, also known as retinol) and vitamin A_2 (3,4-didehydroretinol, also known as dehydroretinol). Vitamin A_2 has about 40% of the activity of vitamin A_1. These vitamins and their biologically active metabolites are known as retinoids. Since mammals do not synthesize retinoids *de novo*, they depend exclusively upon the alimentary supply of retinol, its derivatives, and precursors (provitamins A). Retinoids are found only in animal products. Eggs, dairy products, animal livers, kidneys, and fish liver oils (e.g. cod liver oil and halibut liver oil used as dietary supplements) are particularly rich sources. Provitamins A are widely distributed in plants and microorganisms that are capable of carotenoid biosynthesis. Vitamins A are important metabolites of carotenoids that have at least one non-hydroxylated ring system of the β-type, e.g. carotenes (β-carotene, α-carotene, and γ-carotene) and xanthophyls (β-cryptoxanthin and echinenone). Green vegetables (e.g. spinach) and rich vegetable and fruit sources (carrots, apricots, mangoes) provide adequate levels of provitamins A that are synthesized within the plastids.

In mammals, provitamins A are transformed into vitamin A by oxidative cleavage of the β-type carotenoids taken in the diet. Cleavage of β-carotene [5.1)] occurs in the mucosal

[5.1)] Of the carotenoids, β-carotene is the most potent retinol precursor, yet is six fold less effective on a weight basis than retinol, resulting from incomplete absorption and conversion. One retinol equivalent (RE) is equal to 1 μg of retinol, 6 μg of β-carotene, or 12 μg of mixed carotenes.

cells of the intestine. It is catalyzed by an oxygen-dependent dioxygenase β-carotene 15,15'-dioxygenase (EC 1.14.99.36). This reaction proceeds in three stages, epoxidation of the C15,C15'-double bond, hydration of the double bond leading to ring opening, and oxidative cleavage of the diol formed. This symmetric or centric cleavage can yield two molecules of the intermediate aldehyde all-*trans*-retinal (Figure 5.1). Other provitamins A give only one molecule of all-*trans*-retinal.

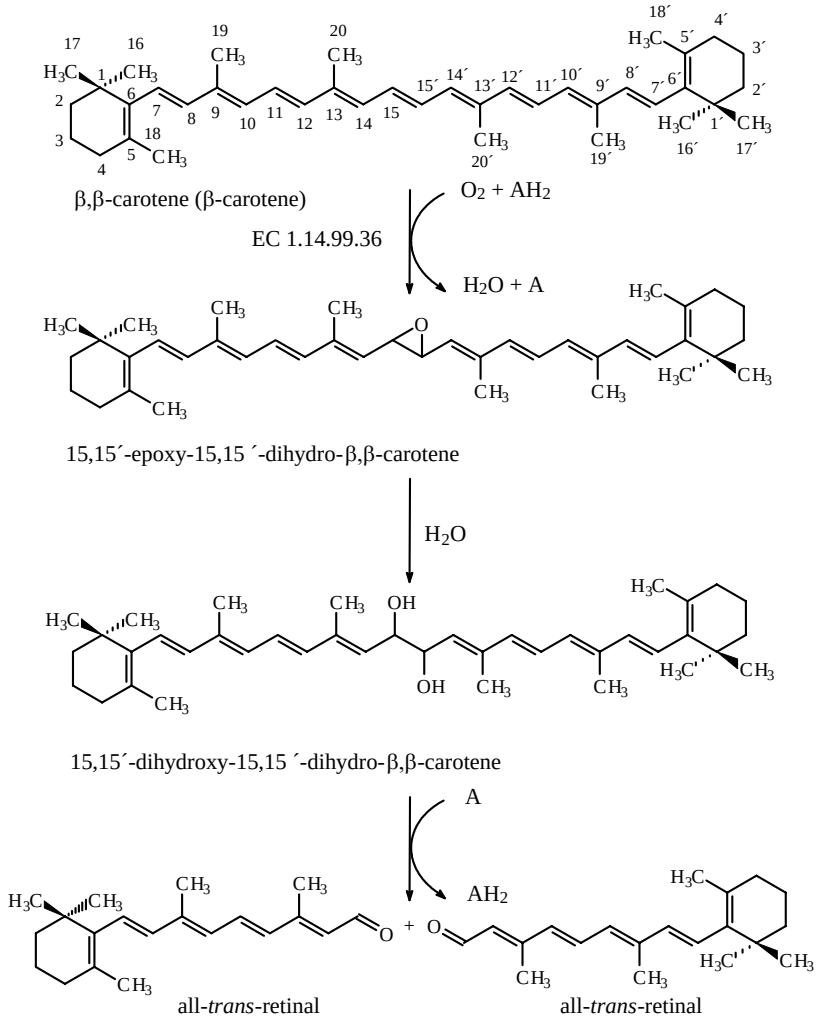


Figure 5.1

Retinal is further metabolized [5.2)] forming reversibly retinol (retinol dehydrogenase, EC 1.1.1.105) and, subsequently, esters with long-chain fatty acids (phosphatidylcholine-

[5.2)] Retinal binds a major intestinal protein, cellular retinol-binding protein, which protects it from oxidation into retinoic acid, but allows it to be reduced into retinol by the microsomal retinol dehydrogenase. Retinol

retinol *O*-acyltransferase, EC 2.3.1.135), the storage forms of retinol [5.3]. Irreversible oxidation of the retinal carbonyl group by the metalloflavoprotein (FAD) (retinal dehydrogenase, EC 1.2.1.36) gives all-*trans*-retinoic acid, oxidation of the β-ring yields 3,4-didehydroretinol, which contains cyclohexadiene ring system (Figure 5.2).

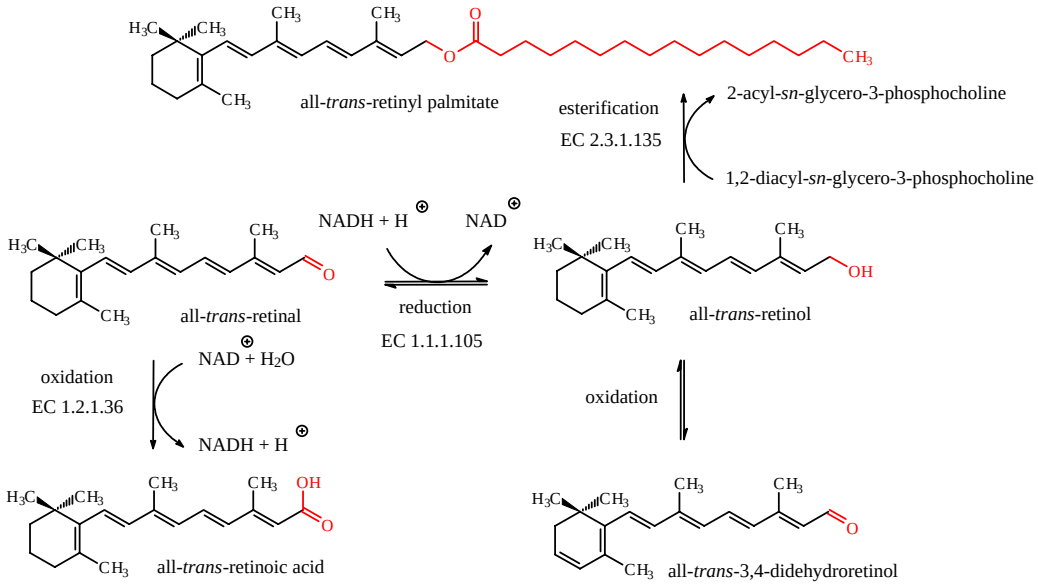


Figure 5.2

A survey of the most common names of the major retinoids and their precursors is given in Table 5.1. Structures of several other important retinoids are given in Figure 5.3.

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formed by hydrolysis of retinyl esters in the intestinal lumen during uptake also complexes with retinol-binding protein. The protein-retinol complex then serves as a substrate for the conversion of retinol into retinyl esters. The retinyl esters (predominantly but not exclusively retinyl palmitate) are incorporated into chylomicrons, along with triacylglycerols, cholesteryl esters, carotenoids, and other fat-soluble vitamins, secreted into lymph, and transported to the livers for storage. Once in the hepatocytes (retinyl esters are also stored in the lungs and bone marrow), retinyl esters undergo hydrolysis to release free retinol, which then binds with retinol-binding protein. This complex is mostly transferred to the hepatic stellate cells and retinol is re-esterified. Mobilization of retinol from these cells involves ester hydrolysis and complexation of free retinol with retinol-binding protein before excretion into plasma. Inside the target cells, retinol (having no direct known biological activity) is converted into hormonally active products, e.g. retinal and retinoic acid.

The functional retinoids fall into two categories. The first category comprises the cofactor (having its role in vision), 11-*cis*-retinal, covalently bound to the protein opsin to form rhodopsin. The second category includes the humoral agents that regulate gene expression. This category includes the retinoids derived from all-*trans*-retinal, i.e. all-*trans*-retinoic acid, 9-*cis*-retinoic acid, and 3,4-didehydroretinoic acid, which is derived from all-*trans*-3,4-didehydroretinol, and 14-hydroxy-*retro*-retinol.

[5.3] To date, two retinol-esterifying enzymes have been described. The first enzyme, phosphatidylcholine-retinol *O*-acyltransferase (EC 2.3.1.135; requires a fatty acyl group from the *sn*-1 position of phosphatidyl-choline as an acyl donor), esterifies free or bound (cellular-retinol-binding-protein) retinol in the majority of tissues. The second enzyme, acyl-CoA:retinol *O*-acyltransferase (EC 2.3.1.76, acts on palmitoyl-CoA and other long-chain acyl derivatives of HS-CoA), catalyzes esterification of free retinol in the mammary gland.

Although β-carotene cleaved at the central double bond is capable of giving rise to two molecules of retinol, there is evidence that cleavage can also occur at other double bonds [5.4)], by the so-called asymmetric or excentric cleavage. This asymmetric cleavage leads to the formation of two molecules of β-apo-carotenals with different chain lengths.

Table 5.1 Trivial, specific, semi systematic, or systematic names of the major carotenoids and retinoids

Name
α-Carotene, β,ε-carotene
β-Carotene, β,β-carotene
γ-Carotene, β,ψ-carotene
β-Cryptoxanthin, 3-hydroxy-β,β-carotene
all-*trans*-3,4-Didehydroretinol, 3,4-didehydroretinol, vitamin A_2, (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)nona-2,4,6,8-tetraen-1-ol
Echinenone, β,β-caroten-4-one
all-*trans*-Retinal, retinal, retinene, vitamin A_1 aldehyde, 15-apo-caroten-15-al, (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenal
all-*trans*-Retinoic acid, retinoic acid, tretinoin, vitamin A_1 acid, (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-carboxylic acid
all-*trans*-Retinol, retinol, vitamin A_1, (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-ol

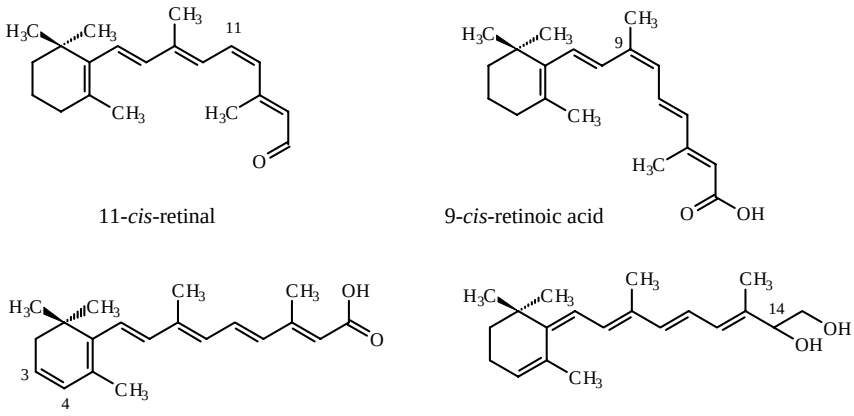


Figure 5.3

The asymmetric cleavage of β-carotene at the C11´,C12´ double bond results in the formation of β-apo-12´-carotenal and β-ionilideneacetaldehyde (11-apo-β-caroten-11-al).

[5.4)] The properties of this enzyme (requires bile salts and Fe^{2+}) have been a subject of controversy because formerly it was considered to be a β-carotene 15,15'-monooxygenase (EC 1.13.11.21). Furthermore, both symmetric and asymmetric cleavage of β-carotene was reported. Recently, a second type of β-carotene dioxygenase has been described, which catalyses exclusively the asymmetric oxidative cleavage at the 9´,10´ double bond of β-carotene. Besides β-carotene, also lycopene can be oxidatively cleaved by this enzyme.

The C9′,C10′ double bond cleavage yields β-apo-10′-carotenal and β-ionone (9-apo-β-caroten-9-one). The C7′,C8′ double bond cleavage gives β-apo-8′-carotenal and β-cyclocitral (7-apo-β-caroten-7-al) (Figure 5.4). Further chain shortening then produces retinal, but only one molecule is produced per molecule of β-carotene. Some of these apocarotenes (e.g. β-ionone and β-cyclocitral) are prominent flavor-active components of many fruits and vegetables.

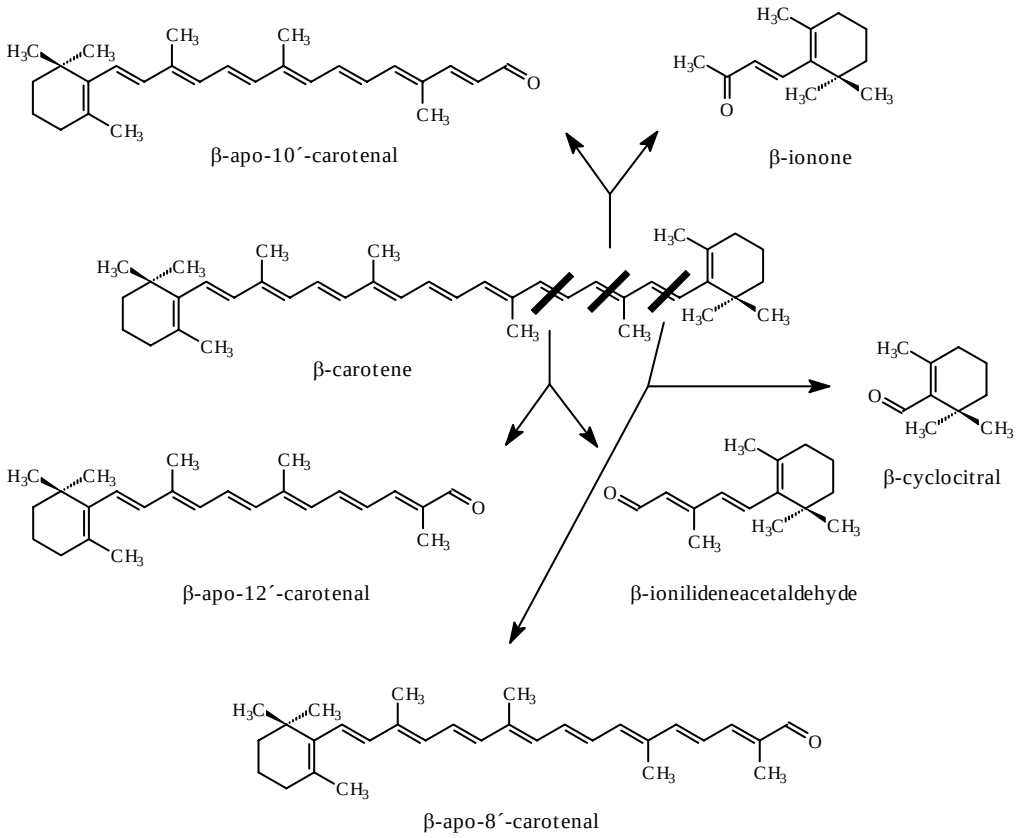


Figure 5.4

β-Apo-carotenals (i.e. β-apo-8′-carotenal, β-apo-10′-carotenal, and β-apo-12′-carotenal) can be oxidized to the corresponding β-apo-carotenoic acids or split at the C15,C15′ double bond to all-trans-retinal. The β-apo-carotenoic acids, in turn, can be degraded either by a mechanism similar to β-oxidation of fatty acids or by splitting at the C15,C15′ position, which produces all-*trans*-retinoic acid.

Fresh-water fish contain considerable amounts of vitamin A_2 in addition to vitamin A_1. In this respect they differ from marine fish and from birds and mammals, which appear to have only vitamin A_1. It was found that, except the above pathway, fresh-water fish are able to convert lutein, also known as 3,3′-dihydroxy-α-carotene or (3*R*,3′*S*,6′*R*)-β,ε-caroten-3,3′-diol, to anhydrolutein in the intestine. Anhydrolutein is then split to all-*trans*-3,4-dehydroretinol and all-*trans*-3-hydroxyretinol, after which the latter can be converted

to all-*trans*-3,4-dehydroretinol (Figure 5.5). β-Carotene is converted to all-*trans*-retinol as in birds and mammals.

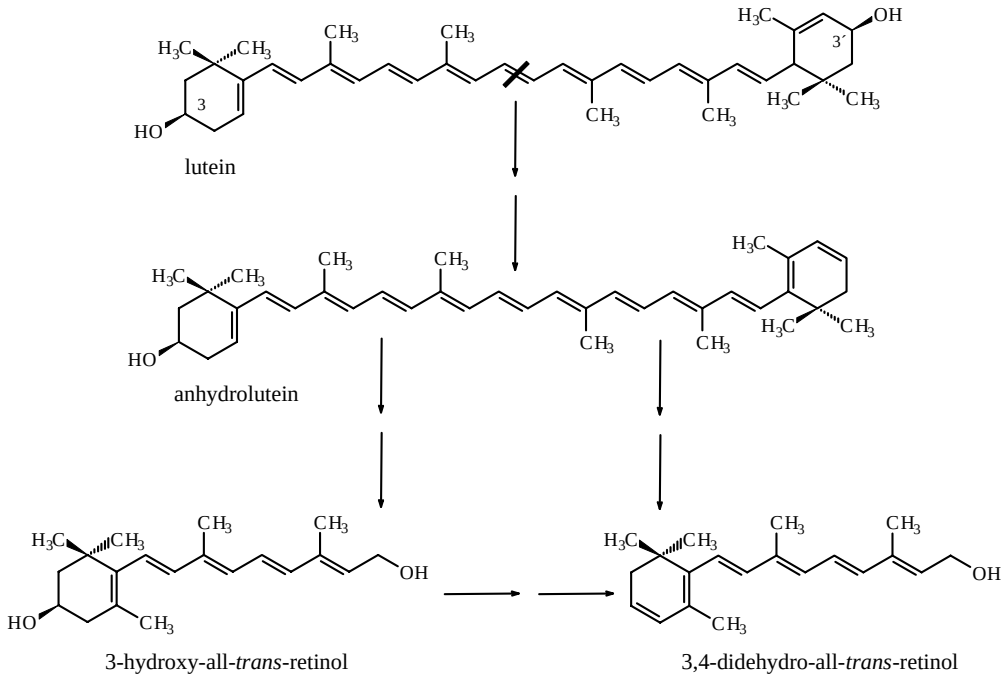


Figure 5.5

5.1.2 Vitamin D

The biological functions of vitamin D, achieved largely through a steroid hormone-like mechanisms, are usually divided into classical roles, which include the regulation of blood calcium and phosphate concentrations by actions at intestine, bone, and kidney, and nonclassical roles, which include cell differentiation and antiproliferative actions on various cell lines, especially bone marrow, skin, and intestine.

Vitamin D_3 (cholecalciferol) is obtained in the diet from liver and dairy products, such as butter, cream, and milk, whilst large amounts can be found in fish liver oils. Further requirements in animals are produced photochemically from the immediate precursor of cholesterol, 7-dehydrocholesterol, by the sun's irradiation of the skin (Figure 5.6). A photochemical reaction (absorption of light energy by π-electron system) allows electrocyclic ring opening of 7-dehydrocholesterol to yield precholecalciferol (previtamin D_3). Once it is formed, it is slowly transformed by a thermal 1,7-hydrogen shift to cholecalciferol. The 1,7-hydrogen shift should be considered as an extended version of an allylic isomerization (Figure 5.7), however, there is intramolecular transfer of the proton rather than employing an external source.

Cholecalciferol is not itself the active form of vitamin D. A specific globulin, known as vitamin D binding protein, transports cholecalciferol from the skin to liver for storage or the first step of activation, i.e. hydroxylation to calcidiol (25-hydroxyvitamin D_3) by the

enzyme cholecalciferol 25-hydroxylase (from the sub-sub class of oxidoreductases, EC 1.14.15.-) (Figure 5.6). Calcidiol is subsequently transported to kidney for the second step of activation, hydroxylation to calcitriol (1α,25-dihydroxyvitamin D_3), which is catalyzed by the 25-hydroxycholecalciferol 1α-hydroxylase enzyme (calcidiol monooxygenase, which is an active part of cytochrome P_{450}, EC 1.14.13.13).

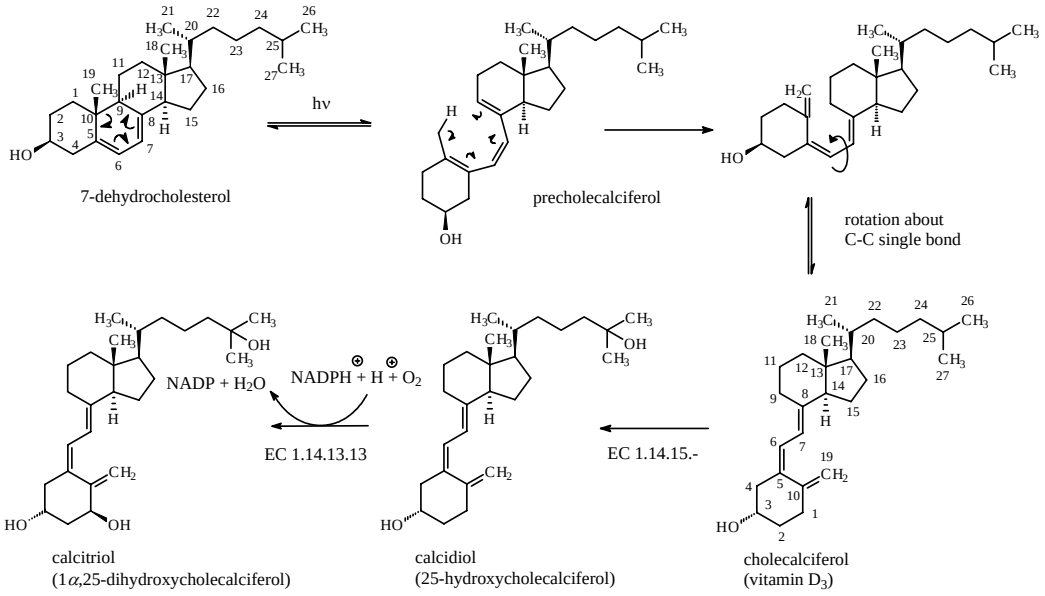


Figure 5.6

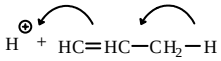


Figure 5.7

Many other vitamin D_3 metabolites have been reported over the years. In the kidney, calcidiol can be transformed into (24*R*)-24,25-dihydroxyvitamin D_3. In the kidney and other target tissues (skin, bone, intestine, and parathyroid gland) this is transformed into (24*R*)-1α,24,25-trihydroxyvitamin D_3 and this compound to calcitroic acid (Figure 5.8).

6.3.1 Monoterpenic hydrocarbons

Deprotonation of the resonance-stabilized linalyl carbenium ion (Figure 6.6) leads to the formation of alicyclic monoterpenes (Table 6.1) that are present in many fruits and essential oils. The linalyl cation can undergo deprotonation at the C-3 methyl group to form myrcene (β-myrcene) (Figure 6.7) [6.6]. Deprotonation at C-4 yields β-ocimene that can exist in two stereoisomeric forms, *cis* and *trans*.

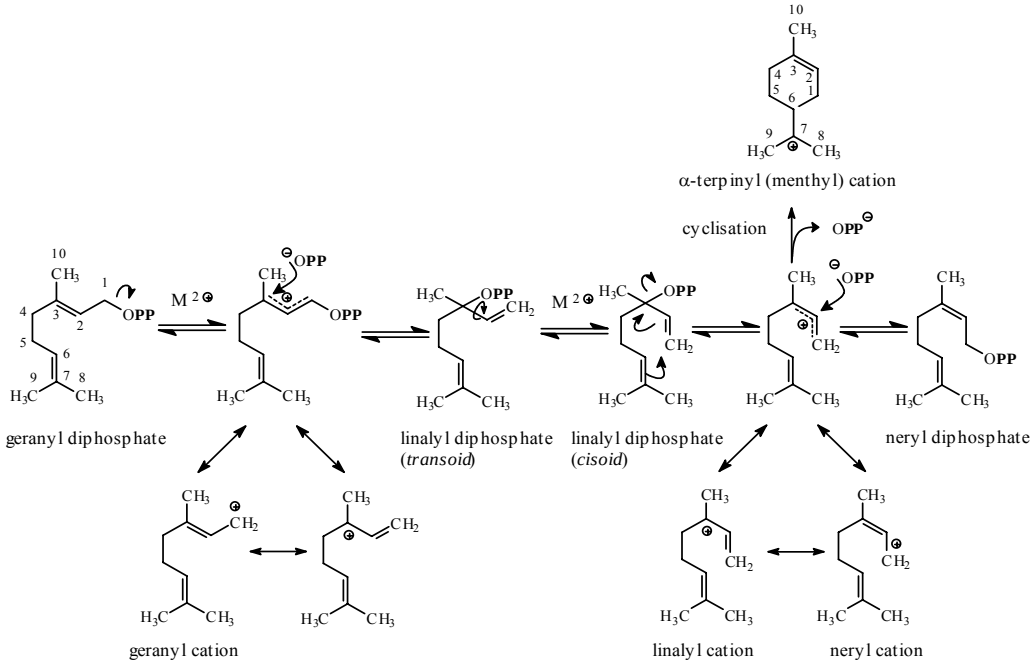


Figure 6.6

Monocyclic hydrocarbons are mainly derived from *p*-menthane (4-isopropyl-1-methylcyclohexane) (Figure 6.8). Bicyclic hydrocarbons are based on seven skeletal types. The bicyclic systems are formed by combination of five-membered and three-membered rings (thujane also known as sabinane), six-membered and three-membered rings (carane), six-membered and four-membered rings (pinane), or two five-membered rings (fenchane, camphane also known as bornylane, isocamphane, and isobornylane).

Formation of mono- and bicyclic hydrocarbons proceeds via the α-terpinyl cation intermediate. This cation can lose the C-8 proton (Figure 6.9) to give limonene (*p*-mentha-1,8-diene) that occurs abundantly in many essential oils (e.g. oils of *Citrus* and *Mentha* species) and conifer turpentine.

[6.6] Only a few monoterpene synthases involved in the biosynthesis of monoterpenoid hydrocarbons have been characterized in detail. Myrcene biosynthesis is catalyzed by myrcene synthase, also known as geranyl-diphosphate diphosphate-lyase (myrcene-forming) (EC 4.2.3.15), pinene synthase also known as (-)-(1*S*,5*S*)-pinene synthase (EC 4.2.3.14), catalyzes the biosynthesis of (-)-α- and (-)-β-pinenes, (-)-(4*S*)-limonene synthase (EC 4.2.3.16) and (+)-(4*R*)-limonene synthase (EC 4.2.3.20) are involved in the biosynthesis of (-)-(4*S*)-limonene and (+)-(4*R*)-limonene, respectively.

Table 6.1 Trivial and IUPAC systematic names of monoterpenic hydrocarbons

Trivial name	IUPAC systematic name
(+)-Camphene	(1*R*,4*S*)-6,6-dimethyl-5-methylidenebicyclo[2.2.1]heptane
(-)-Camphene	(1*S*,4*R*)-2,2-dimethyl-3-methylenebicyclo[2.2.1]heptane
(+)-Car-3-ene	(1*S*,6*R*)-3,7,7-trimethylbicyclo[4.1.0]hept-3-ene
(-)-Car-3-ene	(1*R*,6*S*)-3,7,7-trimethylbicyclo[4.1.0]hept-3-ene
(+)-Limonene,	(4*R*)-1-methyl-4-prop-1-en-2-ylcyclohex-1-ene
(-)-Limonene	(4*S*)-1-methyl-4-prop-1-en-2-ylcyclohex-1-ene
Myrcene	7-methyl-3-methyleneocta-1,6-diene
cis-β-Ocimene	(3*Z*)-3,7-dimethylocta-1,3,6-triene
trans-β-Ocimene	(3*E*)-3,7-dimethylocta-1,3,6-triene
(+)-α-Phellandrene	(5*S*)-2-methyl-5-propan-2-ylcyclohexa-1,3-diene
(-)-α-Phellandrene	(5*R*)-2-methyl-5-propan-2-ylcyclohexa-1,3-diene
(+)-β-Phellandrene	(6*S*)-3-methylene-6-propan-2-ylcyclohex-1-ene
(-)-β-Phellandrene	(6*R*)-3-methylene-6-propan-2-ylcyclohex-1-ene
(+)-α-Pinene	(1*R*,5*R*)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene
(-)-α-Pinene	(1*S*,5*S*)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene
(+)-β-Pinene	(1*R*,5*R*)-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane
(-)-β-Pinene	(1*S*,5*S*)-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane
(+)-Sabinene	(1*R*)-4-methylene-1-propan-2-ylbicyclo[3.1.0]hexane
(-)-Sabinene	(1*S*)-4-methylene-1-propan-2-ylbicyclo[3.1.0]hexane
α-Terpinene	1-methyl-4-propan-2-ylcyclohexa-1,3-diene
γ-Terpinene	1-methyl-4-propan-2-ylcyclohexa-1,4-diene

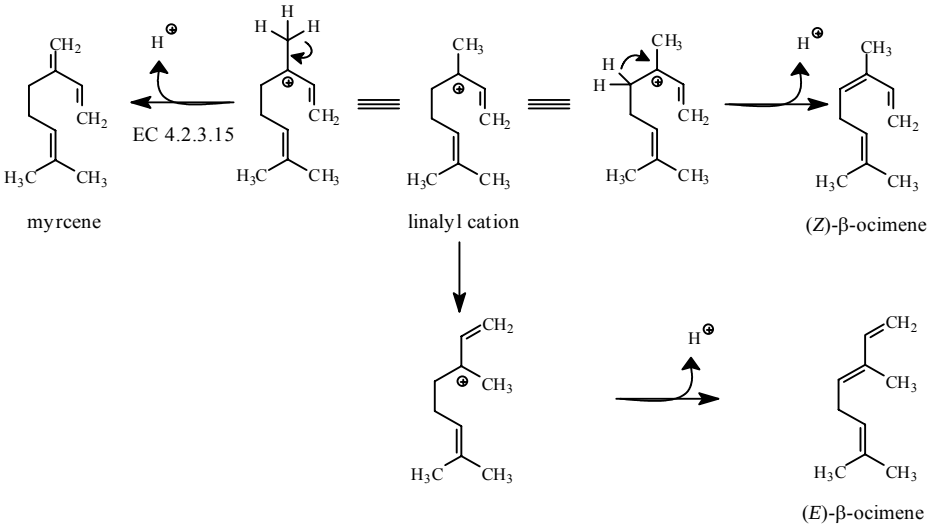


Figure 6.7

Alternatively, folding the cationic side-chain towards the double bond (via the surface characteristics of the enzyme) allows a repeat of the cyclization mechanism and produces pinyl and bornyl cations, according to which end of the double bond was involved in forming the new bond. Discharging the positive charge by loss of different protons from the pinyl cation, producing the double bonds either cyclic or exocyclic, yields α-pinene

(2-pinene) and β-pinene, also known as 2(10)-pinene, nopinene, or pseudopinene, that occur in oils of *Pinus* species and conifer turpentine [6.7] together with the tricyclic monoterpene hydrocarbon tricyclene, i.e. 3,3-dimethyltricyclo[2.2.1.$0^{2,6}$]heptane, and other minor components (Table 6.1). A less common termination step involving loss of a proton is the formation of a cyclopropane ring occurring in car-3-ene and car-2-ene, i.e. 3,7,7-trimethylbicyclo[4.1.0]hept-2-ene found in the Pinaceae needle oils.

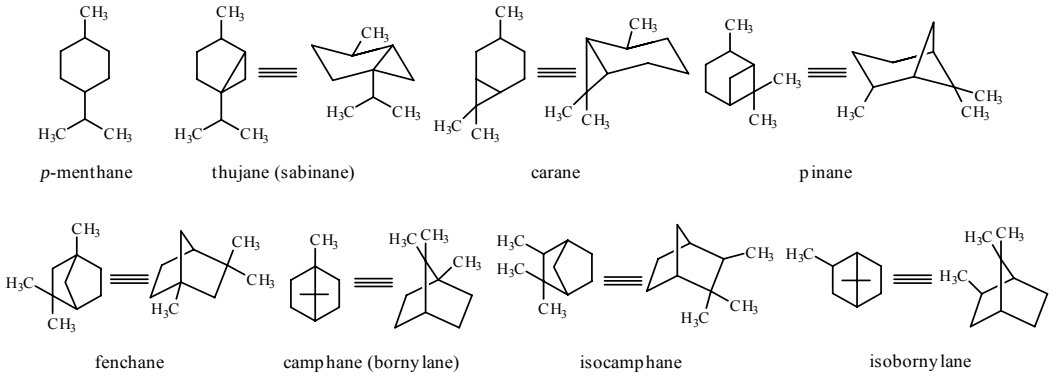


Figure 6.8

Rearrangements of the Wagner-Meerwein type, in which carbon or hydride migrate to achieve enhanced stability for the cation via tertiary against secondary character, or by reduction of the ring strain, are very common reactions. For example, the tertiary α-terpinyl cation may be converted by 1,3-hydride shift to a favorable resonance-stabilized allylic cation, which allows e.g. the formation of α-phellandrene (*p*-mentha-1,5-diene) and β-phellandrene, also known as *p*-mentha-1(7),2-diene. Phellandrenes, formed by deprotonation of the phellandryl cation, occur in e.g. *Eucalyptus* oil, (+)-α-phellandrene is the characteristic impact component in dill (*Anethum graveolens*; Apiaceae). The trans-formation of the bornyl secondary cation (1,2-alkyl shift) produces isocamphyl tertiary carbenium ion, which can be deprotonized to camphene (widespread in e.g. oils of *Pinus* species). The bicyclic pinyl cation, with a strained four-membered ring, is deprotonized to α-pinene and β-pinene and rearranges (1,2-alkyl shift) to the less strained five-membered fenchyl cation (the corresponding fenchene is not found in any naturally occurring products). The 1,2-hydride shift, converting the α-terpinyl cation to terpinen-4-yl cation, only changes one tertiary cation for another, but allows formation of widespread hydrocarbons α-terpinene (*p*-mentha-1,3-diene) and γ-terpinene (*p*-mentha-1,4-diene)

[6.7] The viscous secretion released upon tissue injury of coniferous trees is known as oleoresin. It is involved in the chemical and physical defence of conifers against predators and fungal pathogens. Oleoresin is composed in roughly equal parts of volatile turpentine (a mixture of monoterpenes and sesquiterpenes) and rosin (also known as diterpene resin acids). The exact composition of turpentine and rosin varies within the conifer species.

Tall oil, also called tallol, is obtained as a by-product of wood pulp manufacture. Crude tall oil contains rosins, unsaponifiable sterols (5-10%), resin acids (mainly abietic acid and its isomers), fatty acids, fatty alcohols, and other compounds. By fractional distillation tall oil rosin is obtained, with rosin content reduced to 10-35%. By further reduction of the rosin content to 1-10%, tall oil fatty acids can be obtained, which consists mostly of oleic acid.

that occur in a variety of plant sources. A further cyclization on the terpinen-4-yl cation via thujyl cation yields sabinene, also known as 4(10)-thujene, and β-thujene. Sabinene is found in high concentration e.g. in black pepper essential oil.

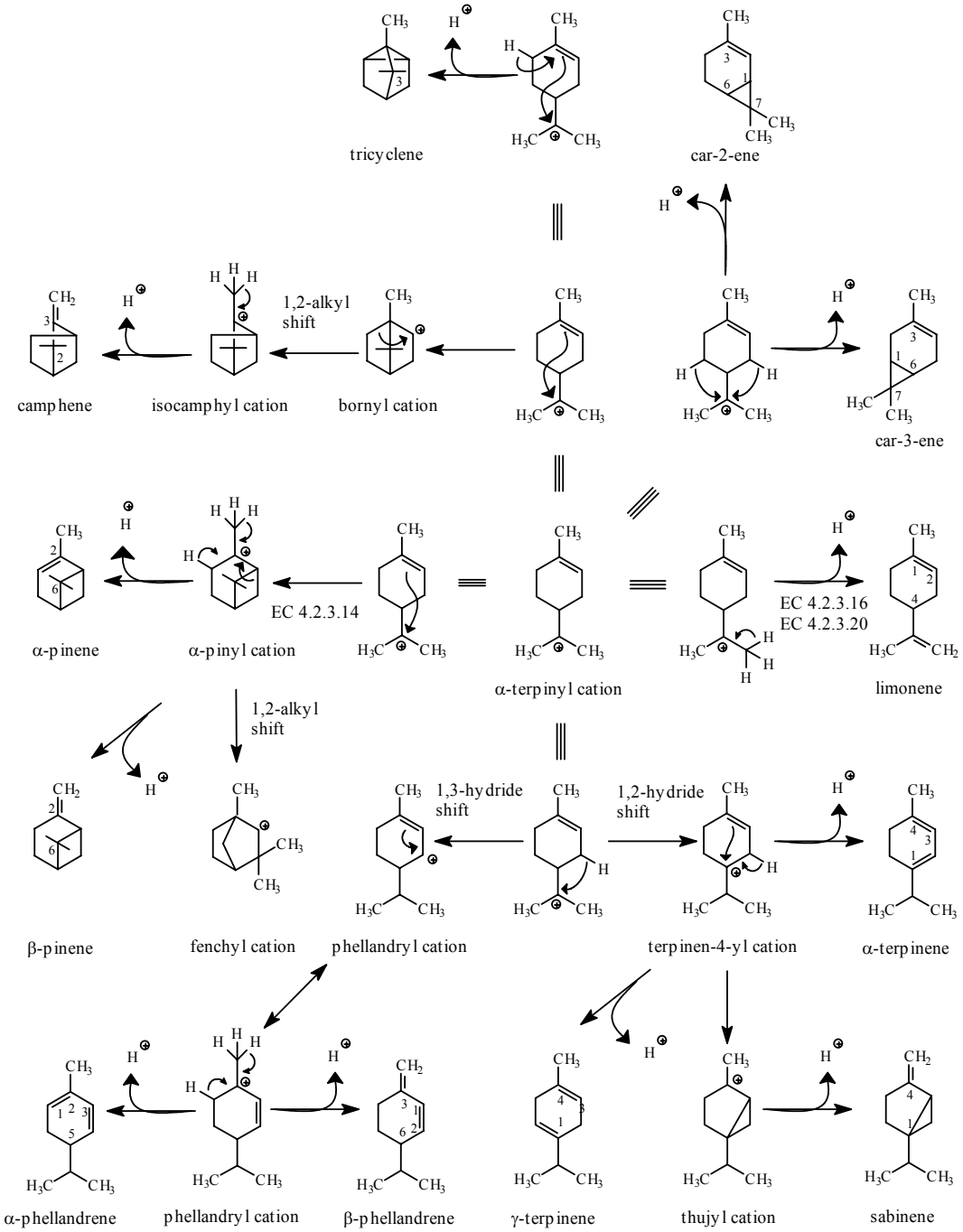


Figure 6.9

Individual enzyme systems present in a particular organism control the initial folding of the geranyl diphosphate molecule and thus define the stereochemistry of the final product. As a result, the preliminary ionization and isomerization of the geranyl diphosphate produces the (+)-(3*R*)- and/or (-)-(3*S*)-linalyl diphosphate (*cisoid* conformers) and their C-6/C-1 ring closure provides (+)-(4*R*)- and/or (-)-(4*S*)-α-terpinyl cation/diphosphate anion pair (Figure 6.10), and the corresponding metabolites depending on the enzyme system involved.

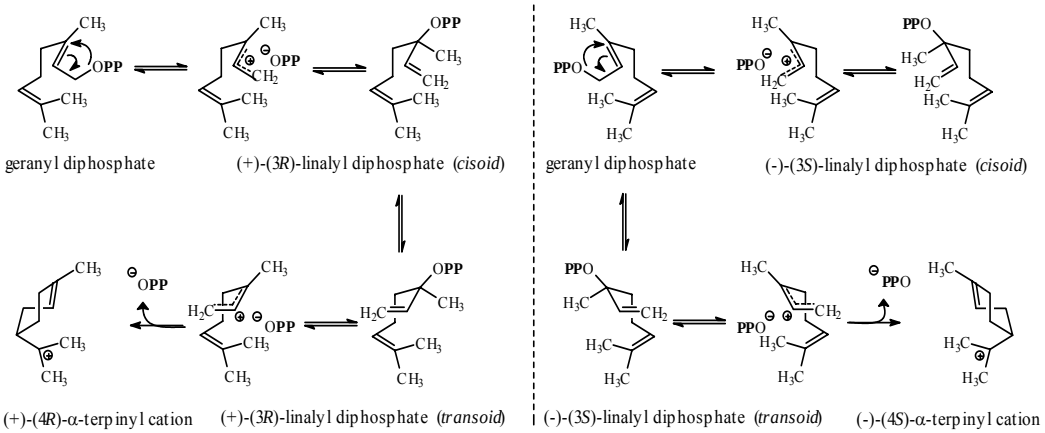


Figure 6.10

Subsequent reactions of the (+)-(4*R*)-α-terpinyl cation intermediate produce e.g. (+)-limonene, (+)-sabinene, (+)-camphene, (+)-α-pinene, (+)-β-pinene, and other (*R*)-enantiomers of monoterpenic hydrocarbons, whereas (-)-(4*S*)-α-terpinyl cation intermediate yields (-)-limonene, (-)-sabinene, (-)-camphene, (-)-α-pinene, (-)-β-pinene, etc. (Figure 6.11). For example, the (+)-isomer of limonene is the major component of citrus peel oils (at a concentration of over 90%) and (-)-limonene is found e.g. in *Mentha* species and conifer oils. The racemate (often referred to as dipentene) occur abundantly in many essential oils.

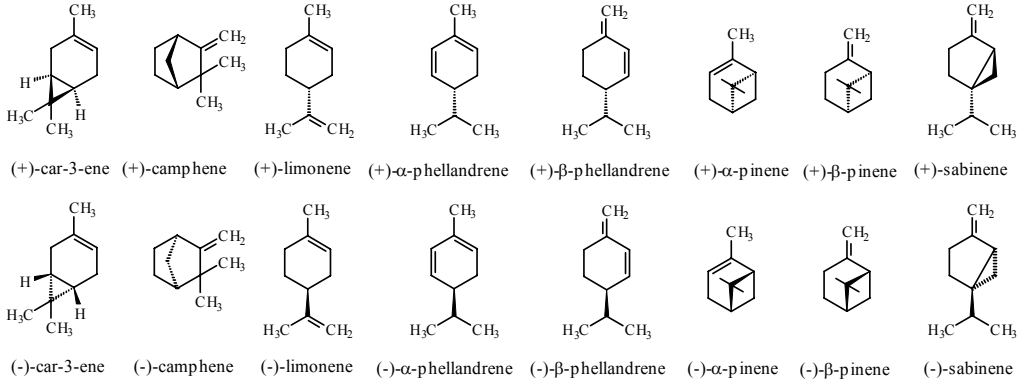


Figure 6.11

6.3.1.1 Wagner-Meerwein rearrangement

The Wagner-Meerwein rearrangement, also known as the Wagner-Meerwein migration, is a class of carbenium ion 1,2-rearrangement reactions (S_N1 and E1 reactions) in which a hydrogen, alkyl, or aryl group migrates from one carbon to a neighboring carbon. Occasionally, 1,3-shift or longer shifts are encountered.

This reaction was originally studied with respect to secondary and tertiary alcohols derived from terpene systems. In a typical case of this reaction, an alcohol substrate protonates and eliminates water to create a carbenium ion species (Figure 6.12). This carbenium ion then undergoes the 1,2-nucleophilic group migration leading to a rearranged more stable carbenium ion, which step constitutes the actual Wagner-Meerwein migration. The new tertiary carbenium ion may then go on to react in various ways, e.g. by loss of a carbon-bonded proton to give olefin or may be deprotonized by a suitable nucleophile (e.g. water) to yield alcohol, etc.

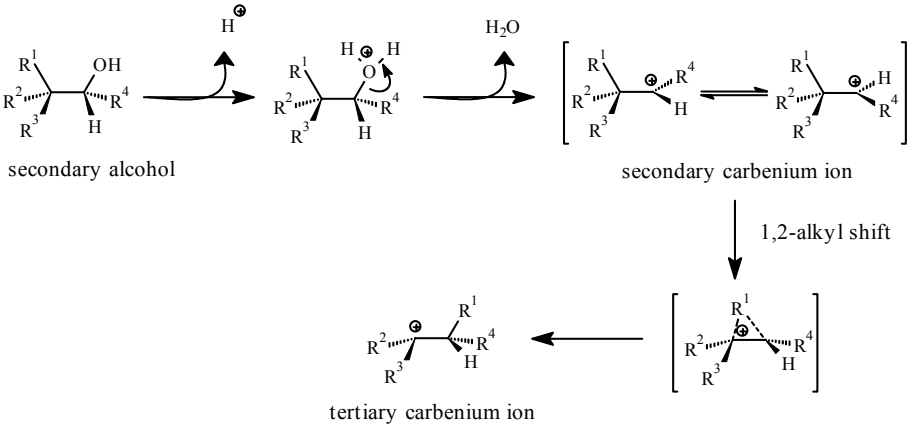


Figure 6.12

6.3.2 Monoterpenic alcohols and other monoterpenoids

Monoterpenic alcohols occur abundantly in many essential oils and constitute the flavor-active components of many spices. They are not generated by the phosphatase activity from the parent diphosphate; instead they form by the addition of a hydroxyl group to a carbenium ion intermediate [6.8)].

The resonance-stabilized allylic cations formed from geranyl diphosphate and the α-terpinyl cation (Figure 6.6) become the precursors of a range of terpenic alcohols (Figure 6.13, Figure 6.14). Their structures and structures of some other important oxygenated monoterpenoids are shown in Table 6.2. The amounts of the major mono-terpenoid components of some common spices are given in Table 6.3, however, they can vary greatly depending on the variety, cultivation conditions, and other factors.

[6.8)] (3*S*)-Linalool synthase (EC 4.2.3.25), (3*R*)-linalool synthase (EC 4.2.3.26), (-)-endo-fenchol synthase (EC 4.2.3.10), sabinene-hydrate synthase (EC 4.2.3.11), (-)-endo-fenchol synthase (EC 4.2.3.10), and bornyl diphosphate synthase (EC 5.5.1.8) have been characterized. At least in some plant species (e.g. in sweet basil, *Ocimum basilicum*, Lamiaceae), nerol can be formed by oxidation of neral. The formation of citronellol from geraniol is catalyzed by a NADPH-dependent oxidoreductase (EC 1.1.1.-).

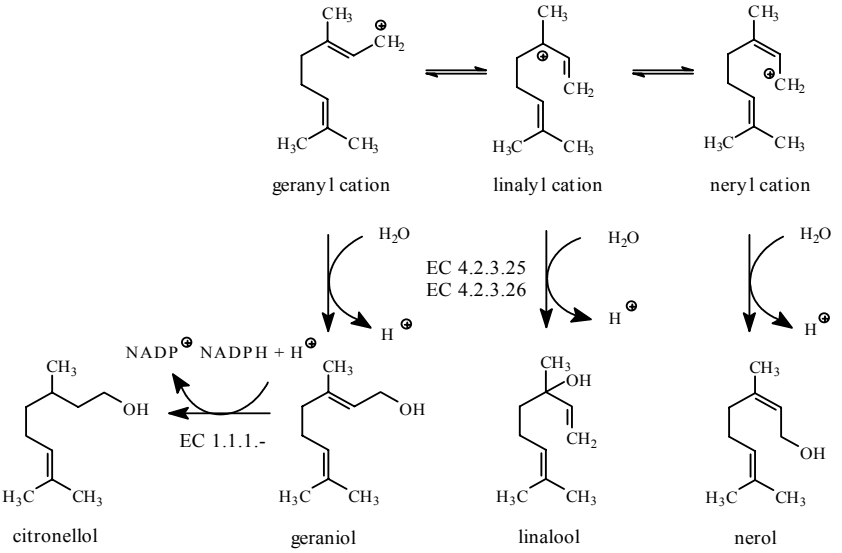


Figure 6.13

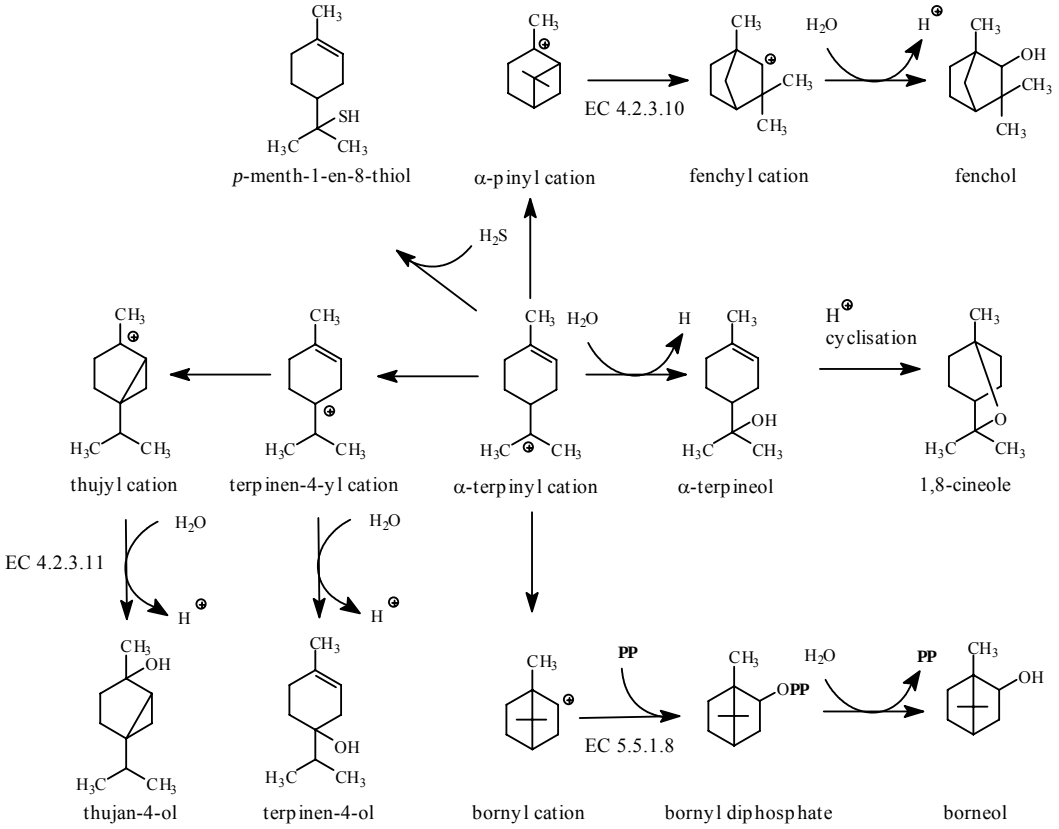


Figure 6.14

In both plants and leaf beetles, iridoids biosynthesis proceeds along the same principal pathway. The iridoid system is derived from geranyl diphosphate by a type of folding, which is different from that already encountered with monoterpenoids and also different is the lack of phosphorylated intermediates and subsequent carbenium ion mechanism in its formation. Geraniol diphosphate is hydrolyzed to geraniol, which undergoes subsequent allylic oxidation, using a P450-dependent mixed-function oxidase geraniol 10-hydroxylase, to produce 8-hydroxygeraniol (also known as 10-hydroxygeraniol) (Figure 6.25). The hydroxylation of geraniol to 8-hydroxygeraniol is followed by subsequent oxidation to 8-oxogeranial, which is catalyzed by a zinc-containing $NADP^+$-dependent oxidoreductase. The fundamental cyclization of 8-oxogeranial to the methylcyclopentanoid nucleus is an enzyme-controlled process and the products arising from this pathway are single stereoisomers.

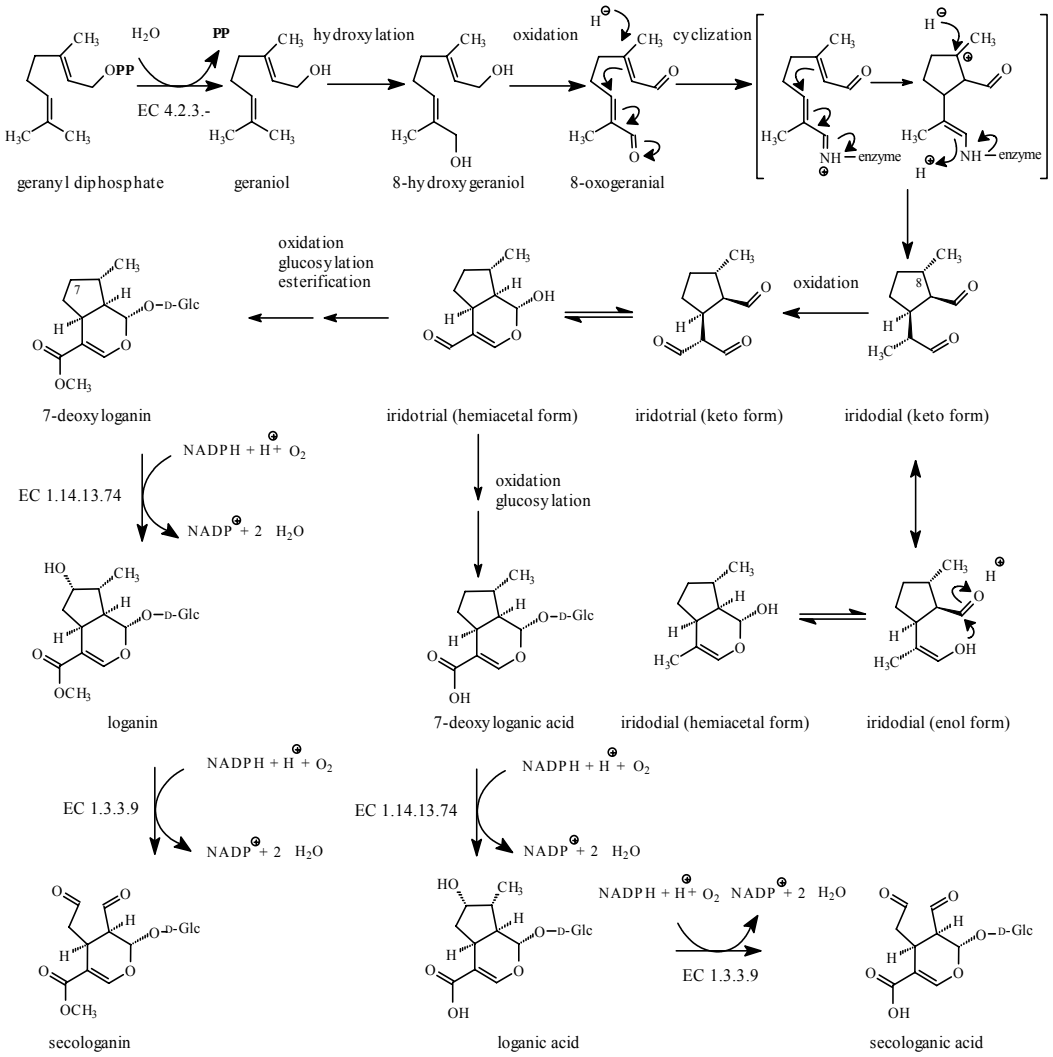


Figure 6.25

In the pathway leading to 7-deoxyloganin, loganin, and further to secologanin, the formation of the next product iridodial, i.e. (1*R*,2*S*,5*R*,8*R*)-iridodial, is formulated as initiated either by electrophilic addition utilizing the unsaturated carbonyl and terminated by addition of hydride or by the Schiff base-assisted mechanism (shown in parentheses in Figure 6.25). Further oxidation yields iridotrial, in which the hemiacetal formation leads to formation of the heterocyclic ring. Oxidation of the formyl group in the iridotrial hemiacetal, *O*-glucosylation (hexosyltransferase, EC 2.4.1.-), and methylation gives 7-deoxyloganin. In the pathway to loganin, 7-deoxyloganin is hydroxylated by the action of the heme-thiolate protein (P450) 7-deoxyloganin 7-hydroxylase (EC 1.14.13.74). A cytochrome P450-dependent monooxygenase secologanin synthase (EC 1.3.3.9) provides the cleavage at the 7,8-bond of the cyclopentane moiety yielding secologanin, a pivotal terpenoid intermediate in the biosynthesis of monoterpenoid indole alkaloids. Alternatively, oxidation of the formyl group in the iridotrial hemiacetal and *O*-glucosylation yields 7-deoxyloganic acid, which is transformed using the same enzymes to loganic acid and further to secologanic acid. Esterification of loganic and secologanic acids with loganate *O*-methyltransferase (EC 2.1.1.50) yields loganin and secologanin, respectively.

Secoiridoid glycosides are often intensely bitter compounds. Loganin occurs e.g. in the root extracts of bog-bean (buckbean; *Menyanthes trifoliata*; Menyanthaceae). The bitter taste of root of great yellow gentian (*Gentiana lutea*; Gentianaceae) is imparted by a number of bitter iridoid glycosides, primarily swertiamarin, gentiopicroside (also known as gentiopicrin; about 1.5% of fresh roots), amarogentoside (amarogentin), and sweroside. Centaury (*Centaurium erythraea*; Gentianaceae) constituents are very similar to those of gentian, also containing gentiopicroside (formerly also known as erytaurin) and other bitter glycosides. The biosynthesis of gentiopicroside starts with hydrolysis of secologanin and formation of the free acid enol tautomer followed by allylic isomerization and lactone formation (Figure 6.26). Amarogentin is one of the bitterest compounds known (Figure 6.27). The biphenylcarboxylic acid moiety in amarogentin is biosynthesized by a polyketide-type pathway, with three units of acetyl-CoA and one unit of 3-hydroxybenzoyl-CoA, this being formed from an early shikimic acid pathway intermediate (Figure 6.28) and not via cinnamic or benzoic acid.

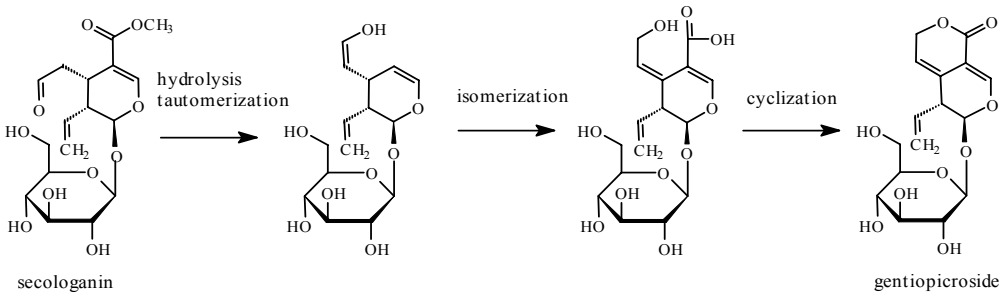


Figure 6.26

Olives (*Olea europea*; Oleaceae) contain a number of unusual phenolics, including various secoiridoids containing an 8,9-exocyclic olefinic functionality that are known as oleosides. Oleosides are predominantly represented by oleuropein, demethyloleuropein, ligstroside, and oleoside (Figure 6.29), whereas verbascoside (see Chapter 7.4.2.2) is the

main hydroxycinnamic derivative of olive fruit. Oleosides can perform a multichemical defense bioactivity against microbe and insect attack onto the olive fruits. Young green fruit contain the oleuropein precursors oleoside and ligstroside that are not bitter. During the ripening process, ligstroside is oxidized to bitter oleuropein. Oleuropein is generally the most prominent phenolic compound in unripe green and yellow fruits and may reach concentrations of up to 140 g/kg (dry matter basis).

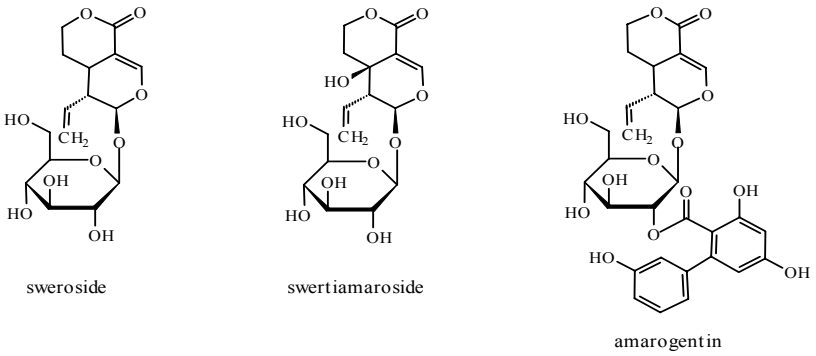


Figure 6.27

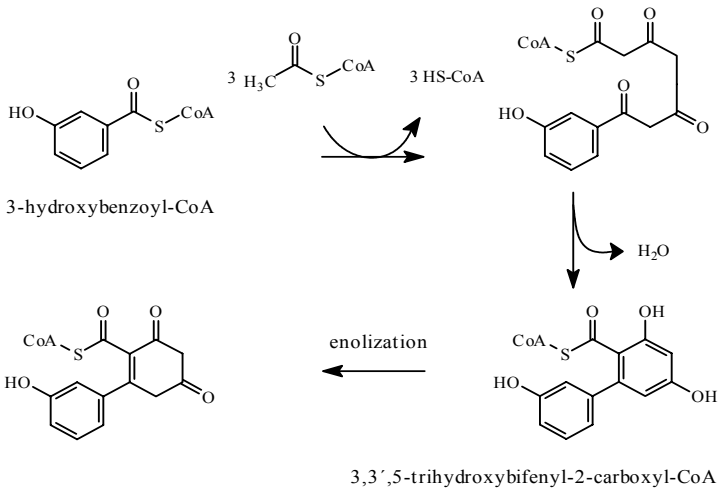


Figure 6.28

The biosynthesis of oleuropein from 7-deoxyloganic acid probably proceeds via 7-epiloganic acid. There may be a pathway between secoxyloganin and oleoside 11-methylester, and this has not been established (Figure 6.29). Oleocanthal (Figure 6.30) is a similar compound isolated from extra virgin olive oil. It is responsible for its slightly peppery bite. Oleocanthal is an tyrosol ester and its chemical structure is related to oleuropein that is also found in olive oil.

Traditionally, the bitterness of olives is removed by lactic acid fermentation (see Chapter 4.4.1) or by an alkaline treatment in NaOH solution. Oleuropein is hydrolyzed to glucose, 3,4-dihydroxyphenylethanol, methanol, and demethylelenoic acid.

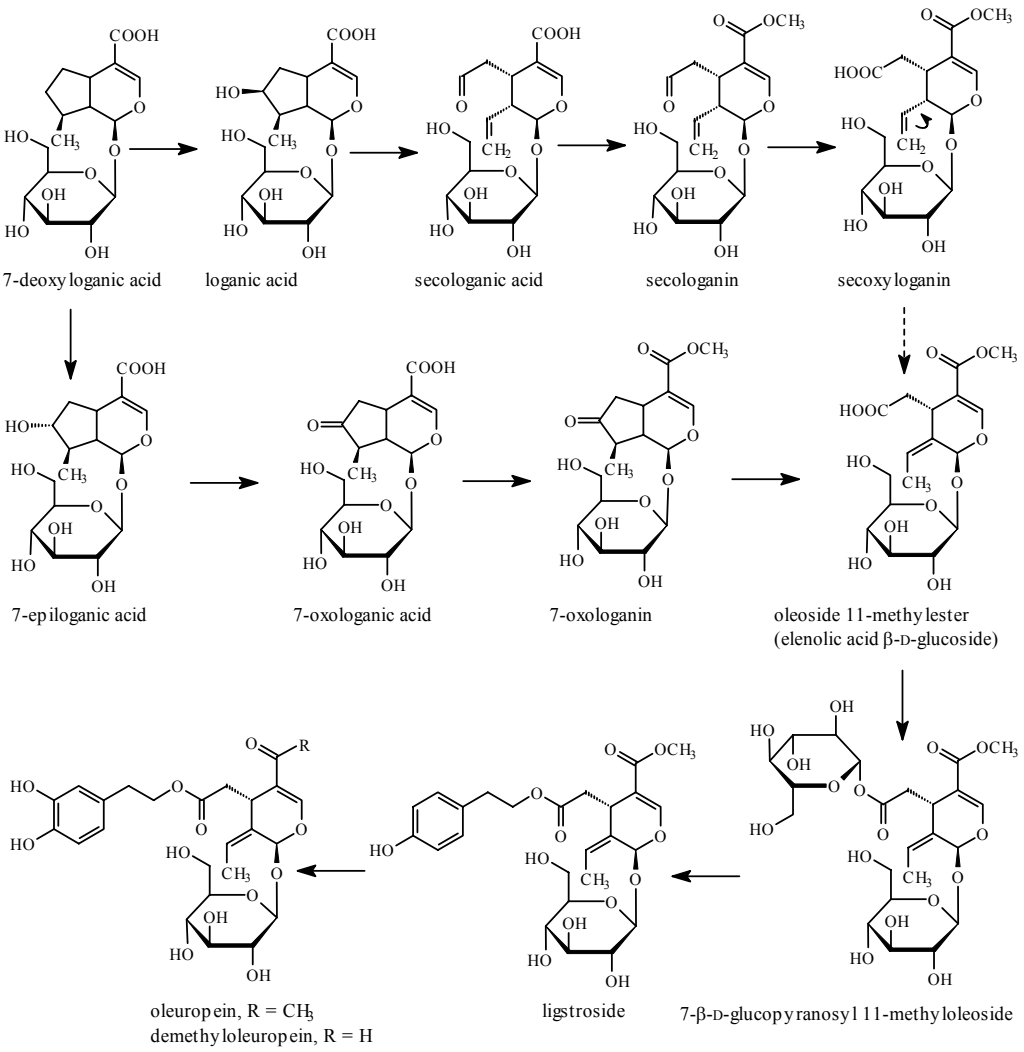


Figure 6.29

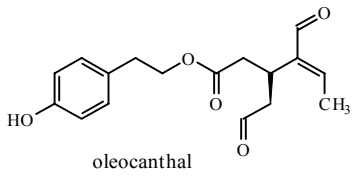


Figure 6.30

6.4 Sesquiterpenoids

Sesquiterpenoids are among the most widely occurring terpenoids. More than 300 distinct sesquiterpene carbon skeletons and more than 7000 of oxidized and otherwise modified derivatives have been identified to date. Sesquiterpenoids display a broad range of

physiological properties, including antibiotic, antiviral, antifungal, antitumor, and hormonal activities. Numerous sesquiterpene hydrocarbons and derived metabolites found in plant essential oils are also highly valued for their desirable flavor characteristics.

6.4.1 Sesquiterpenic hydrocarbons

All linear and cyclic sesquiterpenoids are derived from a single acyclic precursor (2*E*,6*E*)-farnesyl diphosphate. The structural diversity of this class is, however, greater than that of monoterpenes. Because of the increased chain length and additional double bond, a range of linear, monocyclic, bicyclic, and tricyclic structures can result [6.10]. Analogously to the biosynthesis of cyclic monoterpenes from geranyl diphosphate, there is a geometric barrier to direct cyclization of (2*E*,6*E*)-farnesyl diphosphate. Therefore, all later cyclization reactions proceed through the initial ionization (with the assistance of a divalent metal ion) to the allyl cation intermediate and isomerization providing the tertiary (3*R*)-nerolidyl diphosphate or (3*S*)-nerolidyl diphosphate (*transoid*- and *cisoid*-conformers). The isomerization to (2*Z*,6*E*)-farnesyl diphosphate is catalyzed by farnesol 2-isomerase (EC 5.2.1.9) (Figure 6.31).

Directed deprotonation of the cationic intermediates yields the corresponding hydrocarbons α-farnesene and β-farnesene. α-Farnesene exists as four stereoisomers that differ about the geometry of two (C-3 and C-6) of its three internal double bonds. (3*E*,6*E*)-α-Farnesene and (3*Z*,6*E*)-α-farnesene are the most common isomers (Figure 6.32, Table 6.4). (3*E*,6*E*)-α-Farnesene is found in the coating of apples and other fruits. Its oxidation by air to conjugated trienes results in injury to cell membranes, which leads to a storage disorder known as scald. (3*Z*,6*E*)-α-Farnesene has been isolated from the oil of perilla (*Perilla nankinensis*, syn. *P. frutescens*; Lamiaceae). Both isomers are also insect pheromones. The β-form (β-farnesene) can exist as two stereoisomers about the geometry of its central (C-6) double bond. The (6*E*)-isomer is a constituent of various essentials oils, such as the ginger oil. It is also released by aphids as an alarm pheromone. Several plants have been shown to synthesize this pheromone as a natural insect repellent.

In the formation of cyclic sesquiterpenes, the direct deprotonation of the cations to hydrocarbons is preceded by an electrophilic attack of the respective conformer cations on either the central double bond to form six- or seven-membered ring intermediates or on the distal double bond to form 10- or 11-membered ring systems. Thus derived cationic intermediates can be terminated by removal of a proton yielding cyclic sesquiterpene hydrocarbons. These cyclic hydrocarbons can be released by the synthases or may undergo further cyclizations, rearrangements, and terminations depending on the particular plant enzyme systems involved.

Each of the two sesquiterpene synthases of grand fir (*Abies grandis*; Pinaceae), *δ*-selinene synthase (EC 4.2.3.-) and γ-humulene synthase (EC 4.2.3.-), can produce more than 30 different sesquiterpene olefins that have been found as the oleoresin constituents.

[6.10] The following sesquiterpenoid synthases have been characterized: trichodiene synthase (EC 4.2.3.6), pentalenene synthase (EC 4.2.3.7), casbene synthase (EC 4.2.3.8), aristolochene synthase (EC 4.2.3.9), *δ*-cadinene synthase (EC 4.2.3.13), taxadiene synthase (EC 4.2.3.17), abietadiene synthase (EC 4.2.3.18), *ent*-kaurene synthase (EC 4.2.3.19), vetispiradiene synthase (EC 4.2.3.21), germacradienol synthase (EC 4.2.3.22), germacrene-A synthase (EC 4.2.3.23), and amorpha-4,11-diene synthase (EC 4.2.3.24).

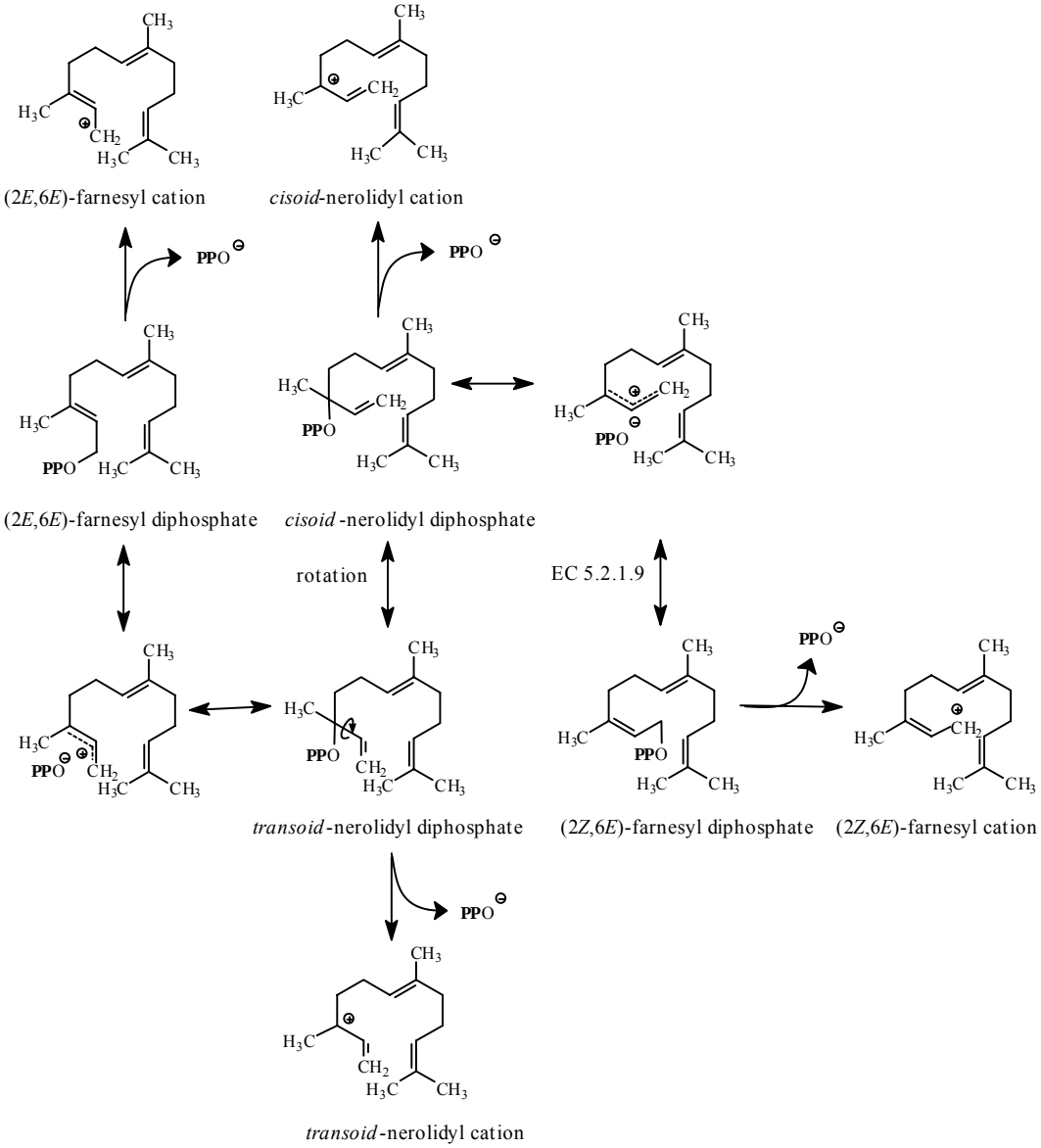


Figure 6.31

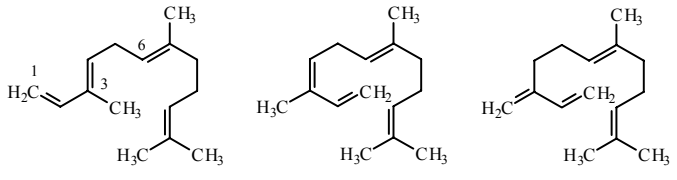


Figure 6.32

The diene is required to be in the s-cis conformation. In solution, the carbon-carbon single bond in the diene that connects the two alkenes is constantly rotating, so at equilibrium there is usually some mixture of dienes in the s-trans conformation and some in the s-cis conformation. The ones that are at that moment in the s-trans conformation do not react. If the dienophile is disubstituted, there is the possibility for stereochemistry in the product. The Diels-Alder reaction ends up with the stereochemistry that started with. In other words, if the substituents started cis on the dienophile, they end up cis in the product. If they started trans on the dienophile, they end up trans in the product. When the diene is in a ring, the product of the Diels-Alder reaction is a bicyclo ring system. When the dienophile that is substituted, there are two possible products that you can form from, the endo product, in which the substituent points down from the top of the bicyclic molecule, and the exo product, where the substituent points towards the top of the bicyclic molecule. In general, the endo product forms preferentially. Some of the Diels-Alder reactions are reversible; the decomposition of the cyclic system is then called the retro-Diels-Alder reaction.

6.5 Diterpenoids

The diterpenoids are widespread in the plant kingdom, and they often are encountered in the resins of conifers, woody legumes, composites, and members of the spurge family (Euphorbiaceae). More than 3000 different diterpenoid structures have been defined, all of which appear to be derived from geranylgeranyl diphosphate. Many diterpenoids are physiologically active compounds.

6.5.1 Diterpenic hydrocarbons

Similarly to monoterpenoids and sesquiterpenoids, most diterpenoids are cyclic, and there appear to be two major and fundamentally different modes of cyclization in this class. The macrocyclic diterpenes, such as cembrene, casbene (casbene synthase, EC 4.2.3.8), and taxa-4,11-diene (taxadiene synthase, EC 4.2.3.17), are formed by cyclizations analogous to those of the monoterpene and sesquiterpene series (Figure 6.43). Cembrene has some importance, being a trail pheromone for termites. Casbene is an antifungal metabolite produced by the castor oil plant (*Ricinus communis*; Euphorbiaceae). The structures of cembrene, casbene, and taxa-4,11-diene are central to a very wide variety of natural products found both in plants and animals.

The second mode of cyclization involves generation of copalyl diphosphate as the initial intermediate. The reaction cascade is initiated by protonation of the terminal double bond of geranylgeranyl diphosphate followed by two internal additions and proton elimination (Figure 6.44). These reactions proceed along parallel routes leading to different stereoisomers of copalyl diphosphate, (+)-copalyl diphosphate and (-)-copalyl diphosphate, also known as *ent*-copalyl diphosphate. Copalyl diphosphate then can be transformed into a variety of tricyclic and tetracyclic diterpenoids by ionization of the diphosphate ester and subsequent internal additions, rearrangements, and terminations. In the biosynthesis of (-)-*ent*-kaur-16-ene, the precursor of the plant hormones gibberellins, *ent*-copalyl diphosphate synthase (EC 5.5.1.13) catalyzes the protonation-initiated cyclization of geranylgeranyl diphosphate to (-)-copalyl diphosphate, whereas a separate enzyme, *ent*-kaurene synthase (EC 4.2.3.19), transforms this intermediate to (-)-*ent*-kaur-16-ene via ionization-dependent cyclization. In the biosynthesis of (-)-abieta-7(8),13(14)-diene, the precursor of abietic acid of conifer resin, a single enzyme, copalyl diphosphate synthase (EC 4.2.3.12), catalyzes the conversion of geranylgeranyl diphosphate to (+)-copalyl diphosphate.

The cyclization of this bound intermediate to the olefin is catalyzed by abietadiene synthase (EC 4.2.3.18).

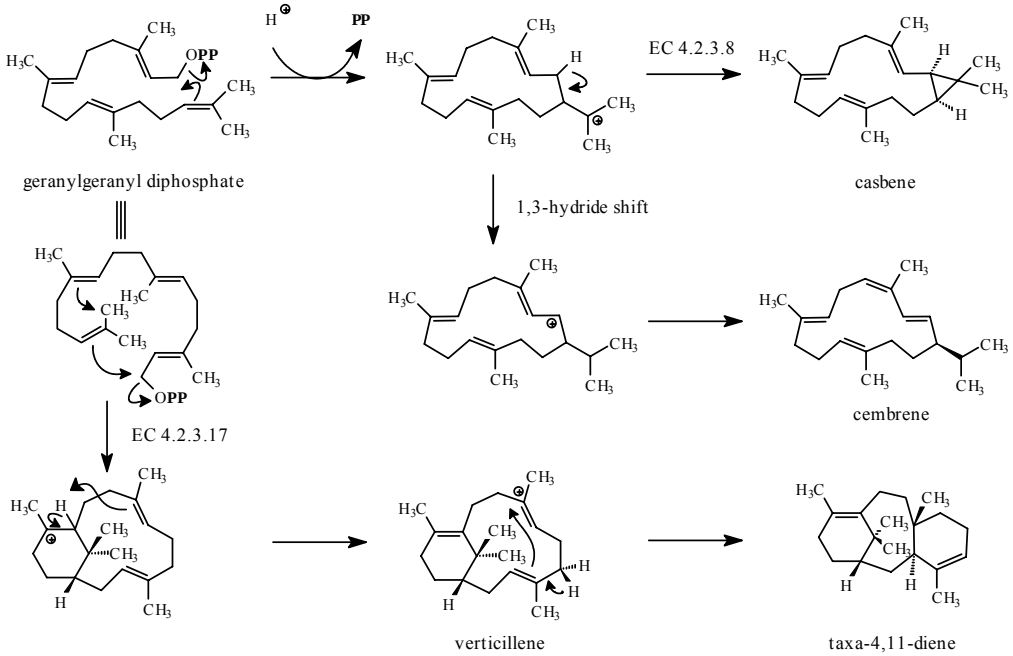


Figure 6.43

6.5.2 Alcohols and other diterpenoids

The most common aliphatic diterpenic alcohol is phytol, (2*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-ol, the lipophilic side-chain of chlorophyll. Geranylgeranyl diphosphate is first reduced by geranylgeranyl reductase (EC 1.1.1.-) to phytyl diphosphate, which is used for the attachment of the phytyl residue (catalyzed by chlorophyll synthase, EC 2.5.1.62) to chlorophyllide yielding chlorophyll. Alternatively, geranylgeranyl diphosphate can serve as a substrate for chlorophyll synthase to form geranylgeranyl chlorophyll a. Geranylgeranyl reductase then reduces geranylgeranyl chlorophyll a to form chlorophyll a. In photosynthetic organisms, free phytol is generated during chlorophyll catabolism by chlorophyllase (EC 3.1.1.14), which is an integral part of leaf senescence, fruit ripening, and corresponds to the loss of green color. Chlorophyll degradation results in the release of chlorophyllide and phytol (Figure 6.45). Chlorophyllide is further decomposed by chlorophyllide oxygenase [6.11].

[6.11] Chlorophyll degradation occurs in the plastids. It is divided into early and final steps. The early steps are common to all plants and lead to the production of a colourless, blue-fluorescing intermediate (primary fluorescent chlorophyll catabolite) via the formation of a red chlorophyll catabolite, also known as red billin. The final steps of chlorophyll catabolism are species-specific. They involve a diversity of modifications of the primary fluorescent chlorophyll catabolite before the catabolites are finally stored in the vacuole in the form of a nonfluorescent chlorophyll catabolite that share a common tetrapyrrolic skeleton with an oxygenolytically opened porphyrin macrocycle, with peripheral, species-specific modification at several side chains. The final conversion of both modified catabolites is a non-enzymatic reaction catalyzed by H^+ ions.

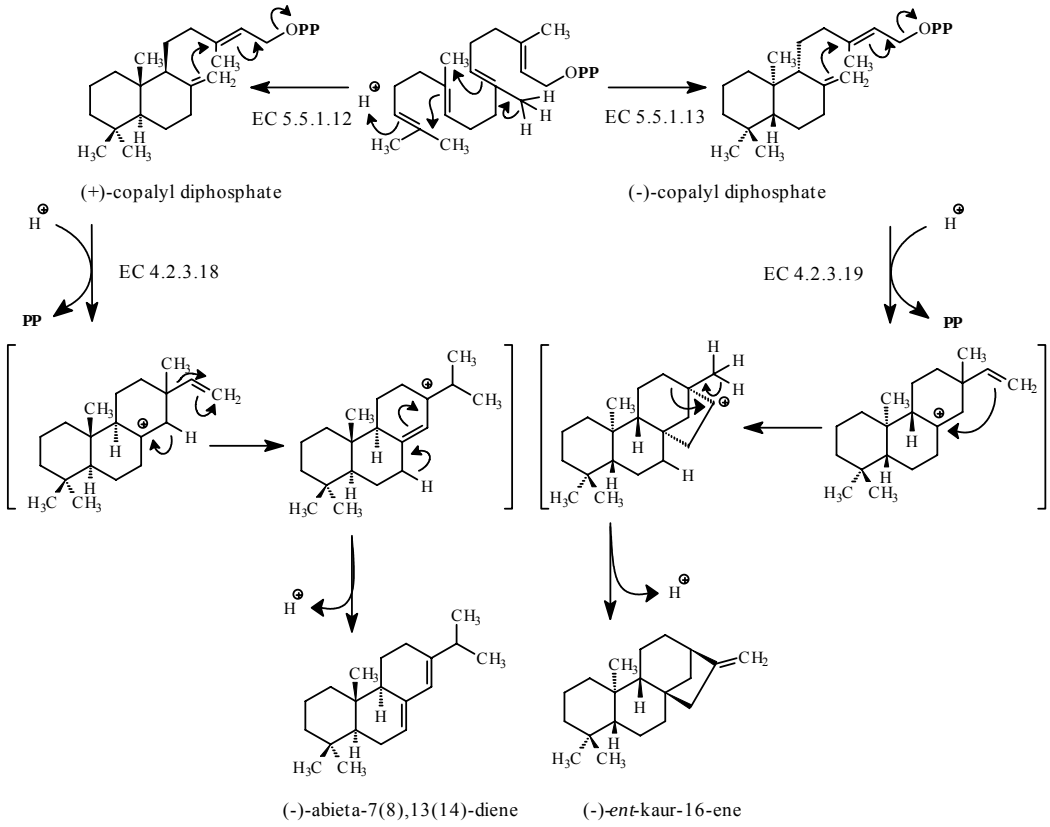


Figure 6.44

Most diterpenoid alcohols and other terpenoids are cyclic. They occur either as free compounds or bound in fatty acid esters and glycosides. They are principally formed by ring closure, oxidation, and further modification of the sesquiterpene molecule as exemplified by the formation of phorbol from casbene (Figure 6.46). Many species of the Euphorbiaceae family are regarded as toxic because their latex can cause poisoning in humans and animals, skin dermatitis, cell proliferation, and tumor promotion. Most of the biological effects are due to fatty acid esters of phorbol, such as the 13-acetyl-12-myristoyl-phorbol. The toxic principle from common yew (*Taxus baccata*; Taxaceae) has been shown to be a mixture of compounds based on the taxadiene skeleton. The diterpenoid alkaloid paclitaxel (taxol; Figure 6.47) is an important anticancer drug, which is biosynthesized from taxadiene using phenylalanine as the nitrogen donor,

Resin acids (abietic acid and its isomers) are usually classified into two main categories: abietanes and pimaranes. Abietane-type diterpenoids have been known to possess a variety of biological activities, such as antibacterial, cardiovascular, and antioxidant.

The released phytol can be, via a salvage pathway, incorporated into tocopherols and phylloquinone. Using CTP, phytyl kinase (EC 2.7.1.-) phytol is esterified to phytyl phosphate, which is transformed, using a trinucleotide and phytyl phosphate kinase (EC 2.7.4.-) to phytyl diphosphate.

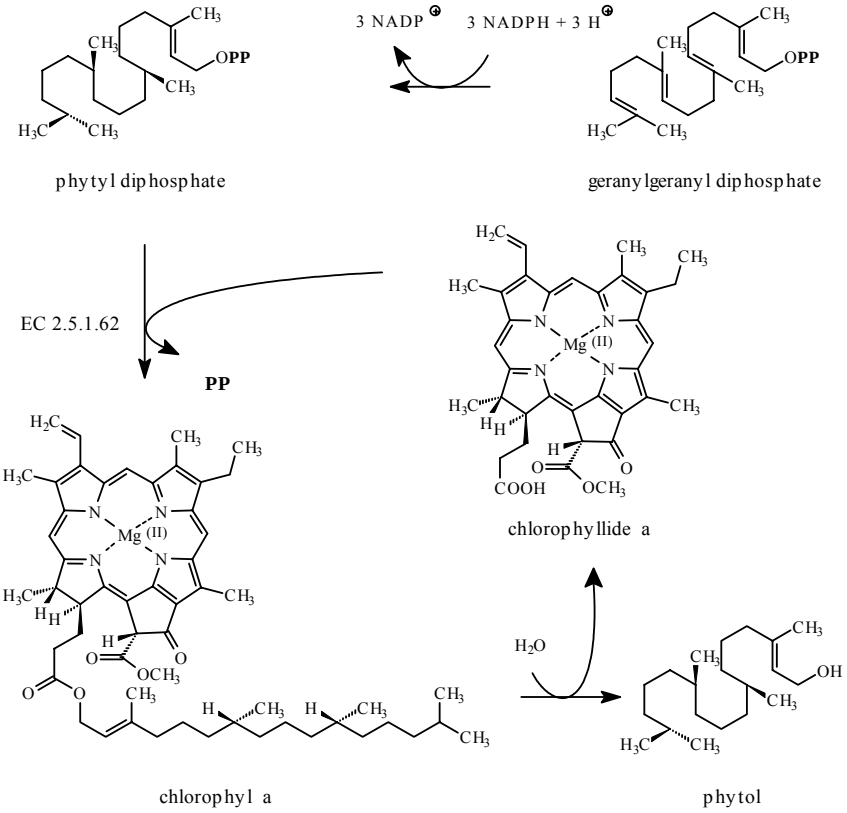


Figure 6.45

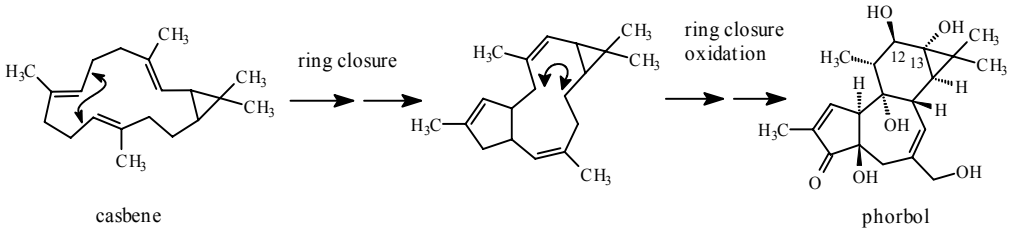


Figure 6.46

Diterpenoids of the abietane family, characterized by the perhydrophenanthrene-type tricyclic ring structure of normal absolute configuration (C-10 β-methyl) with an isopropyl group at C-13 of the C ring, are the principal constituents of the rosin fraction. Pimarene-type diterpenes have been involved in a variety of biological processes: inhibition of platelet formation, antimicrobial, or anti-inflammatory activity. Abietic acid and its isomers form from (-)-abietadiene (Figure 6.48) by a series of oxidations via abietadienol (abietadiene oxidase, EC 1.14.13.-) and abietadienediol (abietadienol oxidase, EC 1,14.13.-), which spontaneously yields abietadienal enzymatically oxidized to abietic acid. Analogous reactions are employed for the biosynthesis of abietic acid isomers (dehydroabietic, neoabietic, palustric, levopimaric, and isopimaric acids).

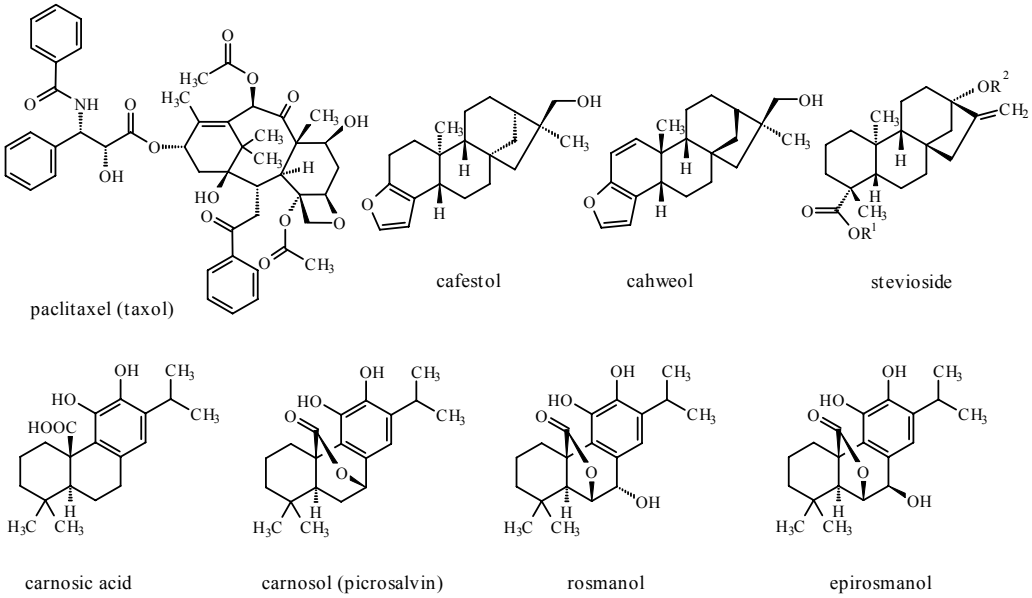


Figure 6.47

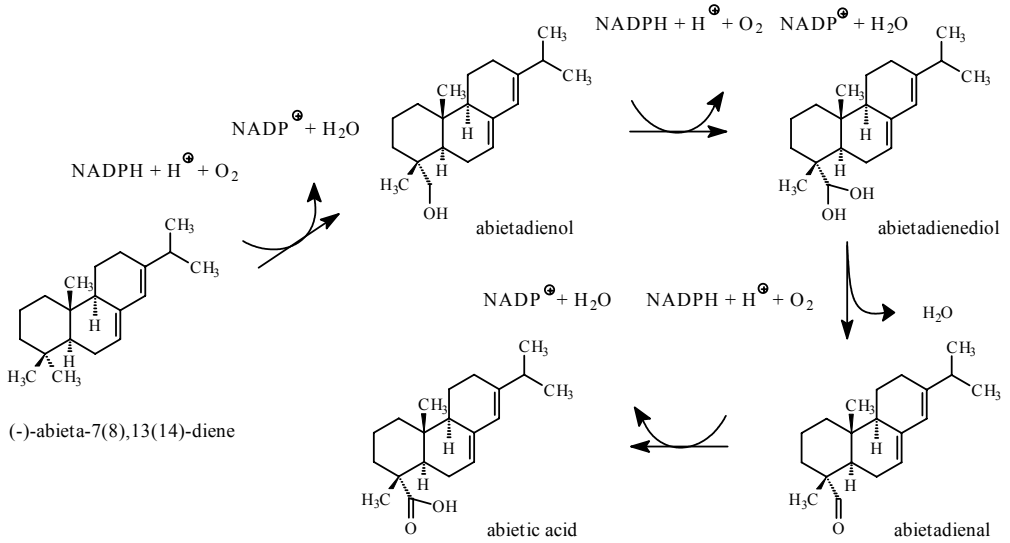


Figure 6.48

ent-Kaurene is the precursor of cafestol, cahweol (Figure 6.47), and other copalyl diphosphate-derived diterpenoids that occur in green coffee beans and unfiltered coffee brews, such as Scandinavian boiled and Turkish coffee. Cafestol is a potent cholesterol-elevating compound in the human diet as its palmitate (and to some extent the palmitate of cahweol) may exert their effect by interacting within membranes. These alcohols partly dehydrate to the so-called dehydroalcohols and oxidize to the corresponding aldehydes during roasting of green coffee beans.

ent-Kaurene is also the precursor of stevioside and related sweet glycosides (Figure 6.47, Table 6.6) occurring in the leaves (3-10%) of *Stevia rebaudiana* (Asteraceae).

Stevioside is biosynthesized by sequentional oxidation of the C-4 methyl group to carboxyl forming *ent*-kaurenoic acid, hydroxylation to steviol, and glycosylation, so that both glucose ester and glycoside linkages (β-sophoroside) are formed (Figure 6.49). Stevioside is some 100-200 times as sweet as saccharose and is used commercially as a sweetening agent in some countries.

Table 6.6 Structures of stevioside and related compounds

Compound name	R^1	R^2
Steviolbioside	H	β-D-Glc*p*-(1→2)-β-D-Glc*p* (β-sophorose)
Stevioside	β-D-Glc*p*	β-D-Glc*p*-(1→2)-β-D-Glc*p*
Rebaudioside A	β-D-Glc*p*	β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-β-D-Glc*p*
Rebaudioside B	H	β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-β-D-Glc*p*
Rebaudioside C (dulcoside B)	β-D-Glc*p*	β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-α-L-Rha*p*
Rebaudioside D	β-D-Glc*p*-(1→2)-β-D-Glc*p*	β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-β-D-Glc*p*
Rebaudioside E	β-D-Glc*p*-(1→2)-β-D-Glc	β-D-Glc*p*-(1→2)-β-D-Glc*p*
Rebaudioside F	β-D-Glc*p*	β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-β-D-Xyl*p*
Dulcoside A	β-D-Glc*p*	β-D-Glc*p*-(1→2)-α-L-Rha*p*

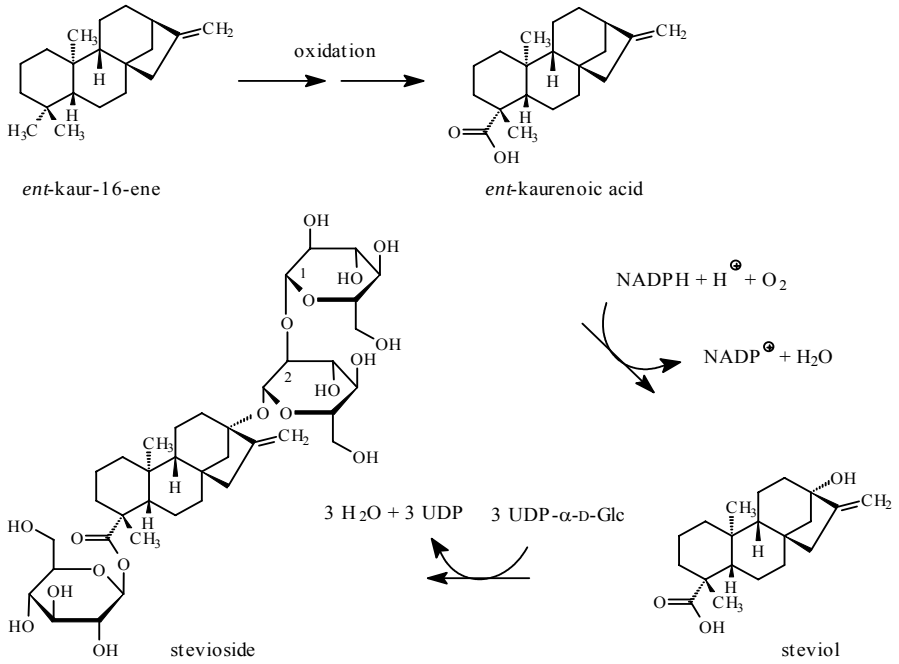


Figure 6.49

Medical plants, spices, and other herbs contain a variety of diterpenoids derived from *ent*-kaurene. Some of them display antioxidant and anti-inflammatory effects against reactive

oxygen species and free radical attacks in food, as well as in biological model systems. Carnosic acid (rosmaricine) and carnosol (picrosalvin) derived from rosemary (*Rosmarinus officinalis*; Lamiaceae) have been shown to exert strong antioxidant activities (Figure 6.47). Carnosic acid, the main component of fresh rosemary (1-2%), is unstable and transforms enzymatically to bitter carnosol. These two diterpenoids represent about 15% of commercial leaf extracts and show about 90% of their antioxidative activity. Other transformation products of carnosic acid are rosmanol (7α-hydroxy derivative), epirosmanol (7β-isomer) and similar compounds. The same diterpenoids occur also in the common sage (*Salvia officinalis*; Lamiaceae) extracts.

6.6 Triterpenoids and steroids

The triterpenoids are a large and structurally diverse group of molecules that contain 30 carbon atoms. They are generated by the polymerization of six isoprene units, although a number of derivatives, some with more, but most with less carbon atoms are also counted among this group. Nearly 200 different skeletons are known in several thousands different types of molecules isolated and characterized from very different animals, plants, and microorganisms. Some have vital functions in living organisms (e.g. vertebrate steroidal hormones, plant steroidal hormones brassinoids, steroidal glycoalkaloids, phytoanticipins, and phytoalexins); some have been used in medicine (e.g. cardioactive steroidal glycosides). Function for many specific compounds still remains to be determined. Most terpenoids are C_6-C_6-C_6-C_5 tetracycles, C_6-C_6-C_6-C_6-C_5 pentacycles, or C_6-C_6-C_6-C_6-C_6 pentacycles, but acyclic, monocyclic, bicyclic, tricyclic, and hexacyclic triterpenoids also occur in natural sources. Despite their varied structures, all of them are synthesized from one precursor, the alicyclic C_{30} hydrocarbon squalene or related acyclic C_{30} precursors, by only a few pathways.

6.6.1 Squalene and 2,3-epoxysqualene

Triterpenoids do not form by an extension of the process of adding further C_5 isopentenyl diphosphate unit to the growing chain. Instead, all higher organisms produce C_{30} hydrocarbon squalene (all-*trans*-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene), the universal precursor of all triterpenoids and steroids [6.12)].

Squalene forms from two molecules of (2*E*,6*E*)-farnesyl diphosphate joined together "tail to tail" and reduced by the enzyme squalene synthase (EC 2.5.1.21) (Figure 6.50). The reaction proceeds by the attack of the C-2/C-3 double bond of farnesyl diphosphate on to the farnesyl carbenium ion, analogously to the formation of prephytoene diphosphate from geranylgeranyl diphosphate in the pathway leading to carotenoid pigments. The resultant tertiary cation is discharged by loss of a proton and formation of cyclopropane ring of presqualene diphosphate. Loss of diphosphate gives a primary cation, which can, via Wagner-Meerwein rearrangement (1,3-alkyl shift), generate a tertiary cation and

[6.12)] The name originates from the Latin name for the shark (*Squalus*), since shark liver oil is considered the richest source of squalene that comprises up to 85% of the oil. However, squalene is abundant in many plant oils, including olive oil, palm oil, wheat germ oil, amaranth oil, and rice bran oil. In humans, about 60% of dietary squalene is absorbed and transported in serum to be distributed ubiquitously in different tissues, with the greatest concentration in the skin.

achieves the required C-1/C-1´bond. Breaking the C-1/C-2´ bond produces alkene and a favorable allylic cation that is quenched by supply of hydride from NAD(P)H.

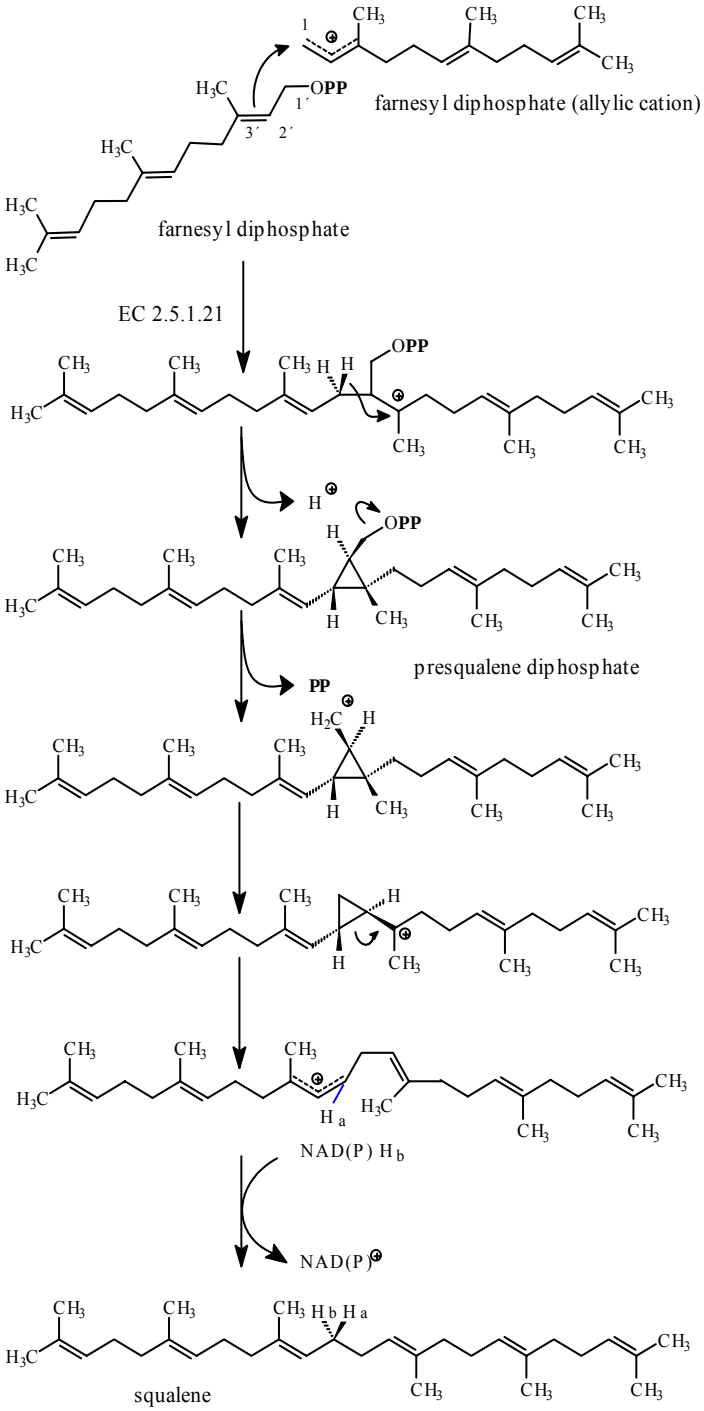


Figure 6.50

Squalene can be transported for incorporation into tissues or bound in an appropriate orientation and further metabolized. On the pathway to triterpenoids, the enzyme squalene monooxygenase (EC 1.14.99.7) generates (3*S*)-2,3-epoxysqualene, also known as squalene oxide or oxidosqualene (Figure 6.51), that becomes the precursor of most triterpenoids.

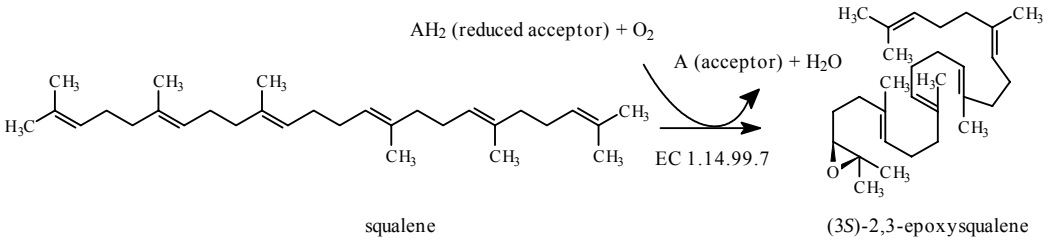


Figure 6.51

6.6.2 Triterpenoids

The parent hydrocarbon from which triterpenoids and steroids occurring in foods and feeds are mostly derived is the hydrocarbon 5α-cholestane, a tetracyclic cyclopenta[*a*]phenanthrene structure with a (20*R*)-configuration (Figure 6.52).

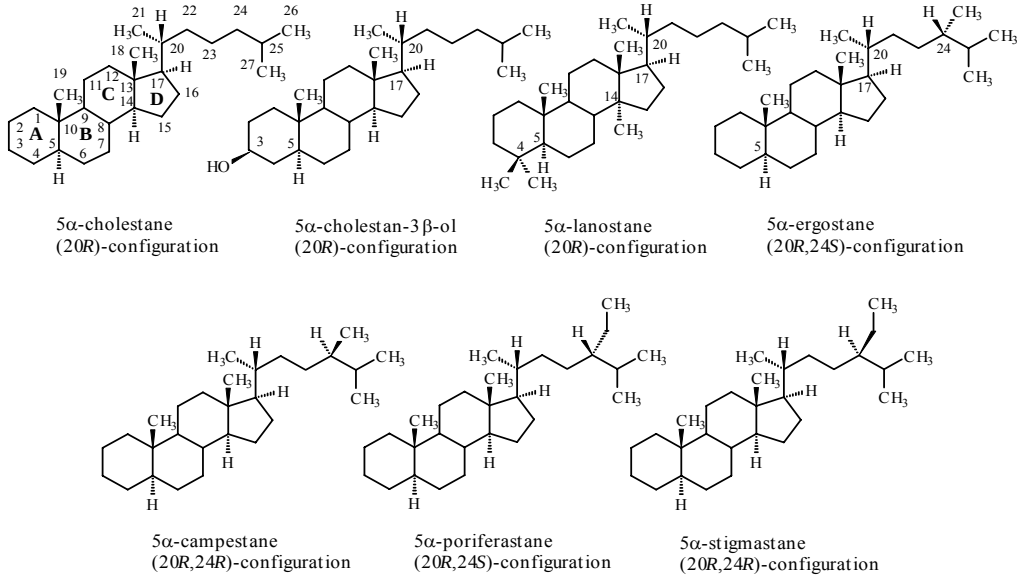


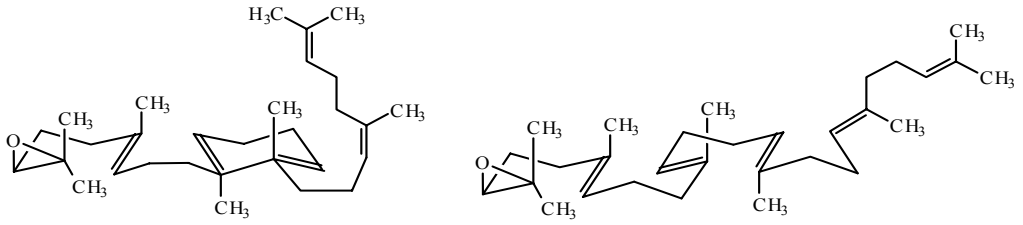
Figure 6.52

The numbers 28, 29, and 30 are assigned to the additional methyl groups at C-4 and C-14 in triterpenoids. This basic structure has seven chiral centers (on C-5, C-8, C-9, C-10, C-13, C-14, C-17). The four rings (A, B, C, D) have *trans* ring junctions and the molecule is almost planar, having the chair-boat-chair-boat conformation, with the individual substituents above (β substituents) or below (α substituents) the plane. The C-17 side-chain, methyl groups at C-18 and C-19, and H-8 are at an angle to the rings above the

plane with β stereochemistry, the hydrogen atoms H-5 and H-9 are at an angle below the plane with α stereochemistry.

Triterpenoids carrying a β-hydroxyl group at C-3 and most of the skeleton of cholestane (5α-cholestan-3β-ols) are known as sterols. They may be regarded as being derived either from cholestane or the hydrocarbon with unsaturation and substitution in the side-chain attached at C-17 (Figure 6.52) [6.13]. The common categories of steroids according to their occurrence in nature are animal sterols (zoosterols), plant sterols (phytosterols), and fungus sterols (mycosterols) [6.14]. Sterols can be further classified on a structural or biosynthetic basis as 4,4-dimethylsterols, 4-monomethylsterols, and 4-desmethylsterols (i.e. with no substituent on C-4). Steroids of the first two groups that contain 30 carbon atoms are also known as triterpenic alcohols. In addition, the 4-desmethyl sterols may be subdivided into Δ^5-sterols, Δ^7-sterols, and $\Delta^{5,7}$-sterols depending on the position of the double bonds in the B ring. At trace levels, phytosterols are accompanied by fully saturated phytosterols known as phytostanols that are relatively abundant in cereal grains.

The fate of 2,3-epoxysqualene is different in different organisms. The biosynthetic pathways leading to most triterpenoid structures proceed via protosteryl cation and depend on the type of cyclase enzyme and its folding on the enzyme surface (Figure 6.53).



(3*S*)-2,3-epoxysqualene
(chair-boat-chair-boat conformation)

(3*S*)-2,3-epoxysqualene
(chair-chair-chair-boat conformation)

Figure 6.53

The chair-boat-chair-boat conformer of 2,3-epoxysqualene undergoes a series of cyclizations followed by a sequence of concerted Wagner-Meerwein migrations of methyl groups and hydrides (Figure 6.54).

6.13) Lanosterol may be regarded as 4,4,14-trimethyl-5α-cholesta-8,24-dien-3β-ol (5α-lanosta-8,24-dien-3β-ol), the parent hydrocarbon lanostane is 4,4,14-trimethyl-5α-cholestane. Analogously, cycloartenol is 4,4,14-trimethyl-9,19-cyclo-5α,9β-cholest-24-en-3β-ol, campesterol is (24*R*)-24-methylcholest-5-en-3β-ol or campest-5-en-3β-ol, stigmasterol is (24*S*)-24-ethylcholesta-5,22-dien-3β-ol or (22*E*)-stigmasta-5,22-dien-3β-ol, β-sitosterol is (24*R*)-24-ethylcholest-5-en-3β-ol or stigmast-5-en-3β-ol, ergosterol is (22E, 24*R*)-24-methylcholesta-5,7,22-trien-3β-ol or (22*E*)-ergosta-5,7,22-trien-3β-ol, etc.

6.14) Some bacteria take up cholesterol and other sterols from host animals for use as membrane constituents and a few species of prokaryotes are able to synthesize sterols *de novo*. Among the eubacteria, certain methylotrophs (*Methylobacterium* and *Methylosphaera* species) produce 4-mono- and 4,4-dimethyl sterols, including lanosterol. Similarly, some soil bacteria produce 4-desmethylsterols. The place of sterols in many more species of bacteria takes hopanoids, the pentacyclic triterpenoids based on the C_6-C_6-C_6-C_6-C_5 hopane skeleton with a cyclopentane E-ring. The five rings have chair-chair-chair-chair-chair conformations in comparison to sterols, which have chair-boat-chair-boat conformations.

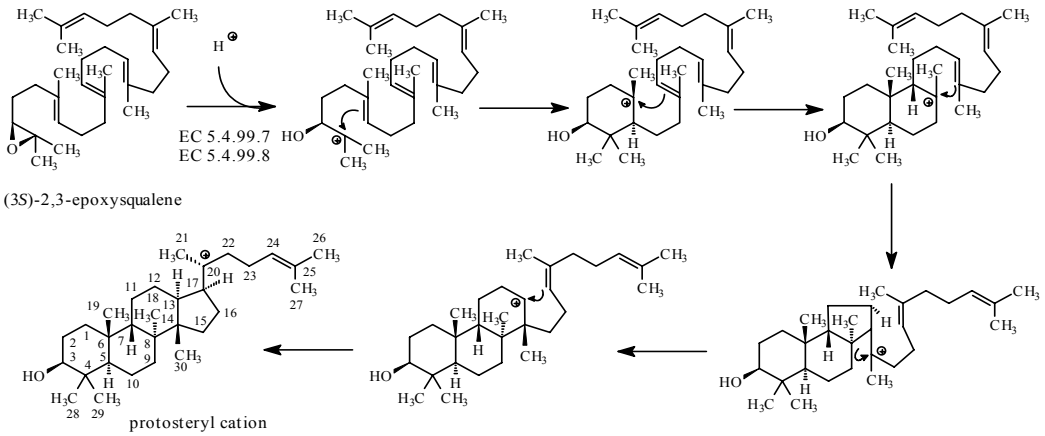


Figure 6.54

The cyclizations are carbenium ion mediated and proceed in a step-wise sequence. Protonation of the epoxide group of 2,3-epoxysqualene allows ring opening and yields a tertiary carbenium ion that allows electrophilic addition (Markovnikov addition) to a double bond, formation of a six-membered ring, and production of a new tertiary carbenium ion. This process is repeated generating a new tertiary carbenium ion and a new six-membered ring. Finally, the same process produces another carbenium ion and a five-membered ring that is expanded to a six-membered one via a Wagner-Meerwein 1,2-alkyl shift. A further electrophilic addition yields the transient tertiary protosteryl cation.

In animals and fungi (the reaction takes place in the endoplasmic reticulum), the protosteryl cation becomes the substrate for lanosterol synthase (EC 5.4.99.7) that generates the first steroidal intermediate lanosterol. This is the main gateway for the synthesis of all other sterols in these organisms, including cholesterol. In plants, 2,3-epoxysqualene processed by cycloartenol synthase (EC 5.4.99.8) generates the intermediate cycloartenol that can be converted to many different steroids. In both cases, 2,3-epoxysqualene is folded in its chair-boat-chair-boat conformation (Figure 6.55).

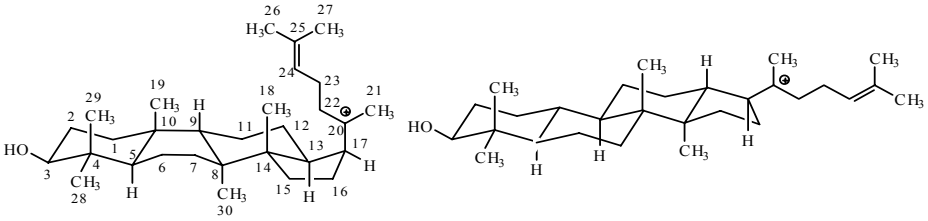


Figure 6.55

In the pathway to lanosterol in animals and fungi (Figure 6.56), the protosteryl cation undergoes a series of Wagner-Meerwein 1,2-hydride shifts and 1,2-methyl shifts. A loss of H-9 creates the C-8/C-9 double bond. In plants, the H-9 of the same conformer

migrates to C-8, the carbenium ion so formed is quenched by the cyclopropane ring formation. The loss of one proton from C-10 methyl yields cycloartenol.

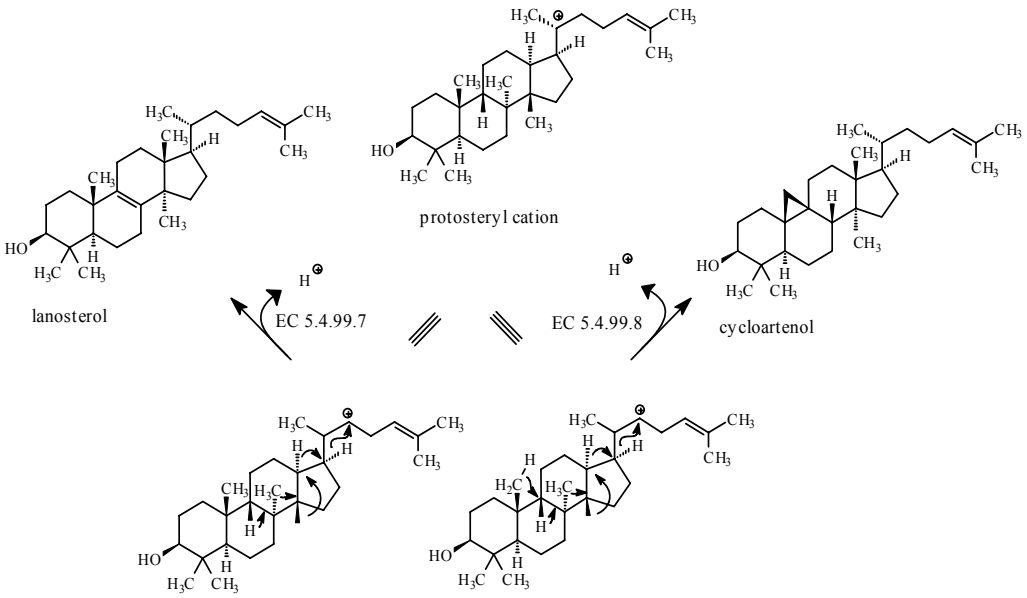
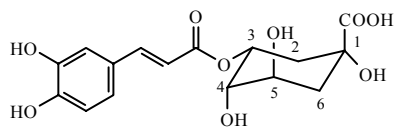


Figure 6.56

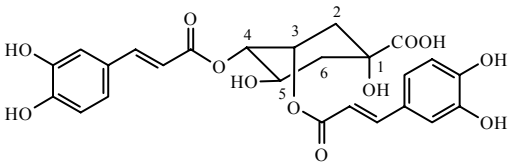
In some plants, 2,3-epoxysqualene, folded onto another type of cyclase enzymes, e.g. the multifunctional α-amyrin/β-amyrin synthase (EC 5.4.99.-) and lupeol synthase, (EC 5.4.99.-) in its chair-chair-chair-boat conformation (Figure 6.53), produces the transient dammarenyl cation (Figure 6.57) that has different stereochemical features to the protosteryl cation. The Wagner-Meerwein migrations can occur, but they are relatively little since these would invert stereochemistry and invert the already favorable conformation. Instead, the dammarenyl cation typically undergoes further carbenium ion promoted cyclizations, without any major changes to the ring system already formed. Subsequent reactions produce in varying amounts a variety of triterpene compounds, such as euphol (widely distributed in e.g. *Euphorbia* spp.; Euphorbiaceae), lupeol (from *Lupinus* spp.; Fabaceae), and the C_6-C_6-C_6-C_6-C_6 pentacyclic triterpenoids α-amyrin, β-amyrin (widely distributed in plants), and taraxasterol (found in dandelion; *Taraxacum officinale*; Asteraceae).

6.6.2.1 Cucurbitacins

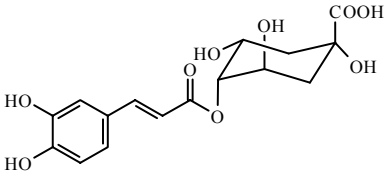
The C-10 methyl and the H-5 of the chair-boat-chair-boat conformer of the protosteryl cation share an *anti*-axial position and are also susceptible to the Wagner-Meerwein rearrangement catalyzed by the enzymes producing cucurbitacins. The first sequence of Wagner-Meerwein 1,2-hydride shifts and 1,2-methyl shifts yields C-9 cation that may initiate further sequence of 1,2-methyl shifts and 1,2-hydride shifts. The cation is finally terminated by the formation of the C-5/C-6 double bond. Subsequent oxidations and esterifications then produce cucurbitacin A (Figure 6.58) and its analogues (Figure 6.59).



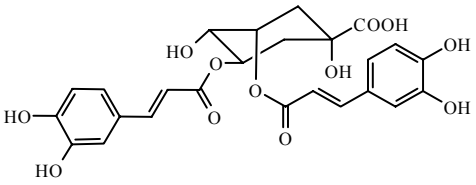
3-*O*-caffeoyl- L-quinic (chlorogenic) acid



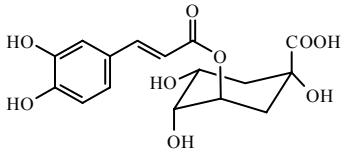
3,4-di-*O*-caffeoyl- L-quinic (isochlorogenic a) acid



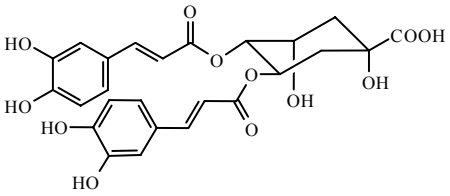
4-*O*-caffeoyl- L-quinic (cryptochlorogenic) acid



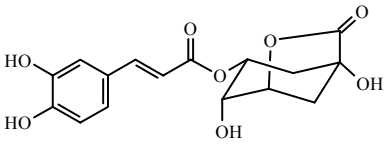
3,5-di-*O*-caffeoyl- L-quinic (isochlorogenic b) acid



5-*O*-caffeoyl- L-quinic (neochlorogenic) acid



4,5-di-*O*-caffeoyl- L-quinic (isochlorogenic c) acid



3-*O*-caffeoyl- L-quino-1,5-lactone

Figure 7.11

The starting compounds for the biosynthesis of caffeoylquinic, feruloylquinic, and 4-coumaroylquinic acids are the corresponding cinnamoyl acids and their coenzyme A esters, respectively. Shikimate *O*-hydrocinnamoyltransferase (EC 2.3.1.133) and/or quinate *O*-cinnamoyltransferase (EC 2.3.1.99) then produce the cinnamoylquinic acid as it is outlined in Figure 7.12.

7.4.2.2 Rosmarinic acid and other caffeic acid derivatives

Various caffeic acid esters frequently occur in various fruits and herbs and many have been shown to be useful compound with regard to medicine virtue and, for their anti-oxidative properties, as food additives. Rosmarinic acid, an ester of caffeic acid and (*R*)-(+)-3,4-dihydroxyphenyllactic acid, is widely spread in species of the Lamiaceae and Boraginaceae families that are used commonly as medical plants and culinary herbs, such as rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*), but also occurs in lower plants, such as ferns.

4-Coumaroyl-CoA, derived from L-phenylalanine in three well characterized enzymatic steps in plants, is one of the essential components being used for the assembly of

rosmarinic acid (Figure 7.13). These steps (shown in Figure 7.1) are catalyzed by phenylalanine ammonia lyase (EC 4.3.1.5), cinnamate 4-hydroxylase (EC 1.14.13.11), and 4-coumaroyl coenzyme A synthetase (6.2.1.12). The other metabolic branch contributing to the biosynthesis of rosmarinic acid originates from L-tyrosine. It results in the formation of 4-hydroxyphenylpyruvic acid (tyrosine transaminase, EC 2.6.1.5), which is reduced to (*R*)-(+)-4-hydroxyphenyllactic acid (hydroxyphenylpyruvate reductase, EC 1.1.1.-). The two moieties of the parallel pathways are connected by rosmarinic acid synthase (EC 2.3.1.140) catalyzing a transesterification reaction that leads to 4-coumaroyl-4'-hydroxyphenyllactic acid. The last steps in this pathway are catalyzed by two cytochrome P450-dependent monooxygenases (hydroxycinnamoyl-4´-hydroxyphenyllactate 3´-hydroxylase and 4-coumaroyl-4´-hydroxyphenyllactate 3-hydroxylase; EC 1.14.13.-) introducing hydroxyl groups to the 3- and 3'-position of 4-coumaroyl-4'-hydroxyphenyllactate.

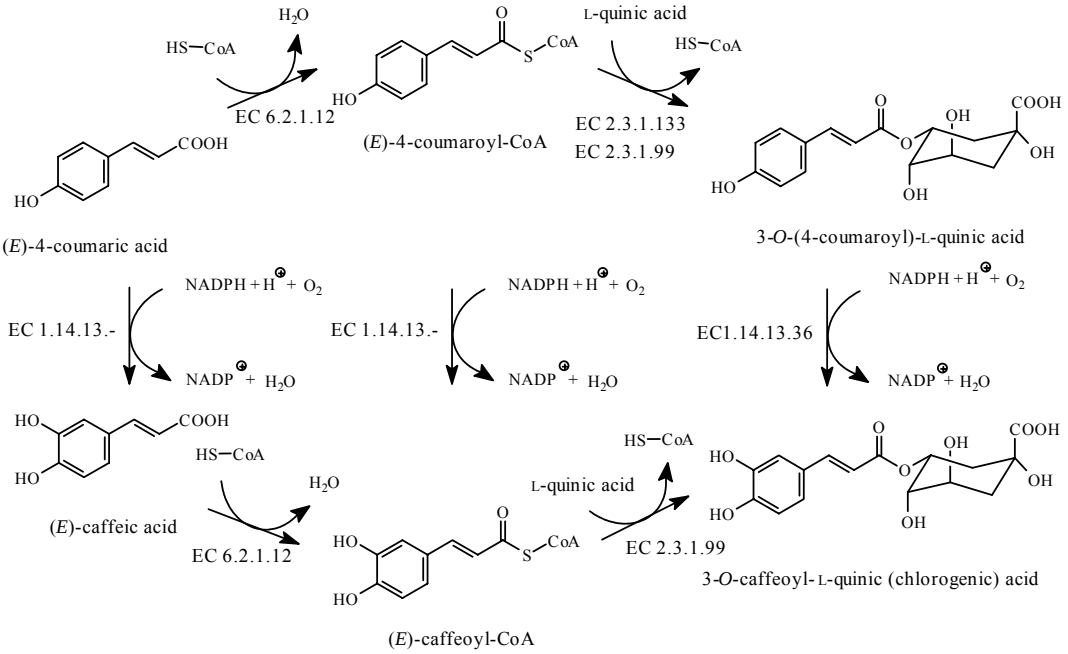


Figure 7.12

Another potential pathway uses 3,4-dihydroxy-L-phenylalanine (dopa). The enzyme catalyzing the entry step into this pathway is tyrosine hydroxylase (EC 1.14.16.2, see Chapter 1.7.4.1). Dopa is then transformed to 3,4-dihydroxyphenylpyruvic acid (dihydroxyphenylalanine transaminase, EC 2.6.1.49), which is reduced to (*R*)-3,4-dihydroxyphenyllactatic acid (hydroxyphenylpyruvate reductase, EC 1.1.1.237) and esterified with caffeoyl-CoA (rosmarinic acid synthase, EC 2.3.1.140) producing rosmarinic acid.

Rosmarinic acid and many other rosmarinic acid derivatives of biological importance occur in herbs. For example, about 25 caffeic acid derivatives have been isolated from *Salvia miltiorrhiza*, one of the most popular traditional herbal medicines in some Asian

countries [7.4)]. Most caffeic acid derivatives were also found in other species of the same genus. Examples of caffeic acid oligomers are a dimer prolithospermic acid (also known as przewalskinic acid), a trimer lithospermic acid, and a tetramer lithospermic acid B (also known as salvianolic acid B) (Figure 7.14). These acids are dihydrobenzofuran-type caffeic acid oligomers. In lithospermic acid, the C-9 carboxyl group is esterified with 3,4-dihydroxyphenyllactic acid and in lithospermic acid B also the carboxyl at C-9a is esterified with the same acid.

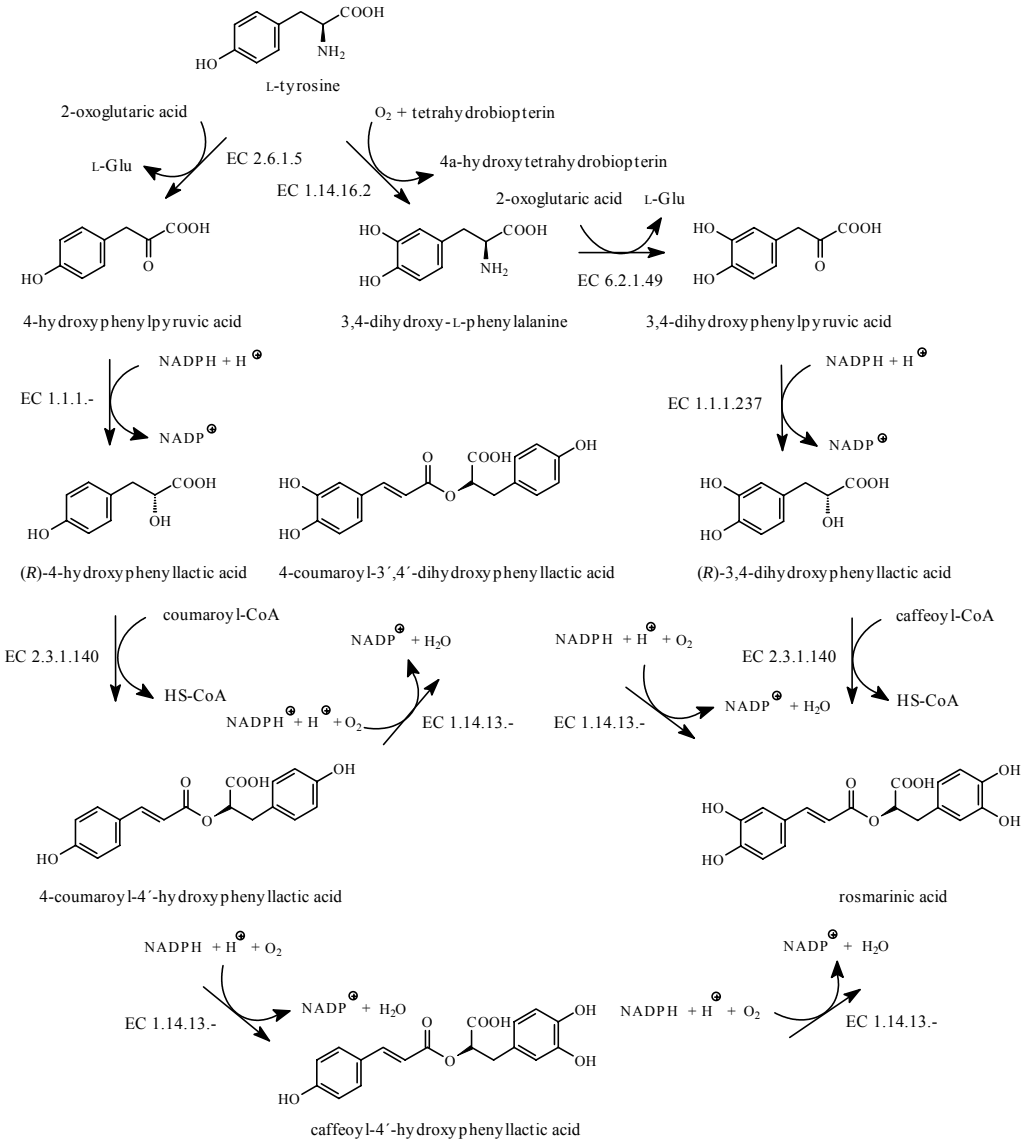


Figure 7.13

[7.4)] The plant is used extensively for the treatment of coronary artery diseases, angina pectoris, myocardial infarction, cerebrovascular diseases, and various types of hepatitis, chronic renal failure, and dysmenorrhea.

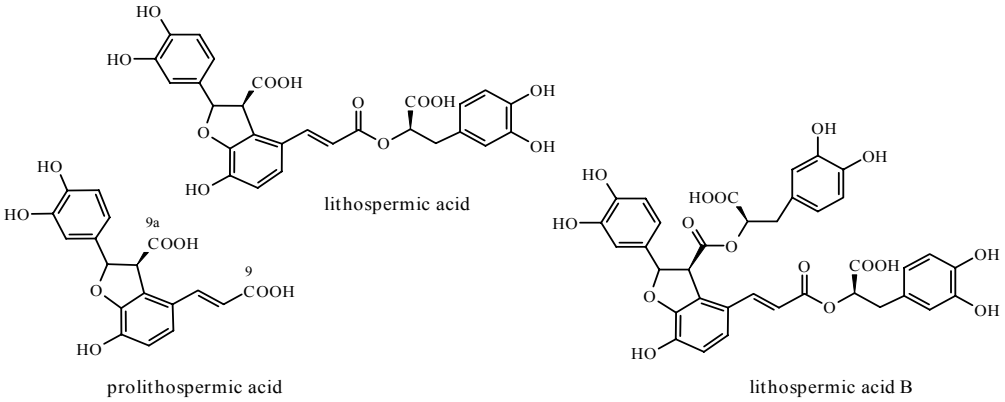


Figure 7.14

Several other depsides of caffeic acid occur in foods of plant origin (Figure 7.15). Dactyliferic acid (3-*O*-caffeoylshikimic acid) and its isomers (4-*O*-caffeoylshikimic acid and 5-*O*-caffeoylshikimic acid) are found in dates (*Phoenix dactylifera*; Arecaceae). Esters of cinnamic acids with (2*S*)-2-hydroxybutanedioic acid, better known as L-(-)-malic acid, e.g. 4-coumaroyl)-, caffeoyl-, feruloyl-, and sinapoyl-L-malic acids, occur in the Brassicaceae plants. Esters with (2*R*,3*R*)-2,3-dihydroxybutanedioic, L-(+)-tartaric or L-(+)-threaric acid, e.g. 4-coumaroyl-L-tartaric (cutaric) acid and caffeoyl-L-tartaric (caftaric) acid, occur in musts and wines. Dicaffeoyl-L-tartaric acid (cichoric acid) occurs in the leaves of some leaf vegetables belonging to the Asteraceae family, such as chicory (*Cichorium intybus*), endive (*C.* endivia), and lettuce (*Lactuca sativa*). The potent anti-oxidant verbascoside is a glycoside of 2,3-dihydroxyphenylethanol (known as hydroxy-tyrosol). It occurs in olives (*Olea europea*; Oleaceae) and wavyleaf mullein (*Verbascum sinuatum*; Scrophulariaceae).The disaccharide quinovose, i.e. 6-deoxy-β-D-gluco-pyranosyl-(1→3)-β-D-glucopyranose, is esterified with caffeic acid.

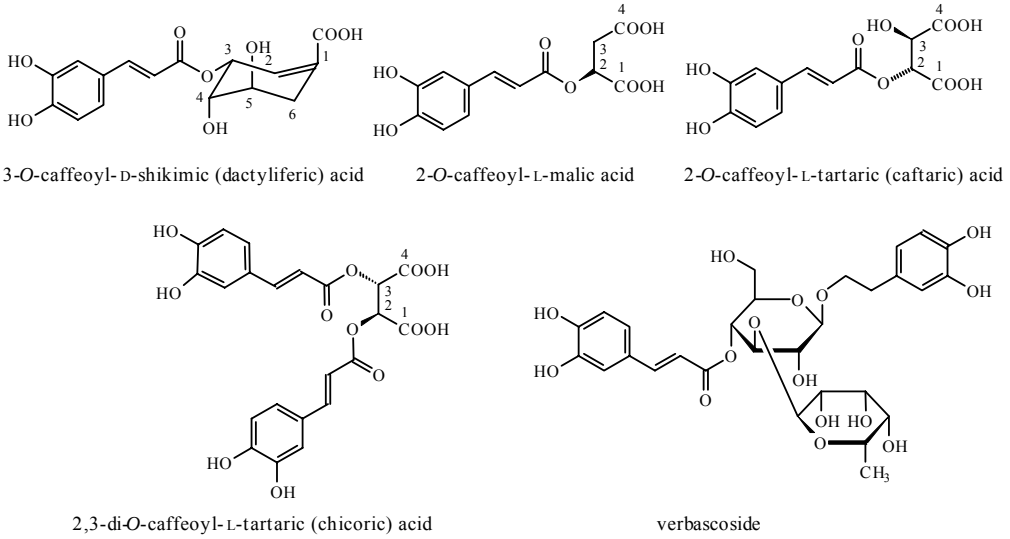


Figure 7.15

7.4.2.3 Sinapic acid esters

Sinapoylesters (sinapoylmalate, sinapoylcholine) have been found predominantly in the Brassicaceae family, where those esters are among the major phenylpropanoid metabolites to be accumulated. While the function of sinapoylcholine as such is not yet elucidated, sinapoylmalate has been found to act as an UV-B (280-320 nm) protectant in vegetative plant tissues. Sinapoylesters are considered as antinutritive compounds, contributing to bitter taste, forming complexes with proteins during oil refining, and overall diminishing the value of seeds for human consumption.

The sinapoyl esters are biosynthesized (Figure 7.16) and utilized at different times in the course of the plant's development. During the seed germination, the formation of sinapoylglucose (sinapate-1-glucose transferase, EC 2.4.1.120) and sinapoylcholine (sinapine) (sinapine synthase, EC 2.3.1.91) prevails. The sinapine esterase (EC 3.1.1.49) hydrolyzes sinapine producing sinapic acid and choline, which has been proposed to be needed for the biosynthesis of phosphatidylcholine (see Chapter 3.3.2.1.1) in the growing seedlings. In this development stage, the seedlings use the released sinapic acid to produce sinapoylmalate via the intermediate sinapoylglucose (sinapoylglucose:malate sinapoyltransferase, EC 2.3.1.92) to increase their UV-B tolerance. The dark-grown seedlings contain considerably less sinapoylmalate and accumulate another sinapate ester (1,2-disinapoylglucose) instead. The latter reaction is catalyzed by sinapoylglucose-sinapoylglucose *O*-sinapoyltransferase (EC 2.3.1.103). Several other sinapoyl metabolites, including gentiobiose and soforose derivatives, have been recently described.

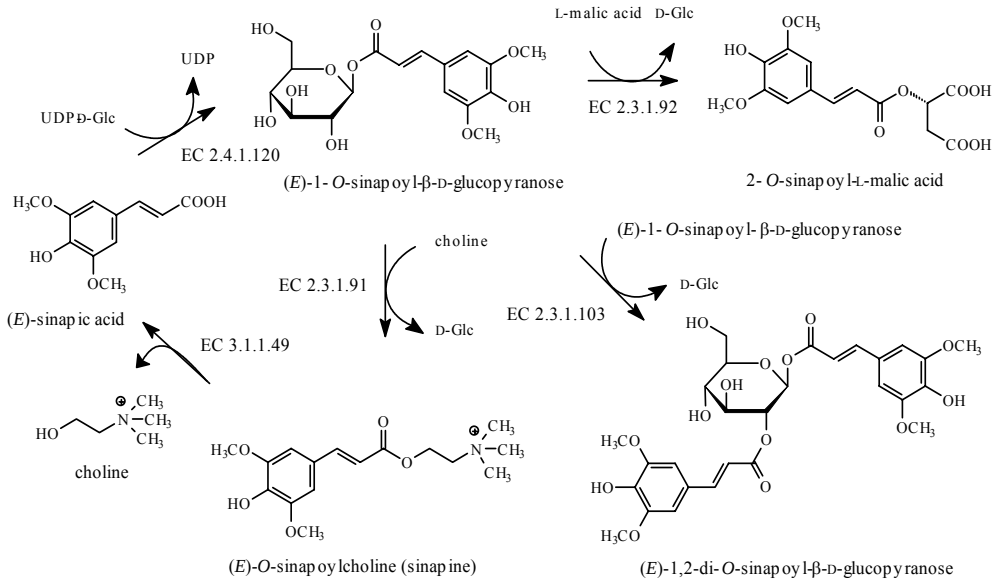


Figure 7.16

7.5 Cinnamic acid amides

Amino acid amides with hydroxycinnamic acids belong to the group of amides that are wide-spread in higher plants as constituents of their cell wall. For example, the

incorporation of hydroxycinnamic acid tyramine amides into cell walls is believed to enhance the efficiency of cells to act as a barrier against pathogens by increasing the rigidity and decreasing the digestibility of the cell wall.

Amino acid amides with hydroxycinnamic acids are synthesized by the condensation of hydroxycinnamoyl-CoA thioesters and aromatic amines. The hydroxycinnamoyl-CoA thioesters include cinnamoyl-CoA, 4-coumaroyl-CoA, caffeoyl-CoA, feruloyl-CoA, and sinapoyl-CoA. The aromatic amines include tyramine, octopamine, tryptamine, serotonin, dopamine, and norepinephrine (see Chapter 1.8.1). Figure 7.17 exemplifies the biosynthesis of tyramine amides that form from hydroxycinnamoyl-CoA thioesters and tyramine under the catalysis of tyramine *N*-hydroxycinnamoyltransferase (EC 2.3.1.110). Tyramine is produced by decarboxylation of tyrosine (tyrosine decarboxylase, EC 4.1.1.25).

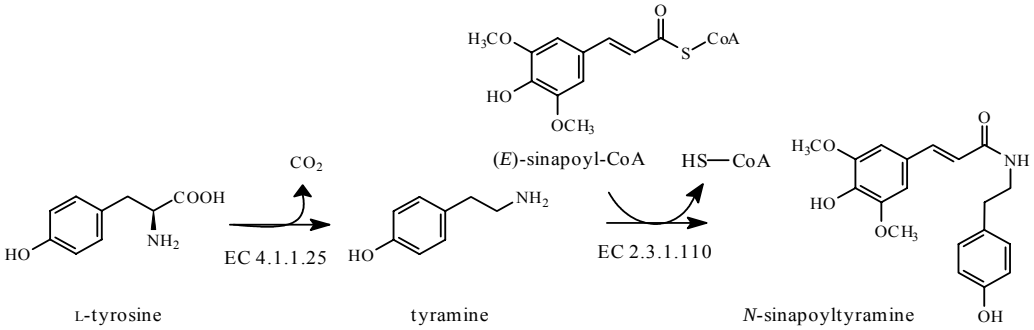


Figure 7.17

Avenanthramides, a group of about 40 *N*-substituted cinnamoylanthranilic acids in oat grains (*Avena sativa*; Poaceae), are often classified as alkaloids. In addition to being antioxidants, they are also known to be phytoalexins, i.e. compounds produced by plants *de novo* as a defense against microorganisms. Avenanthramides consist of an anthranilic acid derivative linked to a hydroxycinnamic acid derivative. The most abundant avenanthramides are derived from the tryptophan metabolite 5-hydroxyanthranilic acid linked to ferulic (40-132 mg/kg), 4-coumaric, and caffeic acids and to the oat-specific avenlumic acid. Avenlumic acid forms in the acetic acid pathway from a cinnamoyl-CoA starter.

Avenanthramides seem to be synthesized by the condensation of corresponding substituted anthranilic acid, substituted cinnamoyl-CoA, and avenlumoyl-CoA thioesters. The proposed biosynthetic pathway of the most common avenanthramides is given in Figure 7.18. The enzymes involved are 4-coumaroyl-CoA 3-hydroxylase (EC 1.14.13.-), caffeoyl-CoA 3-*O*-methyltransferase (EC 2.1.1.104), and hydroxyanthranilate hydroxycinnamoyltransferase.

7.6 Lignans and lignin

The structural biopolymers lignans and lignins accomplish different tasks in plants. Lignans have been attributed to protection and defense, lignins are involved in structural support as their primary physiological function. Taken together lignins and lignans count for about 30% of carbon in vascular plants.

Coumarins are thought to play some role in plant defense as phytoalexins (they show antibacterial activities) due to the induction of their biosynthesis following various stress events (wounding, fungal elicitors, acid fog, etc.). Coumarins are also known to exhibit various pharmacological and physiological effects, such as anti-inflammatory and antioxidant activities. Moreover, extensive research into their pharmacological and therapeutic properties for many years has resulted in the acknowledgment of their therapeutic role in the treatment of cancer. However, linear coumarins can cause photosentization towards UV light, resulting in sunburn and serious blistering [7.7)].

7.8.1 Coumarin and its derivatives

Coumarin is found in a variety of plants, such as sweet clover (*Melilotus* spp.; Fabaceae), tonka bean (the seed of *Dipterix odorata*; Fabaceae), lavender (*Lavandula* spp., Lamiaceae), and liquorice (*Glycirrhyza glabra*; Fabaceae), but also occurs in food plants, such as strawberries, apricots, cherries, and cinnamon. Coumarin is best known for its fragrance, described as vanilla-like odor or the aroma of freshly mowed hay, and is used to make perfumes and flavorings. Used mainly as a flavoring substance in tobacco products and artificial vanilla substitutes, coumarin has been banned as a food additive in numerous countries since the mid-20th century because it is toxic to the liver and kidneys.

The formation of coumarin proceeds from cinnamic acid via 2-coumaric acid formed by the catalysis of cinnamate 2-hydroxylase (EC 2.4.1.14) (Figure 7.29). 2-Coumaric acid is then glycosylated (2-coumarate *O*-β-glucosyltransferase, EC 2.4.1.114) and the glucoside isomerized to glucoside of coumarinic acid by 2-coumarate β-D-glucoside isomerase (EC 5.2.1.-). The *trans-cis* isomerization occurs also spontaneously by means of UV-light. Hydrolysis then provides free coumarinic acid (coumarinic acid glucoside β-glucosidase, EC 3.2.1.21) and the last step of the pathway is the spontaneous lactonization of coumarinic acid in acid medium forming coumarin. Coumarin is produced only when plants are wounded as the glucosylated coumarin derivatives (glucosides of coumaric and coumarinic acids) accumulate in the vacuole while the β-glucosidase is located to the extraplasmatic space. Hence, the physical contact of the enzyme and its substrate only occurs after the breakup of the cell and its organelles. As a consequence, coumarin is liberated. It is readily further metabolized either as the free compound or coumarinic acid glucoside being converted to melilotic acid (or its glucoside) as the main product via an intermediate dihydrocoumarin (dihydrocoumarin hydrolase, EC 3.1.1.35).

The hemorrhagic factor dicoumarol (also known as dicumarol, dicoumarin, and melitoxin) caused the epidemic bleeding disease in cattle in the 1920s acts as an anticoagulant (vitamin K antagonist). In spoiled sweet clover hay that had been improperly cured and infected with molds, dicoumarol is formed from 2-coumaric acid CoA thioester by the action of microbial enzymes (Figure 7.30).

[7.7)] Handling of e.g. celery and petroselinum plants infected by fungi and the direct contact with fresh fig sap can cause acute dermatitis in case the concentration of psoralens exceeds 18 mg/kg. Handling of giant hogweed (*Heracleum mantegazzianum*; Apiaceae) can cause serious blistering by direct contact with the sap. Plants containing linear furanocoumarins have been used to promote skin pigmentation and sun-tanning. An important use of xanthotoxin (methoxsalen) is in UV treatment for skin problems, such as psoriasis and idiopathic depigmentation vitiligo.

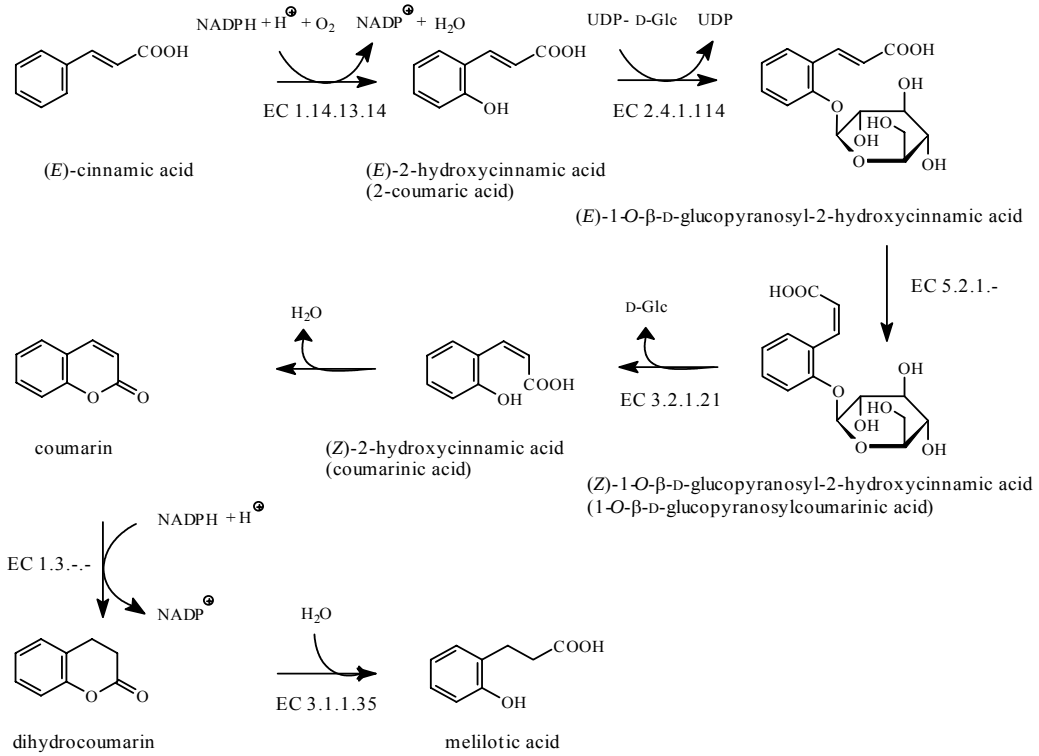


Figure 7.29

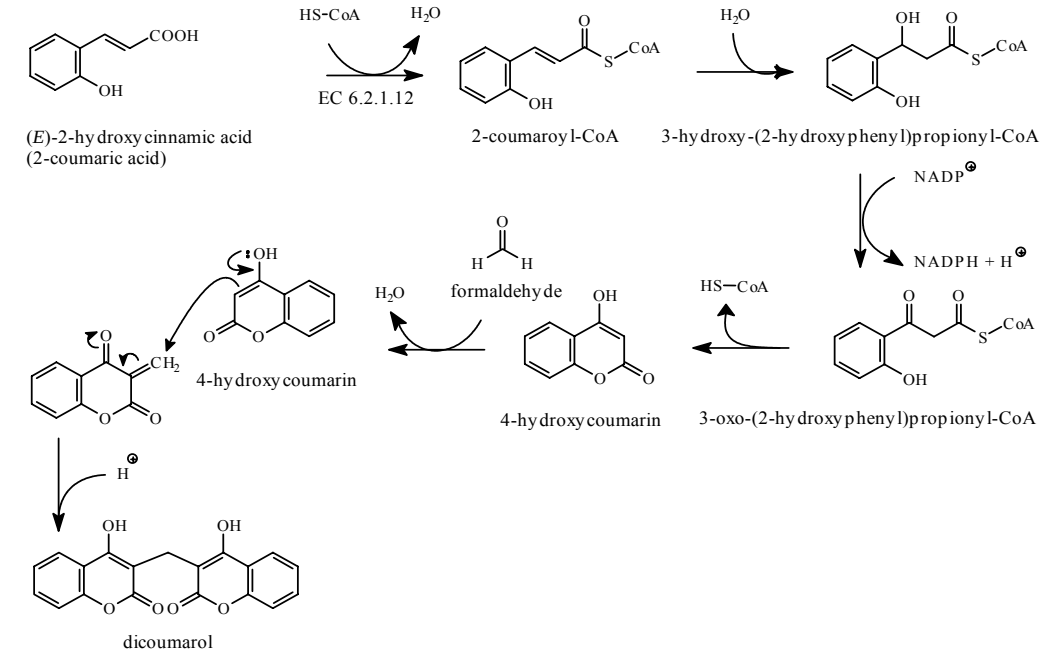


Figure 7.30

In contrast to coumarin, which is biosynthesized through 2-coumaric acid, the bio synthesis of many hydroxysubstituted, methylated, prenylated, and other more complex coumarins proceeds via 4-coumaric acid and 7-hydroxycoumarin (umbelliferone), rather than by a general cinnamic acid to coumarin pathway (Figure 7.31). These compounds mainly occur as glycosides. For example, skimmin is 7-*O*-β-D-glucopyranoside of umbelliferon, esculin (or esculoside) 6-*O*-β-D-glucopyranoside of esculetin, cichoriin 7-*O*-β-D-glucopyranoside of esculetin, scopolin 7-*O*-β-D-glucopyranoside of scopoletin. In small amounts not exceeding 1 mg/kg, esculetin and scopoletin occur in some vegetables (carrot, celery) and fruits (apricot). Cichorin also occurs in the root and chicon of Belgian endive (witloof cichory, *Cichorium intybus*; Asteraceae) and it contributes to its bitter taste. Scoparon occurs in citrus fruits exposed to UV-irradiation or temperature oscillation. Hemiarin and ayapin occur e.g. in tubers of Jerusalem artichoke, also known as topinambur (*Helianthus tuberosus*; Asteraceae).

Umbelliferon is probably the precursor of a series of other coumarins. Limettin, occurring in citrus fruits, is an example of coumarins substituted in other positions of the aromatic ring. The coumarins with an isoprenoid substituent (formed under catalysis of prenyl-transferases, EC 2.5.1.-) are exemplified by osthenol, osthol, and auraptenol that occur in citrus fruits and accompany many other coumarins in garden angelica (*Angelica archangelica*, syn. *Archangelica officinalis*; Apiaceae). The component of sandalwood red santalin AC (see Chapter 8.6.5) is an example of isoflavonoid pigments. 4-Phenylcoumarins belonging to neoflavonoids are represented by e.g. dalbergin (Figure 7.32) that occurs in tropical trees, shrubs, and lianas of the genus *Dalbergia* (Fabaceae). The most famous of these are the rosewood, so-named because of the smell, but several other valuable woods are yielded by this genus.

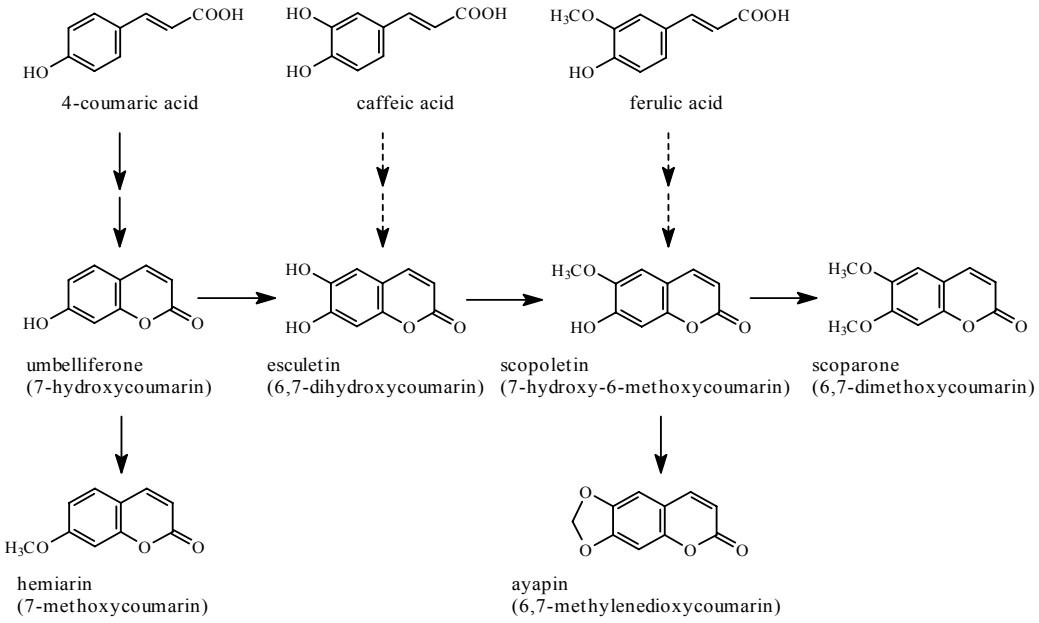


Figure 7.31

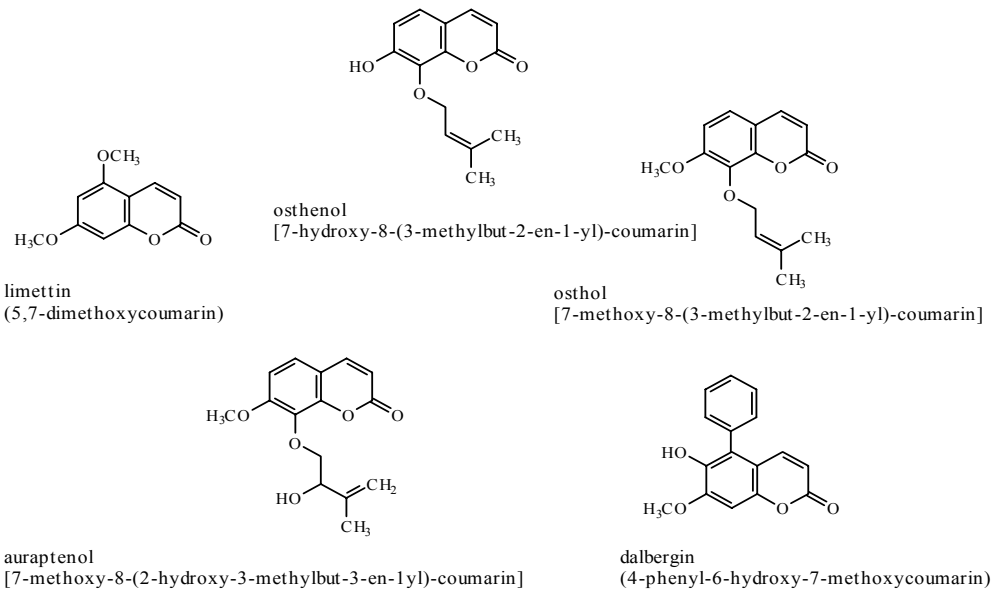


Figure 7.32

7.8.2 Furanocoumarins

Linear and angular furanocoumarins are synthesized from umbelliferone. The crucial first step in the biosynthesis of linear furanocoumarins is its prenylation by dimethylallyl diphosphate at C-6, catalyzed by prenyltransferase (EC 2.5.1.-), to form demethylsuberosin (Figure 7.33).

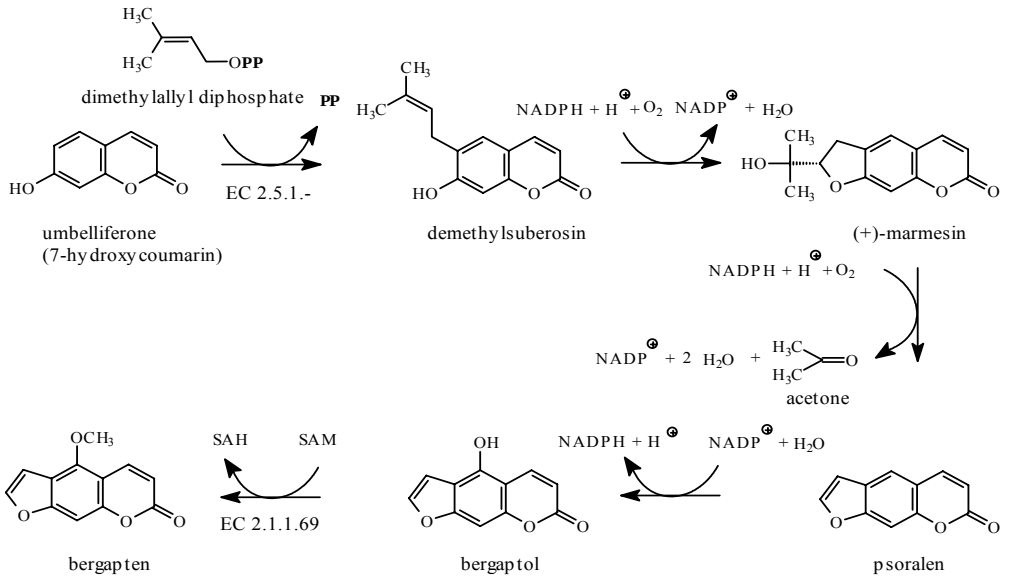


Figure 7.33

The newly introduced dimethylallyl group in demethylsuberosin is able to cyclize with the phenol group yielding the first furancoumarin (+)-marmesin under the marmesin synthase catalysis. Psoralen synthase then removes the C-3′-H_S, together with the *syn* oriented isopropyloxy substituent of (+)-marmesin yielding psoralen, acetone, and water (Figure 7.34). A sequence of oxidation (at C-5, psoralen 5-monooxygenase) and methylation reactions (bergaptol-*O*-methyltransferase, EC 2.1.1.69) generates bergapten. Biosynthesis of bergapten is the last fully elucidated step in this biosynthetic pathway.

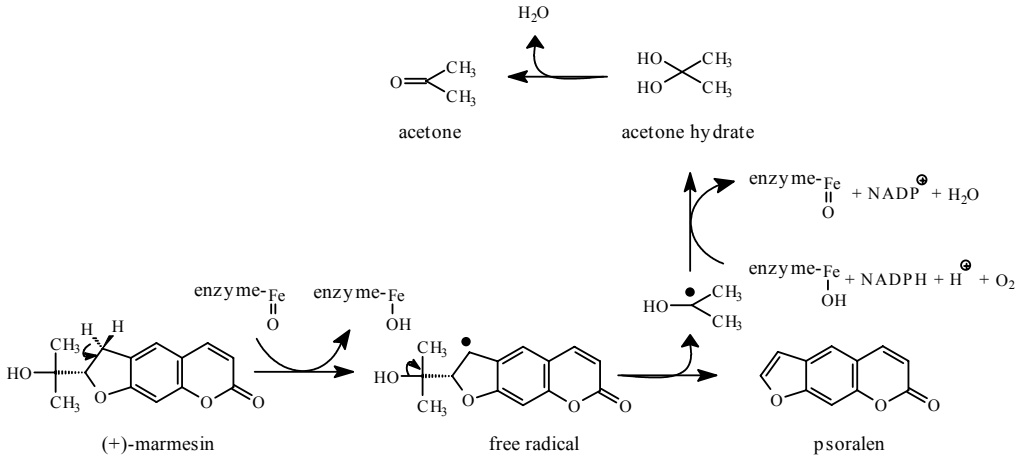


Figure 7.34

Angular furanocoumarins can arise by a similar sequence of reactions, but these reactions involve prenylation at the alternative position *ortho* to the hydroxyl group in umbelliferone. Many other hydroxylated, methylated, and prenylated furanocoumarins and pyranocoumarins are known to be produced through a series of yet not unidentified reactions (Figure 7.35). Their content in citrus essential oils and the Apiaceae vegetables is given in Table 7.3.

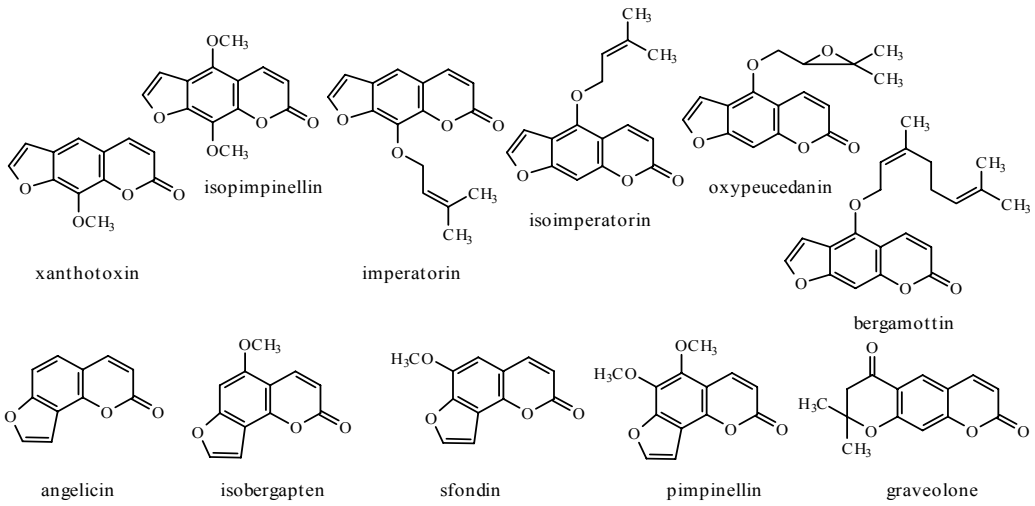


Figure 7.35

Table 7.3 Furanocoumarins of selected essential oils and vegetables

Product	Furanocoumarins (mg/kg)
Orange oil	bergaptol (0.5)
Lemon oil	bergapten (33)
Grapefruit oil	bergapten (120)
Carrot (root)	bergapten (0.01), xanthotoxin (0.01)
Parsley (root)	oxypeucedanin (26), bergapten (12), isoimperatorin (5.6), psoralen (1.4), imperatorin (1.4), xanthotoxin (0.1), graveolone
Parsnip (root)	xanthotoxin (48), angelicin (47), bergapten (7), psoralen (7)
Celeriac (root)	bergapten (0.6), xanthotoxin (0.5), psoralen (0.04)
Celery (haulm)	xanthotoxin (7.2), psoralen (1.0), bergapten (0.6)
Anise, coriander, caraway (seeds)	xanthotoxin (0.01), bergapten (0.01)

7.8.3 Isocoumarins

The biosynthesis of isocoumarins, related stilbenes (see Chapter 7.9), chalcones (see Chapter 8.6.1), and a variety of other phenolics proceeds via the acetate-polymalonate (polyketide) pathway. This pathway is provided by the multienzyme protein complexes termed polyketide synthases (the type III PKSs; see Chapter 7.8.3.1) that synthesize a surprisingly large variety of products. The diversity is based on the use of different starter units (e.g. CoA esters of phenylpropanoids, benzoic acids, and aliphatic acids), different number of condensation reactions, different types of folding after the condensation, and subsequent transformation of reaction products.

Chalcone synthase (EC 2.3.1.70) is a plant specific polyketide synthase that uses a starter CoA-ester from the phenylpropanoid pathway as a substrate. For example, 4-coumaroyl-CoA performs three sequentional condensation reactions with malonyl-CoA and folds the resulting tetraketide intermediate to a new aromatic system of chalcone (Figure 7.36). A linear diketide intermediate, which has been elongated, but not cyclized, can be hydrolyzed to yield benzalacetone. Analogously, a linear triketide intermediate can be either extended by malonyl-CoA to yield a linear tetraketide intermediate or transformed to styrylpyrones (styrylpyrone synthase, EC 2.3.1.-). The tetraketide intermediate becomes the precursor of either stilbenes (stilbene synthase, EC 2.3.1.-) or isocoumarins.

Isocoumarins (3,4-benzo-2-pyrones) are rare phytoalexins synthesized *de novo* in plants. The biosynthesis of isocoumarins in *Hydrangea macrophylla* var. *thunbergii* (Hydrangeaceae) proceeds analogously to the biosynthesis of stilbenes to form a tetraketide intermediate, but includes a reduction step. The carboxylic acid intermediate stilbenecarboxylic acid does not eliminate carbon dioxide. Instead, hydrangeic acid forms isocoumarin hydrangenol. Hydrangeol is further transformed to phyllodulcin, (3*R*)-8-hydroxy-3-(3-hydroxy-4-methoxyphenyl)isochroman-1-one that was formerly used as a sweetener (Figure 7.37).

6-Methoxymellein, a bitter isocoumarin showing estrogenic activity, is produced in carrots upon fungal infection by *Ceratocystis fimbriata*. The biosynthetic formation of 6-methoxymellein proceed via the immediate tetraketide precursor 6-hydroxymellein produced via the acetate-malonate pathway (6-hydroxymellein synthase, EC 2.3.1.-).

The biosynthesis of pterocarpans is salient by the stereospecificity of many of the enzymes involved as exemplified by the biosynthesis of pterocarpans from formononetin and daidzein (Figure 7.50). The enzyme involved in the first reaction step is 4´-methoxyisoflavone 2´-hydroxylase (EC 1.14.13.53) that produces the intermediate 2´-hydroxyformononetin. Pterocarpan synthase (EC 2.1.1.246), the enzyme converting 2'-hydroxyisoflavanones, e.g. (-)-vestitone, to the corresponding pterocarpans, e.g. (6a*R*,11a*R*)-medicarpin or (-)-medicarpin, consists of two enzymes, a reductase (2´-hydroxyisoflavone reductase, EC 1.3.1.45) and a dehydratase (pterocarpan synthase or vestitone reductase, EC 1.1.1.246). The precedent enzyme, 2´-hydroxyisoflavone reductase, catalyzes the stereospecific formation of the 2'-hydroxyisoflavanones (6a*S* or 6a*R* configuration), which determines the stereochemistry of the subsequent pterocarpans, e.g. (+)-maackiain in pea and (-)-maackiain in chickpea (Figure 7.51).

Daidzein becomes the precursor of coumestrol and glyceollins in soybeans. These reactions are catalyzed by 2´-hydroxydaidzein reductase (EC 1.3.1.51), trihydroxypterocarpan dimethylallyl transferase (EC 2.5.1.36), and glyceollin synthase (EC 1.3.1.51). Coumestrol is the main pterocarpan in soybean seeds (0.05-0.2 mg/kg). During sprouting, its concentration increases 70-150 times and its estrogenic activity is about 30-40 times as high as that of isoflavones. Coumestrol is also the main pterocarpan of fodder plants being accompanied by medicarpin, lucernol, sativol, and other compounds.

The initial enzymatic steps of the glyceollin biosynthesis parallel the biosynthesis of medicarpin (and maackiain). In contrast to those simple pterocarpans, the complex prenylated pterocarpans glyceollins (and phaseollins) also derive from the 5-deoxyisoflavone daidzein. Glycinol, the final compound in this part of the glyceollin pathway, is stereospecifically hydroxylated at the 6a position resulting in the (-)- or (6a*S*,11a*S*)-3,6a,9-configuration. The remaining enzymatic reactions maintain the given configuration as demonstrated for other pathways that involve a pterocarpan-6a-hydroxylase in their biosynthesis (e.g. pisatin). The final enzymatic steps in glyceollin biosynthesis include the introduction of dimethylallyl groups to (-)-glycinol yielding two prenylated precursors (glyceollidin I and II). The subsequent cyclization of those intermediates produces the three glyceollins (glyceollin I, II, and III) identified in soybean. The synthesis of phaseollin parallels the synthesis of glyceollins.

7.13 Flavonolignans

Milk thistle (*Silybum marianum*; Asteraceae) is typically used to treat liver cirrhosis, chronic hepatitis (liver inflammation), and gallbladder disorders. The active principle is silymarin, 70-80% of which is a mixture of closely related flavonolignans. The main component of the silymarin complex is silybin (also known as silibinin), which is a mixture of two diastereomers A and B in approximately 1:1 proportion.

Flavonolignans are formed by oxidative coupling processes between a flavonoid (dihydroflavonol), such as taxifolin (dihydroquercetin) and a phenylpropanoid, usually coniferylalcohol. One electrone oxidation of taxifolin yields a free radical, which may combine with the free radical generated from coniferylalcohol (Figure 7.52). This would lead to an adduct, which could cyclize by attack of the phenol nucleophile on to the quinone methide system provided by coniferylalcohol.

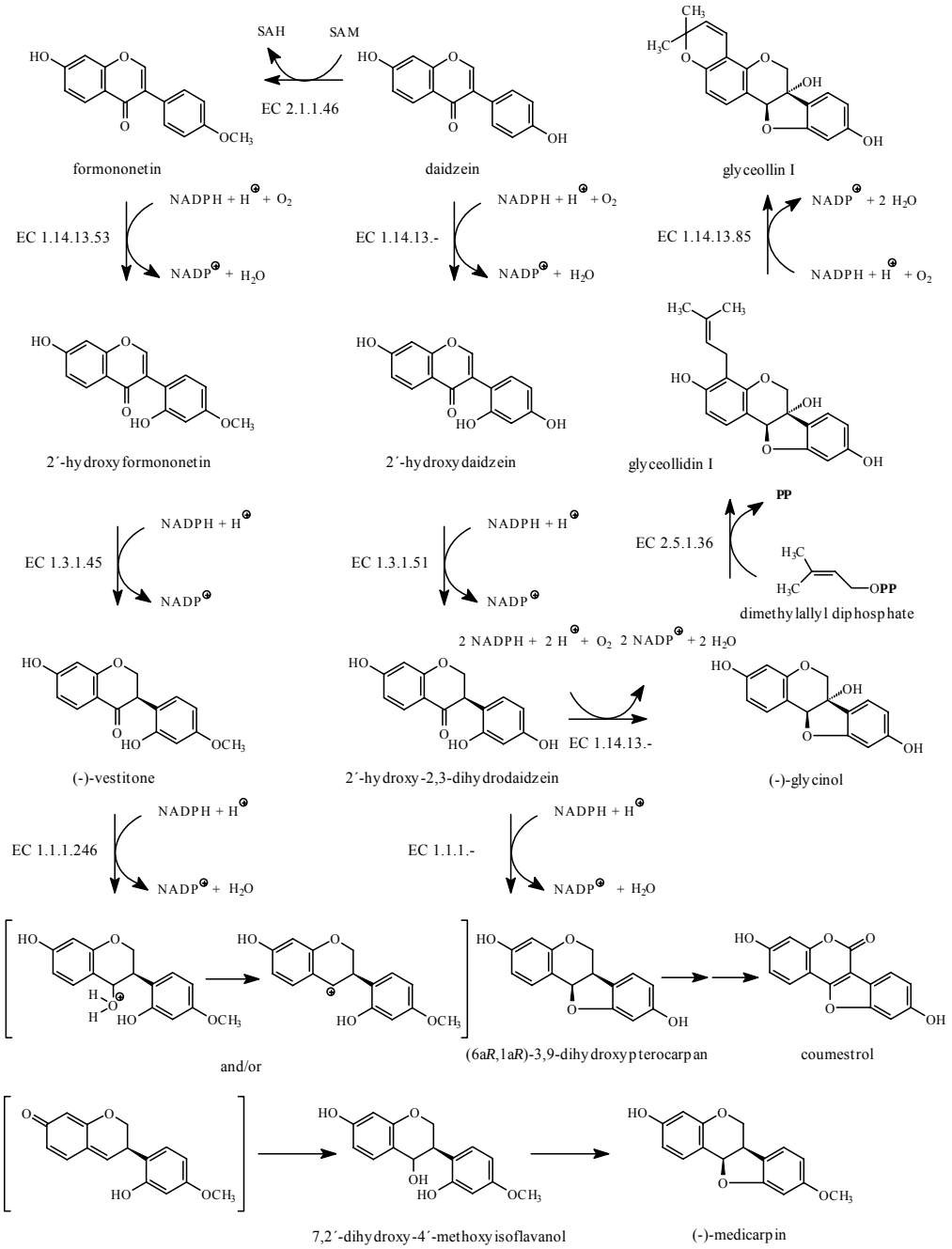


Figure 7.50

7.14 Miscelanous compounds

A range of aromatic compounds are polyketides biosynthesized from aliphatic precursors by sequential reactions catalyzed by polyketide synthases. Such compounds are

phloroglucinols and phthalides that belong to prominent flavor-active food constituents. Their biosynthesis uses acyl-CoA esters derived from amino acids as the primers and malonyl-CoA as the extender unit.

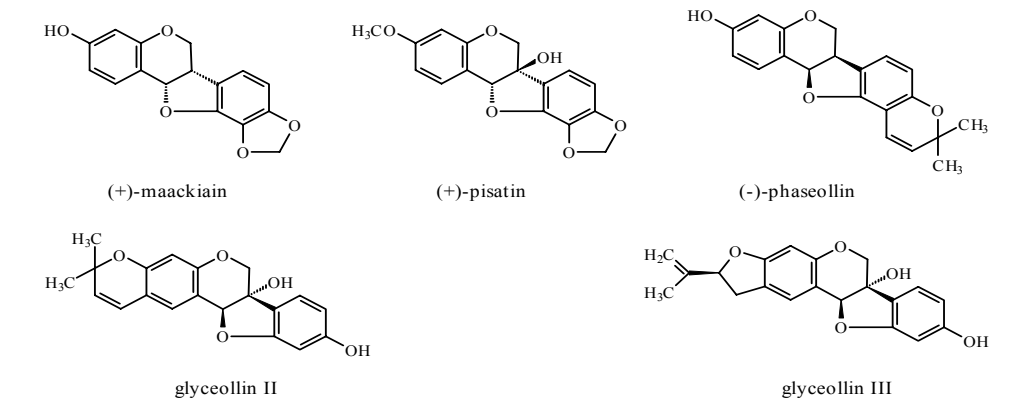


Figure 7.51

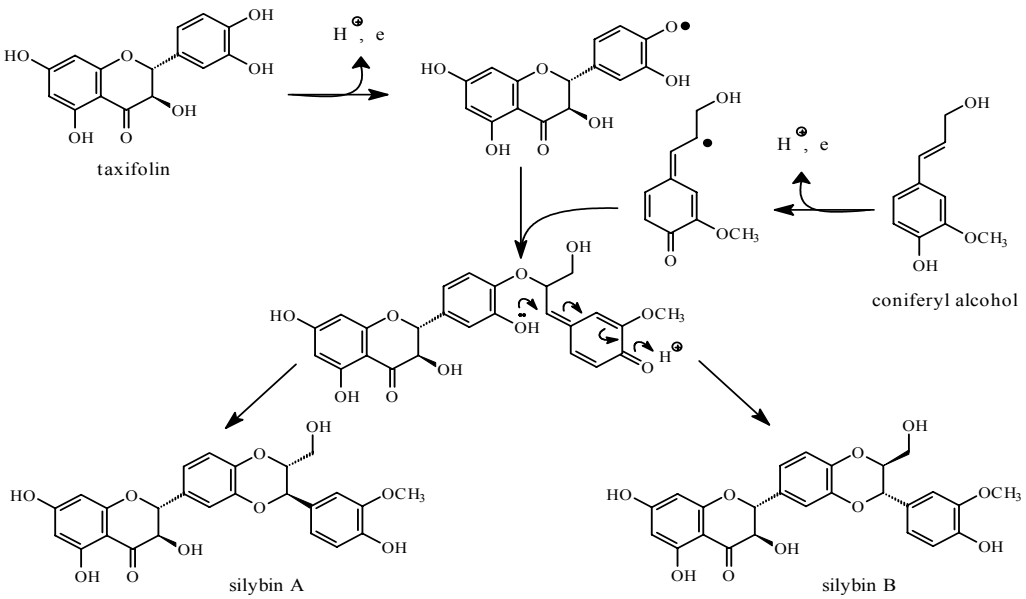


Figure 7.52

7.14.1. Phloroglucinols

The ripe cones of the hop plant (*Humulus lupulus*; Cannabaceae) accumulate in their glandular hairs substituted 1,3,5-benzenetriols, known as phloroglucinols (about 18% dry weight). These compounds, known as bitter acids, contribute to the final aroma and taste of beer. They are also appreciated for their antimicrobial, antifungal, and antifeedant activities. The bitter acids are principally divided into the humulone derivatives and lupulone derivatives. The humulone derivatives are better known as α-acids. The main α-acids are humulone, cohumulone, adhumulone, prehumulone, and posthumulone. The

main lupulone derivatives, also known as β-acids, are lupulone, colupulone, adlupulone, prelupulone, and postlupulone (Figure 7.53, Table 7.4). The group of α-acids is of particular importance for the brewing industry as humulones are spontaneously transformed into iso-α-acids (e.g. isohumulone, isocohumulone, isoadhumulone, isoprehumulone, and isoposthumulone) during the brewing process (Figure 7.54), which are involved in the bitter taste of beer.

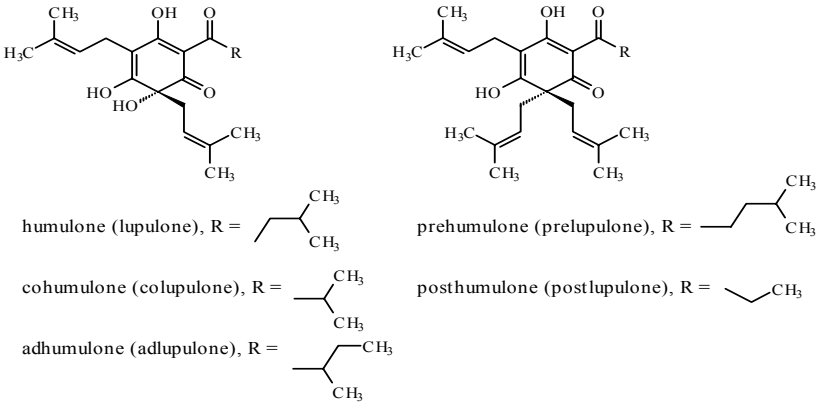


Figure 7.53

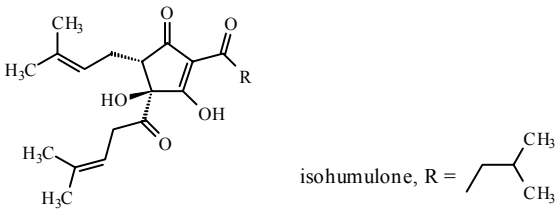


Figure 7.54

Table 7.4 Average content of bitter acids in hops

α-Acids	Total α-acids in %	β-Acids	Total β-acids in %
Humulone	46-67	Lupulone	21-51
Cohumulone	21-41	Colupulone	37-68
Adhumulone	12-14	Adlupulone	11-12
Prehumulone	1-10	Prelupulone	1-5

The first step in the biosynthesis of humulone is the condensation of malonyl-CoA with isovaleryl-CoA, derived from L-isoleucine, which leads to the formation of the phloroglucinol derivative phlorisovalerophenone. The reaction is catalyzed by a chalcone synthase-like enzyme phlorisovalerophenone synthase (EC 2.3.1.156). The prenyl moieties of humulone are derived from dimethylallyl diphosphate and prenyltransferase (EC 2.5.1) activity. Isomerization of diprenylphlorisovalerophenone and its hydroxylation at C-4 catalyzed by deoxyhumulene oxidase (EC 1.1.1.-) yields humulone (Figure 7.55), while isomerization and prenylation of diprenylphlorisovalerophenone at C-4 leads to lupulone. Analogously, some branched- and medium straight-chain 2-oxoacid-CoA

involved in amino acid and fatty acid metabolism yield other humulone and lupulone derivatives. The condensation of malonyl-CoA with L-valine derived isopropyl-CoA yields cohumulone, etc.

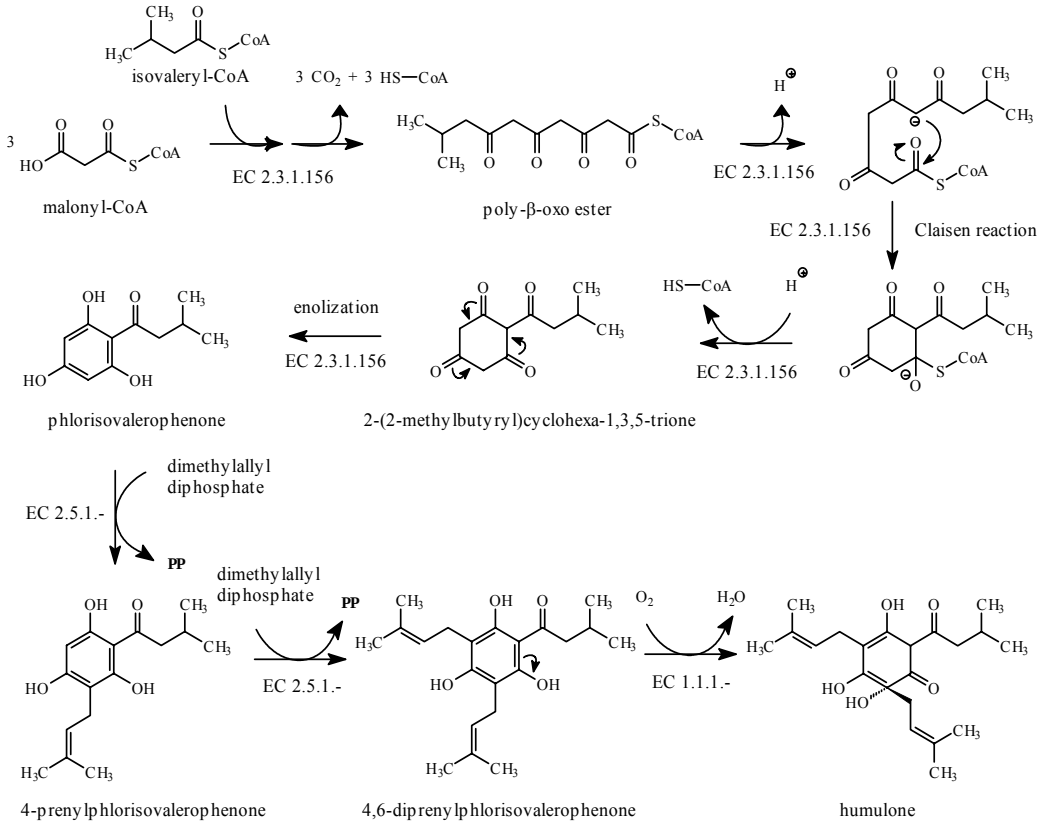


Figure 7.55

A range of more complex phloroglucinols can be found in St. John´s wort (*Hypericum perforatum*; Clusiaceae). The major active compounds, hyperflorin (formed using valine as the precursor) and its derivative adhyperflorin (derived from isoleucine), were shown to inhibit various neurotransmitter receptors. They also posses antidepressant activities (Figure 7.56).

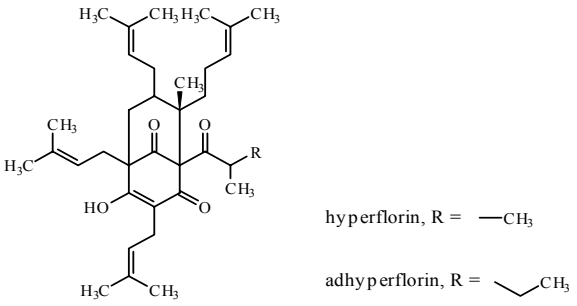


Figure 7.56

7.14.2 Phthalides

Numerous (3*H*)-isobenzofuran-1-ones, known as phthalides, derived hexahydrophthalides, tetrahydrophthalides, and dihydrophthalides occur as the prominent flavor components in some vegetables of the Apiaceae family, such as celery, celeriac (*Apium graveolens*), and lovage (*Levisticum officinale*). Smaller amounts of phthalides also occur in fennel (*Foeniculum vulgare*), parsley (*Petroselinum crispum*), dill (*Anethum graveolens*), and coriander (*Coriandrum sativum*).

The main phthalide in celeriac taproot is 3-butyl-4,5-dihydrophthalide (also known as sedanenolide or senkyunolide). It is accompanied by 3-butyl-3a,4,5,6-tetrahydrophthalide (sedanolide or neoknidilide), 3-butyphthalide, 3-butylidene-4,5-dihydrophthalide, known as (*Z*)-ligustilide, and other minor dihydro-, tetrahydro-, and hexahydrophthalides. The lovage leaves contain mainly (*Z*)-ligustilide, while (*E*)-ligustilide occurs in parsley, together with sedanenolide and 3-butylphthalide. 3-Butylphthalide also occurs in fennel and sedanolide is the main phthalide in the leaves of dill and coriander (Figure 7.57).

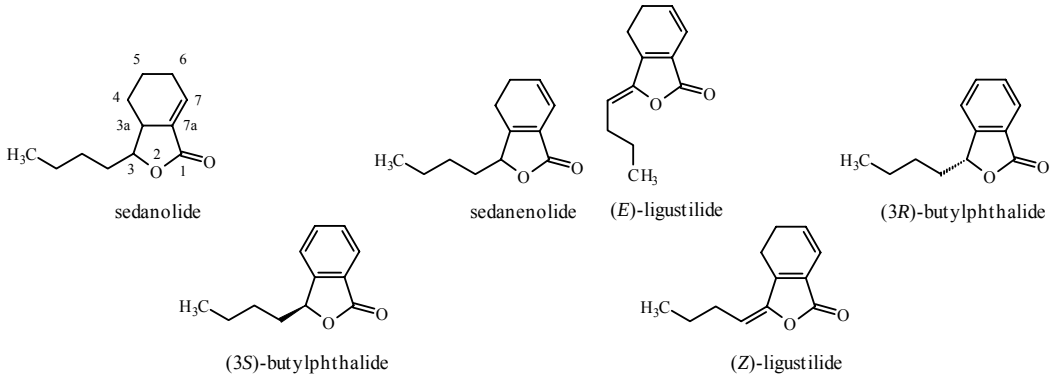


Figure 7.57

The isomeric phthalides have similar flavor reminiscent of celery or Maggi soup seasoning, but they occur in different plants in variable amounts. For example, the content of 3-butylphthalide, one of the predominant phthalides in celeriac taproot, is approximately 4% (fresh weight). About 88% of this amount is (3*S*)-isomer and 12% (3*R*)-isomer that has more intense flavor. 3-Butylphthalide concentration in fennel bulbs is only 0.01%, but the main isomer is (3*R*)-3-butylphthalide (64%).

Phthalides function by different mechanisms at a cellular level. They may induce beneficial enzymes, inhibit enzymes mediating inflammatory processes, and interact with ion channels or cell surface receptors. They also show hypotensive and diuretic effects.

Butylphthalides are probably biosynthesized via the acetate-polymalonate (polyketide) pathway using butyryl-CoA as the starter. Four sequentional condensation reactions with malonyl-CoA, folding of the resulting poly-β-oxo ester intermediate and a series of subsequent reactions can give way to the aromatic 4-butylphthalide backbone. The reaction sequences are schematically shown in Figure 7.58.

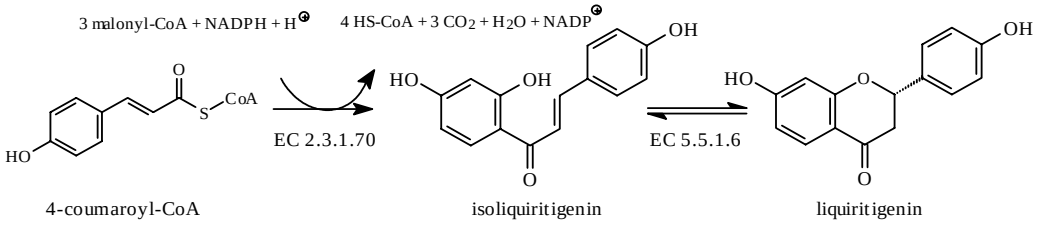


Figure 8.33

8.6.1.1 Quinochalcones

The petals of safflower (*Carthamus tinctorius*; Asteraceae) contain yellow quinochalcone pigments, such as safflor yellow A, safflor yellow B, precarthamin, and red carthamin. Traditionally, safflower has been used for its flowers in cooking as a cheaper substitute for saffron (sometimes referred to as bastard saffron), textile dye, and herbal medicine in oriental countries. Nowadays, the plant is cultivated mainly for the edible oil used quite commonly as an alternative to sunflower seed oil. Its is supposed that these pigments are biosynthesized, analogously to the chalcone naringenin, from the cinnamoyl-CoA starter and three malonyl-CoA extenders yielding first safflor yellow A, which is transformed to safflor yellow B, the precursor of precarthamin (Figure 8.34). Precarthamin forms from safflor yellow B by oxidation of the open-chain D-glucose residue. Oxidative decarboxylation of precarthamin (catalyzed by precarthamin decarboxylase) yields carthamin.

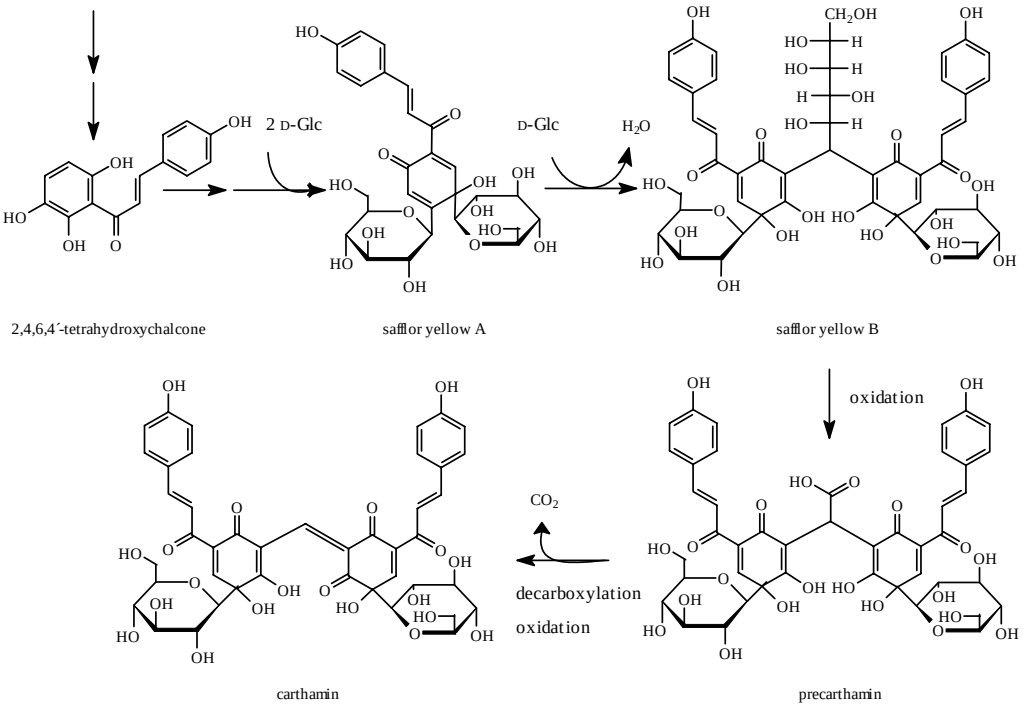


Figure 8.34

8.6.2 Flavones, flavanonoles, flavonols, leucoanthocyanidins, (epi)catechins, and anthocyanidins

Flavanones can give rise to many variants of the basic skeleton, e.g. flavones (flavone synthase, EC 1.14.11.22), flavanonoles (flavanonole 3-hydroxylase, EC 1.14.11.9), flavonols (flavonol synthase, EC 1.14.11.23), leucoanthocyanidins (dihydroflavanol 4-reductase, EC 1.1.1.219), anthocyanidins (anthocyanidin synthase, EC 1.14.11.19), catechins (leucoanthocyanidin reductase, EC 1.17.1.3), and epicatechins [8.16)] (anthocyanidin reductase, EC 1.3.1.77) (Figure 8.35).

Modifications of the hydroxylation patterns in the two aromatic rings may occur, generally at the flavanone, flavanonole, or flavone stage. Methylation, *O*-glycosylation, *C*-glycosylation, and dimethylallylation are also common, increasing the range of compounds enormously. Figure 8.36 shows, as an example, the pathways leading to the most common methylated and glycosylated apigenin derivatives. The enzymes involved are apigenin 4´-*O*-methyltransferase (EC 2.1.1.75), flavone 7-*O*-β-glucosyltransferase (EC 2.4.1.81), flavone 7-*O*-β-glucoside 6´´-*O*-malonyltransferase (EC 2.3.1.115), and flavanone-7-*O*-glucoside 2´´-*O*-β-L-rhamnosyltransferase.

The pathways leading to the most common cyanidin glycosides are given in Figure 8.37. The unstable anthocyanidins are thus coupled to sugar molecules to yield the final relatively stable glycosides anthocyanins. The enzymes involved in these pathways comprise anthocyanidin 3-*O*-glucosyltransferase (EC 2.4.1.115), anthocyanidin 3-aromatic acyl transferase (EC 2.3.1.-), and anthocyanidin 5,3-*O*-glycosyl transferase (EC 2.4.1.-).

8.6.3 Aurones

Aurones belong to a small class of plant flavonoids that, in the glycosylated form, provides the bright yellow color of some important ornamental flowers, such as snapdragon (*Antirrhinum majus*; Scrophulariaceae) and plants belonging to the Asteraceae family (e.g. *Coreopsis*, *Cosmos*, and *Dahlia*).

The pivotal precursors of aurones are chalcones. The minor 4,4´,6-trihydroxyaurone can form from naringenin chalcone by a not yet characterized oxygenase enzyme. The enzyme aureusidin synthase (a copper-containing glycoprotein and a homologue of plant polyphenol oxidase, EC 1.21.3.6) catalyzes the 3-hydroxylation of the ring B and the oxidative cyclization (2´,α-dehydrogenation) of chalcones carrying hydroxyl functions at their C-2´and C-4 positions. Aurone formation from chalcones, having a 4-hydroxy B-ring, must be accompanied by the oxygenation of the ring B, whereas aurones formation from chalcones, with a 3,4-dihydroxy B-ring, is not necessarily accompanied by the B-ring oxygenation.

[8.16)] The products are (2*S*)-flavan-4-ols, (*2R*,3*R*)-dihydroflavonols, *cis*-flavan-3,4-diols) or (2*R*,3*S*,4*S*)-leucoanthocyanidins , catechins (flavan-3-ols), i.e. (+)-catechins or (2*R*,3*S*)-flavan-3-ols, and (-)-epicatechins or (2*R*,3*R*)-flavan-3-ols. Formally, 4´-hydroxyderivatives are derived from 4-hydroxybenzoic (*p*-hydroxybenzoic) acid, 3´,4´-dihydroxyderivatives from protocatechuic acid, and 3´,4´5´-trihydroxyderivatives from gallic acid.

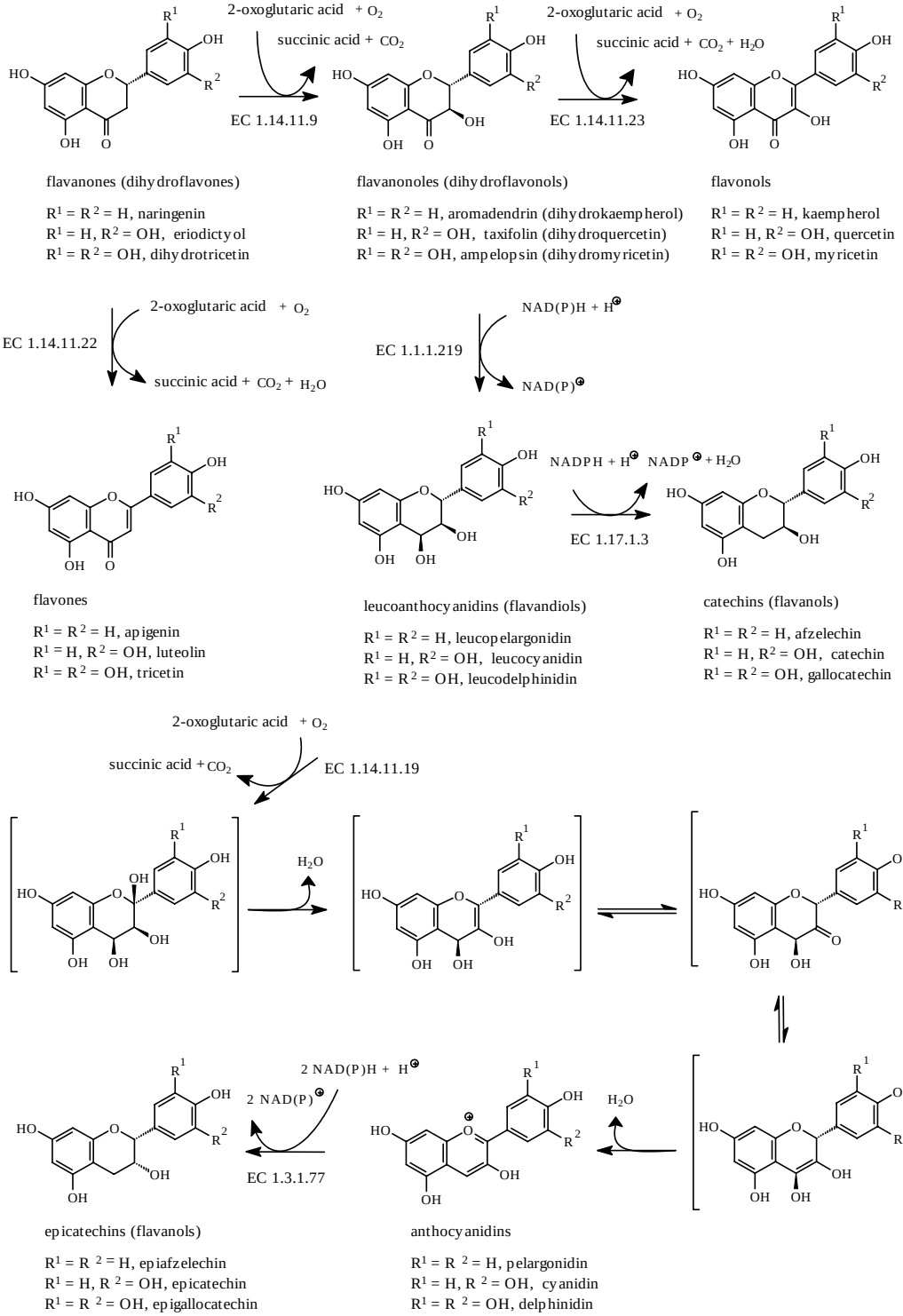

2-oxoglutaric acid + O_2
succinic acid + CO_2
EC 1.14.11.9
2-oxoglutaric acid + O_2
succinic acid + CO_2 + H_2O
EC 1.14.11.23
flavanones (dihydroflavones)
R^1 = R^2 = H, naringenin
R^1 = H, R^2 = OH, eriodictyol
R^1 = R^2 = OH, dihydrotricetin
flavanonoles (dihydroflavonols)
R^1 = R^2 = H, aromadendrin (dihydrokaempherol)
R^1 = H, R^2 = OH, taxifolin (dihydroquercetin)
R^1 = R^2 = OH, ampelopsin (dihydromyricetin)
flavonols
R^1 = R^2 = H, kaempherol
R^1 = H, R^2 = OH, quercetin
R^1 = R^2 = OH, myricetin
EC 1.14.11.22
2-oxoglutaric acid + O_2
succinic acid + CO_2 + H_2O
EC 1.1.1.219
NAD(P)H + $H^\oplus$
NAD(P)$^\oplus$
flavones
R^1 = R^2 = H, apigenin
R^1 = H, R^2 = OH, luteolin
R^1 = R^2 = OH, tricetin
NADPH + $H^\oplus$
NADP$^\oplus$ + H_2O
EC 1.17.1.3
leucoanthocyanidins (flavandiols)
R^1 = R^2 = H, leucopelargonidin
R^1 = H, R^2 = OH, leucocyanidin
R^1 = R^2 = OH, leucodelphinidin
catechins (flavanols)
R^1 = R^2 = H, afzelechin
R^1 = H, R^2 = OH, catechin
R^1 = R^2 = OH, gallocatechin
2-oxoglutaric acid + O_2
succinic acid + CO_2
EC 1.14.11.19
H_2O
2 NAD(P)H + $H^\oplus$
2 NAD(P)$^\oplus$
EC 1.3.1.77
H_2O
epicatechins (flavanols)
R^1 = R^2 = H, epiafzelechin
R^1 = H, R^2 = OH, epicatechin
R^1 = R^2 = OH, epigallocatechin
anthocyanidins
R^1 = R^2 = H, pelargonidin
R^1 = H, R^2 = OH, cyanidin
R^1 = R^2 = OH, delphinidin

Figure 8.35

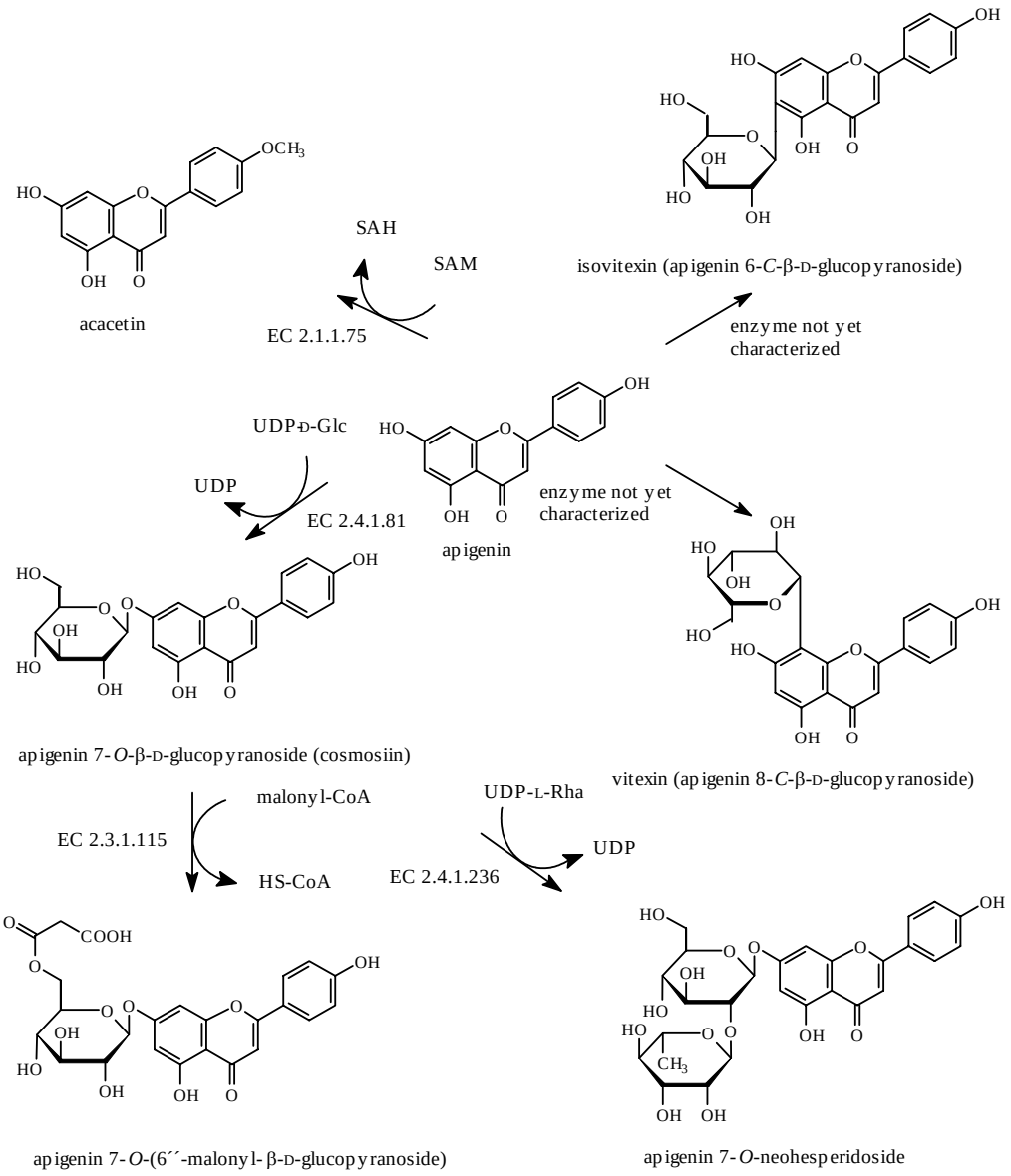


Figure 8.36

Thus, aureusidin may be formed from naringenin chalcone or 3,4,2´,4´,6´-pentahydroxychalcone. It is supposed that 3-hydroxylation proceeds through an intermediate with an *o*-quinone structure, which induces the Michael-type addition of 2´-hydroxyl group. The subsequent two reactions leading to aureusidin may then proceed spontaneously. When 3,4,2´,4´,6´-pentahydroxychalcone becomes the substrate of aureusidin synthase, analogous reactions yield a mixture of aureusidin and bracteatin (Figure 8.38). Aurones with no or only one hydroxyl group in the ring B are produced by alternative mechanisms.

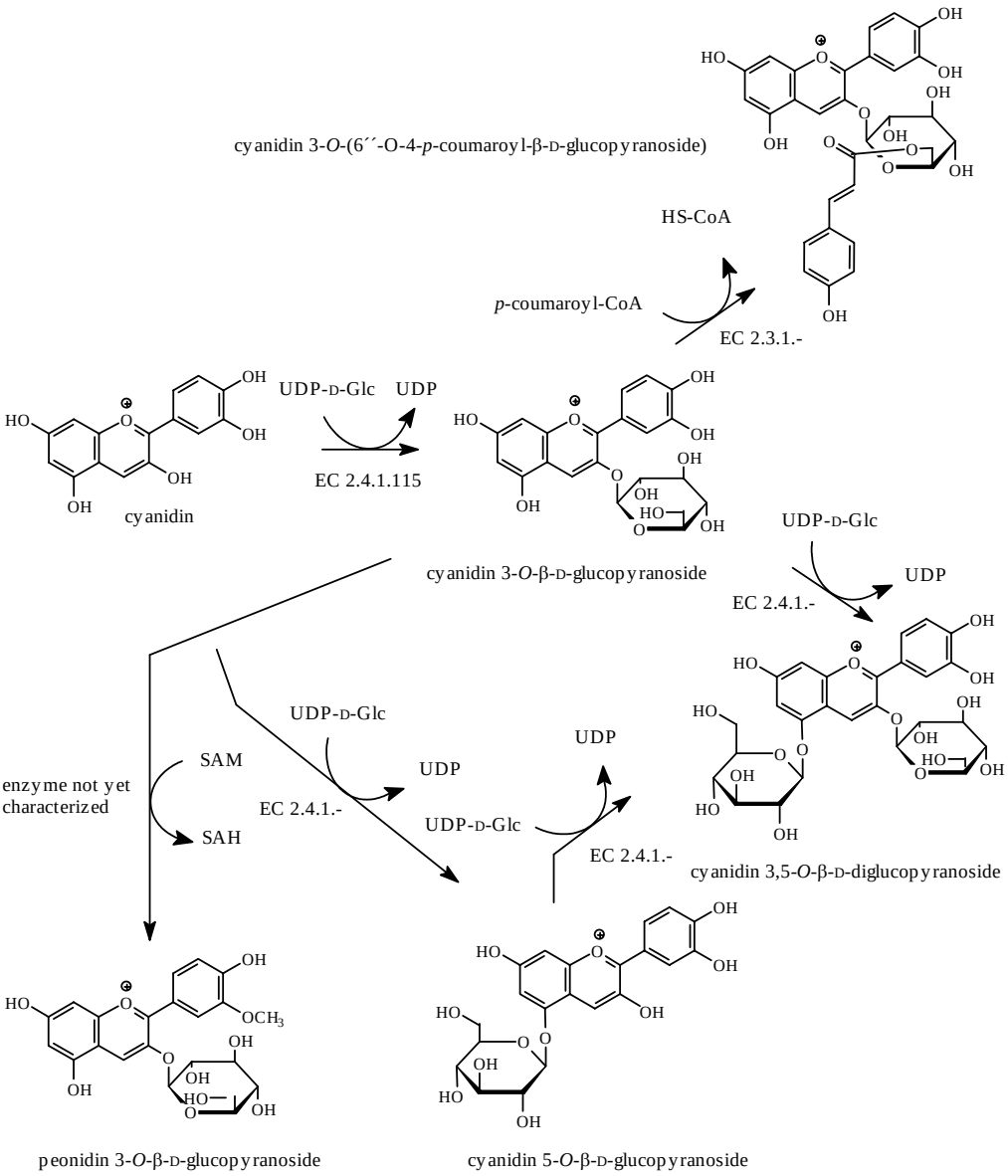


Figure 8.37

8.6.4 Condensed tannins

Condensed tannins, also known as proanthocyanidins, are oligomers or polymers of flavan-3-ol units. Proanthocyanidins are widespread throughout the plant kingdom, where they accumulate in many different organs and tissues to provide protection against predation. At the same time, they impart astringency (dimers and higher oligomers, including decamers) to some fruits and beverages, such as fruit juices, wines, and teas. They are increasingly recognized as having health beneficial effects for humans.

8.9.3 Apocarotenoids

Cleavage of carotenes and xanthophylls leads to various fragments classified as apocarotenoids that exert a number of biological functions. The best known apocarotene produced from β-carotene and some other carotenoids is vitamin A_1 (all-*trans*-retinol). Among the important catabolic products of carotenoids, called diapocarotenoids, is the food color annatto and the coloring principal of the spice saffron.

Annatto from the seeds of the tropical shrub *Bixa orellana* (Bixaceae) is used as an orange food color [8.23)] for a variety of foods (margarines, cheeses, desserts, breakfast cereals, liquors, etc.). It is also still used in the cosmetic industry for body care products. The pigments are found on the surface of the seeds, where they accumulate in a resinous oily substance. This covering contains a variety of pigments, including bixin dimethyl ester and a variety of apocarotenoids, such as all-*trans*-bixin (called just *trans*-bixin) and all-*trans*-norbixin (free dicarboxylic acid); however, the two main constituents of industrial relevance are the water-soluble apocarotenoid norbixin (also known as 9′-*cis*-norbixin) and the oil-soluble apocarotenoid monomethyl ester of bixin (9′-*cis*-bixin).

The biosynthesis of bixin starts with all-*trans*-lycopene, which is split by lycopene cleavage dioxygenase (EC 1.13.12.-) to two apocarotenoids, methyl-(4-methylpent-3-en-1-yl)ketone (6-methylhept-5-ene-2-one) and the residual C_{32} aldehyde called bixin aldehyde (Figure 8.54). Bixin aldehyde dehydrogenase (EC 1.2.1.-) oxidizes bixin aldehyde to the corresponding dicarboxylic acid norbixin, which is methylated in two steps using SAM (norbixin methyltransferase, EC 2.1.1.-) first to bixin and finally to bixin dimethyl ester.

The color, as well as the bitter taste and fragrance, in stigmas of saffron (*Crocus sativus*; Iridaceae) [8.24)], this extremely expensive spice, are due in large part to products of the degradation of the xanthophyll zeaxanthin. The color is mainly due to a number of apocarotenoid glycosides derived from crocetin. Crocetin is a water-insoluble aglycone, which, through sequential glucosylation, is converted to the soluble crocin (also known as α-crocin). Crocin also occurs in the fruit of *Gardenia jasmonoides* (Rubiaceae).

In the biosynthetic pathway leading to crocetin (Figure 8.55), zeaxanthin is split by zeaxanthin cleavage dioxygenase (EC 1.14.99.-) to hydroxy-β-cyclocitral and two molecules of C_{20} dialdehyde (crocetin dialdehyde), which is oxidized by aldehyde dehydrogenase (EC 1.2.99.3) to crocetin. Crocetin is then converted to crocetin monoglucosyl ester and crocetin diglucosyl ester (crocetin 8,8′-glucosyltransferase, EC 2.4.1.-). The crocetin monoglucosyl ester is transformed to crocetin monogentianobiosyl ester (glucosyltransferase, EC 2.4.1.-), which is further glucosylated forming crocetin gentiobiosylglucosyl

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[8.23)] Processing is primarily done by abrading away bixin in a suspending agent (water, vegetable oil), although solvent processing is now also employed. Abrasion may be followed by aqueous alkaline hydrolysis with simultaneous production of all-*trans*-bixin, norbixin, and *trans*-norbixin. Annatto is usually marketed as an extract of the annatto seed, containing amounts of the active pigments that can vary from less than 1% to over 85%.

[8.24)] Picrocrocin is largely responsible for the bitter taste of this spice, whereas safranal is the main constituent of its aroma. These two compounds are produced early in the degradation pathway of zeaxanthin and the volatile safranal is produced non-enzymatically by the action of heat. Traditional drying methods have been developed to generate the aroma of saffron.

ester (crocetin monogentiobiosyl ester glucosyltransferase, EC 2.4.1.-). Crocetin diglucosyl ester gives the same glycoside in a reaction catalyzed by glucosyltransferase (EC 2.4.1.-). Final glucosylation of crocetin gentiobiosylglucosyl ester yields crocin (crocetin digentiobiosyl ester).

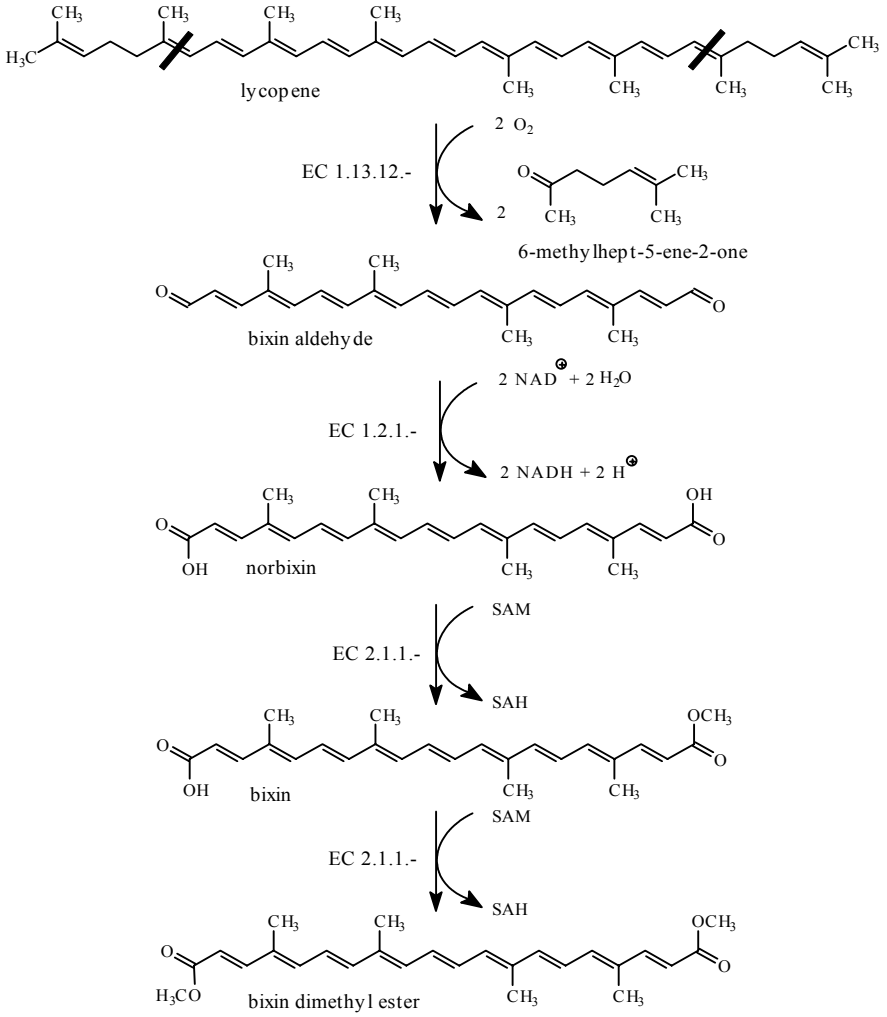

Figure 8.54

8.10 Iridoids

Gardenia jasminoides (syn. *G. augusta*; Rubiaceae), known as common gardenia, cape jasmine, or cape jessamine, is a fragrant flower growing in Southern China, Taiwan, and Japan. Gardenia fruits are used within the traditional Chinese medicine and as a yellow dye for clothes and food (such as the Korean mung bean jelly called *hwangpomuk*). The fruits contain three major pigment types, iridoid pigments, crocins, and flavonoids that have been used as food colorants.

The production of food colorants from gardenia is being investigated at the present time. Most procedures involve extraction of the fruit with water, treatment with enzymes having β-glucosidase activity, and reaction with primary amines from either amino acids or proteins. Manipulation of the reaction conditions, such as time, pH, temperature, oxygen content, etc. enables a series of colorants to be produced that vary from yellow to green, red, violet, and blue.

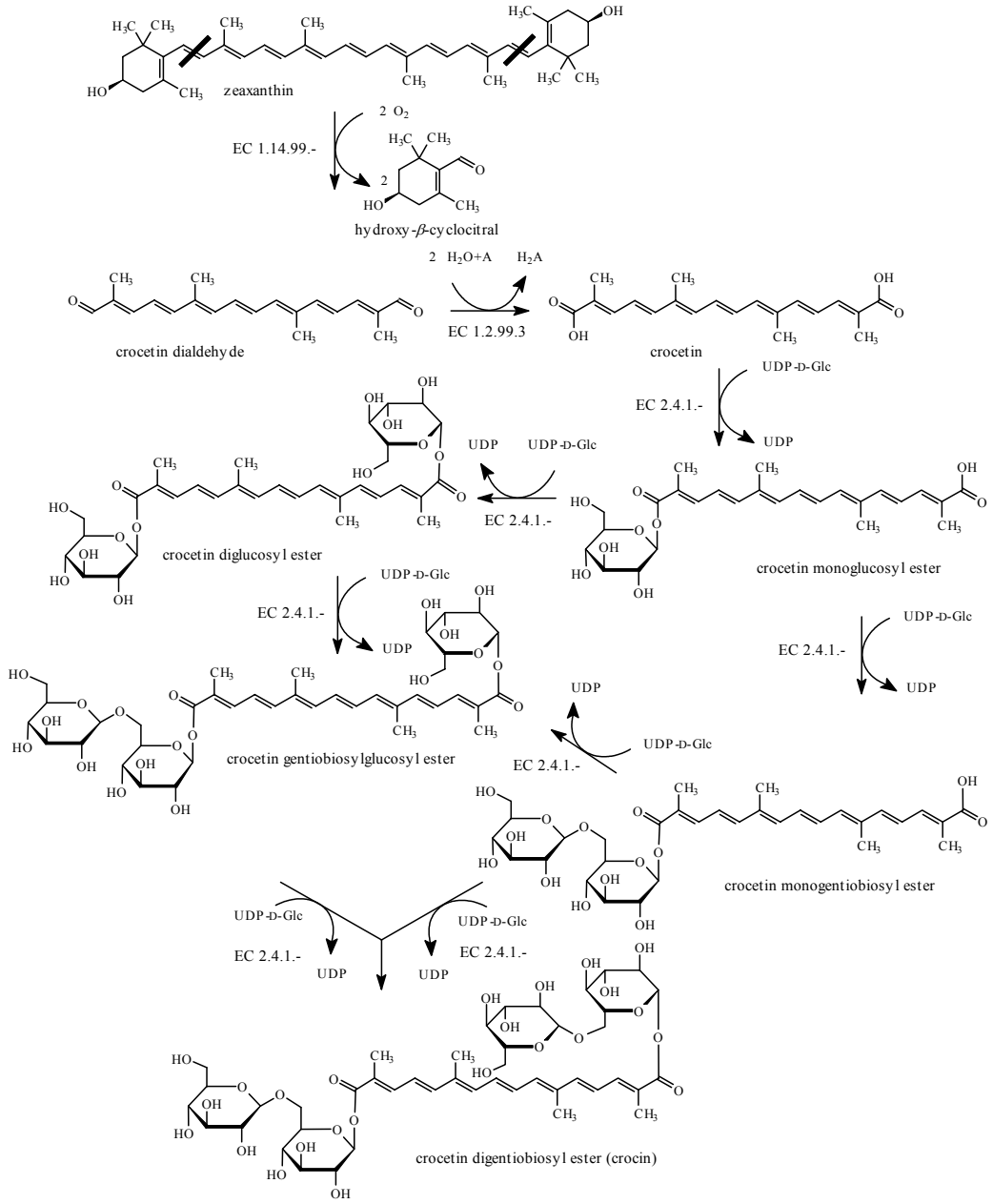

Figure 8.55

The major gardenia pigments, gardenoside and geniposide, are biosynthesized from the iridodial isomer (1*R*,2*S*,5*R*,8*S*)-iridodial, also known as 8-epiiridodial. The biosynthesis proceeds via 8-epiiridotrial, and 8-epi-7-deoxyloganic acid and leads to boschnaloside, tarennoside, geniposidic acid, geniposide, and gardenoside (Figure 8.56). Alternatively, geniposidic acid may be also formed from loganic acid and gardenoside may be produced from 7-deoxyloganic acid. Oxidation and dehydration of loganic acid leads to one of the minor gardenia pigments called geniposidic acid.

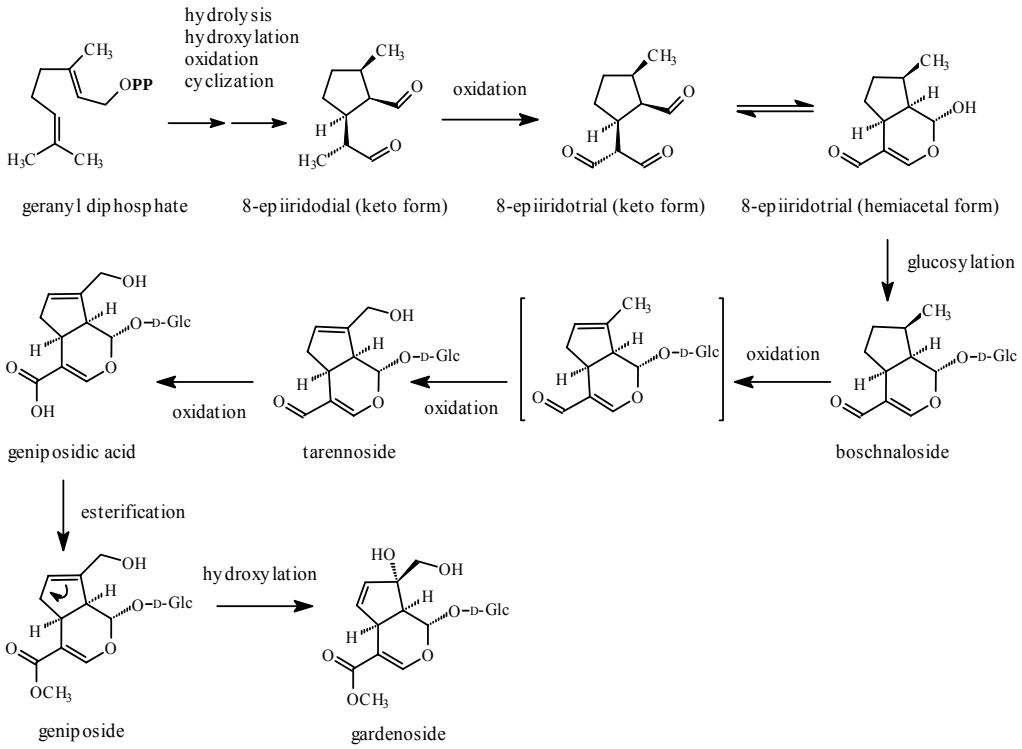

Figure 8.56

8.11 Miscellaneous terpenoids

Gossypol, 2,2′-bis(8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methyl)naphthalene, is a yellow pigment occurring in immature flower buds and seeds of the cotton plant (e.g. *Gossypium hirsutum* and other G. species; Malvaceae) that functions for defense against pathogens and herbivores. Cotton plant is important commercially for its soft fiber cotton that grows around the seeds, cottonseed oil, and other products. Cottonseed oil is used in salad and cooking oils and, after hydrogenation, in shortenings and margarines. The cake (meal), remaining after the oil is extracted, is used in poultry and livestock feeds. Gossypol occurs naturally in the plant in two forms referred to as (+)-gossypol and (-)-gossypol (Figure 8.57) in the ratio of 3:2.

9 ALKALOIDS AND TOXIC GLYCOSIDES

This chapter is devoted to toxic or potentially toxic food constituents that occur in food raw materials, foods, and feeds as a result of their inherent genetic characteristics or form during processing and/or storage. It covers compounds commonly classified as alkaloids, cyanogens, and glucosinolates. Many other compounds showing toxic effects in human beings and animals (e.g. some amino acids, biogenic amines, fatty acids, saponins, phenolics, and other compounds) have been included in other chapters. No attention is paid to toxic compounds that can occur in raw materials and foods due to their contamination.

9.1 Alkaloids

Alkaloids are a diverse group of secondary metabolites containing nitrogen in a negative oxidation state and are of limited distribution among living organisms [9.1)]. They occur in about 15 to 20% vascular plant species, where they exist in the free state or as *N*-oxides, and salts of plant acids, others occur as their esters or amides. Some plant alkaloids are combined with sugars as glycosides. While most alkaloids have been isolated from plants (their leaves, bark, roots, and seeds), a large number have been isolated from mosses, fungi, certain bacteria, and even animal sources [9.2)]. Over 10 000 alkaloids of many different structural types are known; and no other class of natural products possesses such an enormous variety of structures.

Alkaloids are highly reactive substances with biological activity in low doses and, therefore, are thought to play a defensive role in the plant against herbivores and pathogens. Many alkaloids exhibit marked pharmacological activity, some have been exploited as pharmaceuticals and some have been misused as illicit drugs. These alkaloids are not covered in this chapter.

Alkaloids are difficult to differentiate from other types of organic *N*-containing compounds. When not much was known about the biosynthesis of alkaloids, they were grouped by the plants or animals they were isolated from. When more was learned about a certain alkaloid, the grouping was changed to reflect the new knowledge, usually taking the name of a biologically important amine that standed out in the biosynthesis process. Universally adopted is the classification by their fundamental nitrogen-containing ring structure (though the structure of some alkaloids is more complex) and their major biosynthetic precursors. Three main types of alkaloids are thus recognized: true alkaloids, pseudoalkaloids, and protoalkaloids (Table 9.1). True alkaloids are *N*-heterocyclic bases derived from the same precursors, and protoalkaloids are basic aliphatic amino compounds derived from amino acids. Pseudoalkaloids are *N*-heterocyclic bases derived from other precursors (e.g. terpenoids or purines).

9.1) Many other compounds considered alkaloids are treated in other chapters (e.g. trigonelline classified as the pyridine alkaloid, dopamine classified as the phenylethylamine alkaloid, betanidin classified as the indole alkaloid, muscarine classified as the terpenoid alkaloid, etc.).

9.2) For example, they occur in some invertebrates (anthropoids: ants, millipedes, ladybugs, beetles, butterflies, crustaceans), as well as in some vertebrates (amphibians, e.g. frogs, toads, and salamanders).

Table 9.1 Survey of alkaloids occurring in foods

Structural types (basic skeleton)	Precursors	Important groups	Examples
True alkaloids			
Pyridine, piperidine, and pyrrolidine alkaloids	Arg, Lys, Orn, nicotinic acid	tobacco alkaloids	nicotine, nornicotine, anatabine, anabasine
	Lys, Phe	pepper alkaloids	piperine
Pyrrolizidine alkaloids	Arg, Ile, Leu, Orn, Val, Thr	senecio alkaloids	senecionine
Quinolizidine alkaloids	Lys	lupin alkaloids	lupanine, lupinine, sparteine
Quinoline alkaloids	Trp, mevalonic acid	cinchona alkaloids	quinine, quinidine, cinchonidine, cinchonine
Protoalkaloids			
Capsaicinoids (vanillylamides)	Leu, Phe, Val, malonyl-CoA	capsicum (paprika) alkaloids	capsaicin, nordihydrocapsaicin, homodihydro-capsaicin
Pseudoalkaloids			
Purine alkaloids	purines	coffee, tee, and cocoa alkaloids	caffeine, theobromine
Steroidal (terpenoid) glycoalkaloids	mevalonic acid	potato and tomato glycoalkaloids	solanine, tomatine

9.1.1 Pyridine, piperidine, and pyrrolidine alkaloids

9.1.1.1 Nicotine

Alkaloids in tobacco (*Nicotiana tabacum* and *N. rustica*; Solanaceae) are principally derived from pyridine, piperidine, and pyrrolidine (Figure 9.1). They have been widely recognized for their contributions to the organoleptic properties of cigarette smoke. The alkaloid content has been used traditionally as an indicator of tobacco quality. Nicotine, (*S*)-3-(1-methylpyrrolidin-2-yl)pyridine, is the major alkaloid found in tobacco and also found in small amounts in about 24 species of 12 plant families, predominantly in the plants belonging to the Solanaceae family, such as tomato (*Solanum lycopersicum*), potato (*S. tuberosum*), and eggplant (aubergine, *S. melongena*). Nicotine constitutes 0.3 to 5% of the tobacco plant by dry weight. It is always accompanied by (*S*)-3-(pyrrolidin-2-yl)pyridine (nornicotine), (*S*)-3-(1,2,5,6-tetrahydropyridin-2-yl)pyridine (anatabine), (*S*)-3-(piperidin-2-yl)pyridine (anabasine), and other structurally related minor alkaloids. Among the tobacco alkaloids, nicotine accounts for approximately 95% of total alkaloids.

Figure 9.1

Nicotine functions as an antiherbivore chemical, being a potent neurotoxin with particular specificity to insects; therefore nicotine was widely used as an insecticide in the past. In low concentrations (an average cigarette yields about 1 mg of absorbed nicotine), the substance acts as a stimulant in mammals and is one of the main factors responsible for the dependence-forming properties of tobacco smoking. On the other hand, tobacco alkaloids are of great interests due to their combination with nitrate during curing and processing, which leads to the creation of the carcinogenic tobacco-specific nitrosamines.

The biosynthesis of nicotine takes place in the roots, and the alkaloid is accumulated in the leaves. In higher plants [9.3)], putrescine used for the biosynthesis of nicotine is formed from L-arginine (Figure 9.2). Arginine is first converted to agmatine by arginine decarboxylase (EC 4.1.1.19) and agmatine is hydrolyzed by agmatine iminohydrolase (EC 3.5.3.12) into *N*-carbamoylputrescine and ammonia. Putrescine is formed by removal of the ureido group from *N*-carbamoylputrescine by the enzyme *N*-carbamoylputrescine amidohydrolase (EC 3.5.1.53). Putrescine can also be formed directly from L-ornithine by ornithine decarboxylase (EC 4.1.1.17) present in some plant species. In plants, ornithine is formed mainly from L-glutamic acid. In animals, ornithine forms in urea (ornithine) cycle, where it is produced from arginine in a reaction catalyzed by arginase (EC 3.5.3.1).

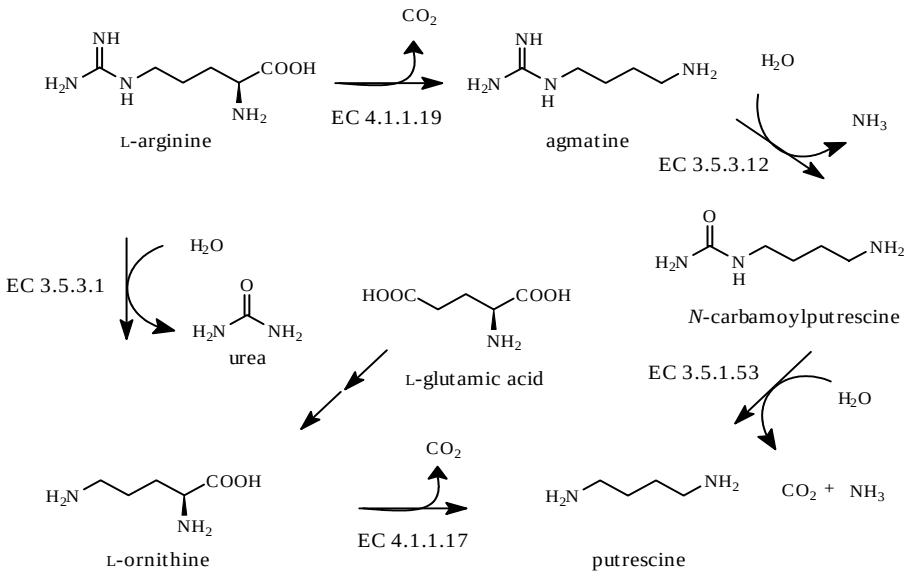

Figure 9.2

Putrescine *N*-methyltransferase (EC 2.1.1.53) diverts the metabolism of putrescine from the polyamine synthesis to alkaloid synthesis by converting putrescine to *N*-methylputrescine, which is oxidized by *N*-methylputrescine oxidase (EC 1.4.3.10) to *N*-methyl-4-aminobutanal. This aldehyde then spontaneously yields *N*-methyl-Δ^1-

9.3) In *Pseudomonas* and *Aeromonas* species and lactic acid bacteria, the biosynthesis of nicotine proceeds by the same pathway as in higher plants. In enterobacteria and mycobacteria, arginine is first converted to agmatine by arginine decarboxylase (EC 4.1.1.19) and agmatine is converted directly to putrescine by the enzyme agmatinase (EC 3.5.3.11).

pyrrolinium cation, which is a major branch-point metabolite in nicotine, as well as in tropane alkaloids biosynthesis. The *N*-methyl-Δ^1-pyrrolinium cation is attached to the pyridine ring, derived from an intermediate in NAD^+ biosynthesis, most likely nicotinic acid, displacing the carboxyl during the sequence (1,4-reduction of pyridine to dihydropyridine and decarboxylation to 1,2-dihydropyridine). The reaction is catalyzed by a not-so-well characterized enzyme nicotine synthase. Nicotine may be demethylated to nornicotine by a cytochrome P450 monooxygenase (Figure 9.3). This enzyme catalyzes hydroxylation of *N*-methyl group to *N*-hydroxymethyl group, which spontaneously decomposes under the formation of nornicotine and formaldehyde. Some other alkaloids form during the tobacco curing process as nicotine catabolic products.

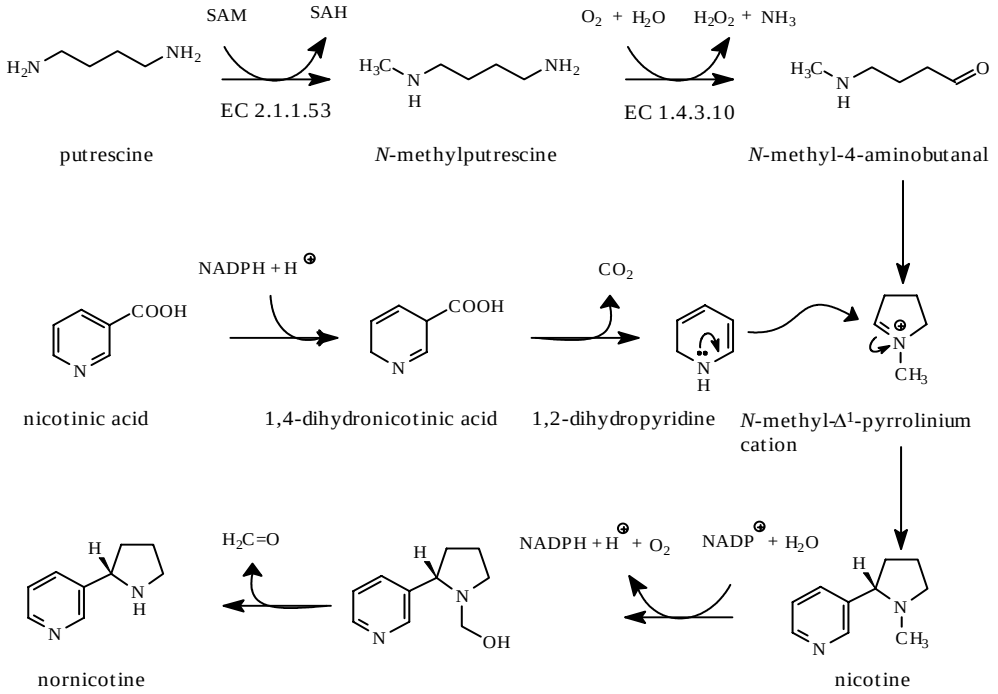

Figure 9.3

Anabasine is produced from nicotinic acid via 1,2-dihydropyridine and from lysine via Δ^1-piperidinium cation in an essentially analogous reaction sequence involving an aldol-type reaction between enamine (1,2-dihydropyridine) and iminium ion (Δ^1-piperidinium cation). Anatabine appears to be only derived by combination of two nicotinic acid units via 1,2-dihydropyridine transformed to 1,4-dihydropyridine (enamine-imine tautomerism). An aldol-type reaction between enamine (1,2-dihydropyridine) and the protonized iminium ion (protonized 1,4-dihydropyridine) yields anatabine (Figure 9.4).

9.1.2 Piperine

Piperine, i.e. (*E*,*E*)-1-piperoylpiperidine or (*E*,*E*)-1-[5-(1,3-benzodioxol-5-yl)-1-oxopenta-2,4-dienyl]piperidine, is found in plants belonging to the Piperaceae family,

such as *Piper nigrum*, commonly known as black pepper, and *Piper longum*, commonly known as long pepper, that are widely used condiments. Piperine, predominantly located in the outer perisperm of the green fruit, is the major pungent substance of the fruits of pepper [9.4]. The minor pungent alkaloids are piperettine, piperyline, piperanine, and piperolein A and B (Figure 9.5).

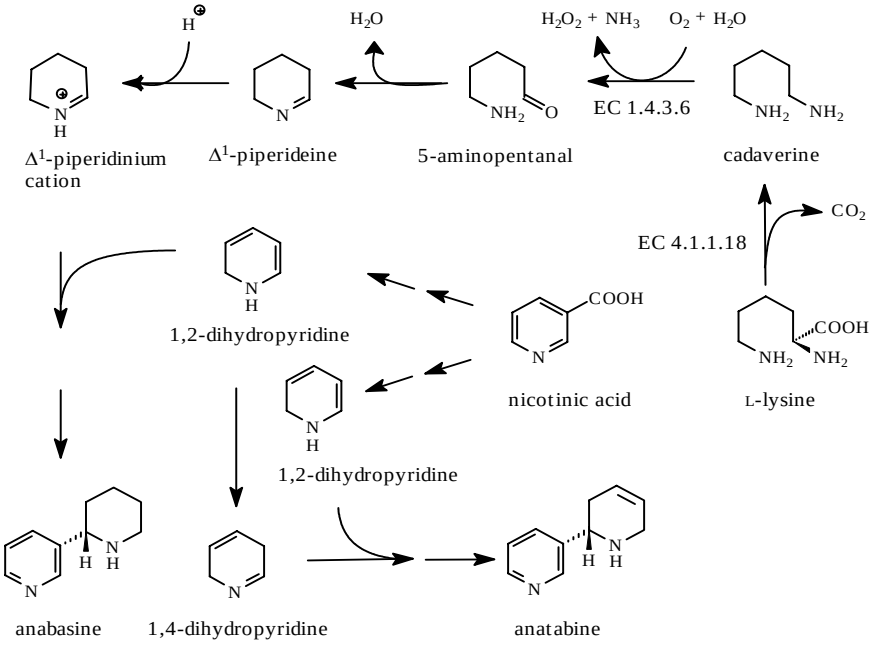

Figure 9.4

Generally, the piperine content of black or white pepper lies within the range of 30 to 80 g/kg, whereas the content of the minor alkaloids piperyline and piperettine have been estimated as 2-3 and 2-16 g/kg, respectively. The piperine content in oleoresins is commonly 35-40%, which replaces the ground spices at the ratio of about 1:25.

The piperic acid portion of piperine is derived from a ferulic acid-CoA precursor. The methylene bridge formation is mediated by cytochrome P450 protein (EC 1.14.21.-) and the chain extension is achieved using malonic acid. (Figure 9.6). The formed product is reduced and dehydrated to piperic acid CoA ester (piperoyl-CoA), which combines with piperidine to yield the tertiary amide structure of piperine. The piperidine ring is incorpo-

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[9.4] The term black pepper is used both for the plant *Piper nigrum* and the spice, the fruit of the plant. Black pepper is obtained from the unripe, but mature green berries on sun drying, while fully ripe dried fruits devoid of pericarp form the commercial white pepper. The sharp flavor of freshly ground pepper is mainly attributed to the major component piperine. Pepper products lose flavor through evaporation of the flavor-active volatiles and when exposed to light, which leads to light-induced isomerization of piperine into (*Z*,*Z*)-1-piperoylpiperidine (chavicine), nearly tasteless (*E*,*Z*)-1-piperoylpiperidine (isochavicine), and (*Z*,*E*)-1-piperoylpiperidine (isopiperine) (Figure 9.5). Piperine has also been found to inhibit some enzymes important for the metabolism and transport of xenobiotics and enzymes important in drug metabolism.

Piperidine is naturally found also in fire ant (*Solenopsis invicta*) venom, and is the cause of the burning sensation associated with the bite of these insects.

rated via piperidine itself, the reduction product of Δ^1-piperideine (Figure 9.3), which spontaneously forms from 5-aminopentanal (via a Schiff base formation), the product of oxidative deamination of diamine cadaverine (diamine oxidase, EC 1.4.3.6). Cadaverine forms from lysine under the action of lysine decarboxylase (EC 4.1.1.18).

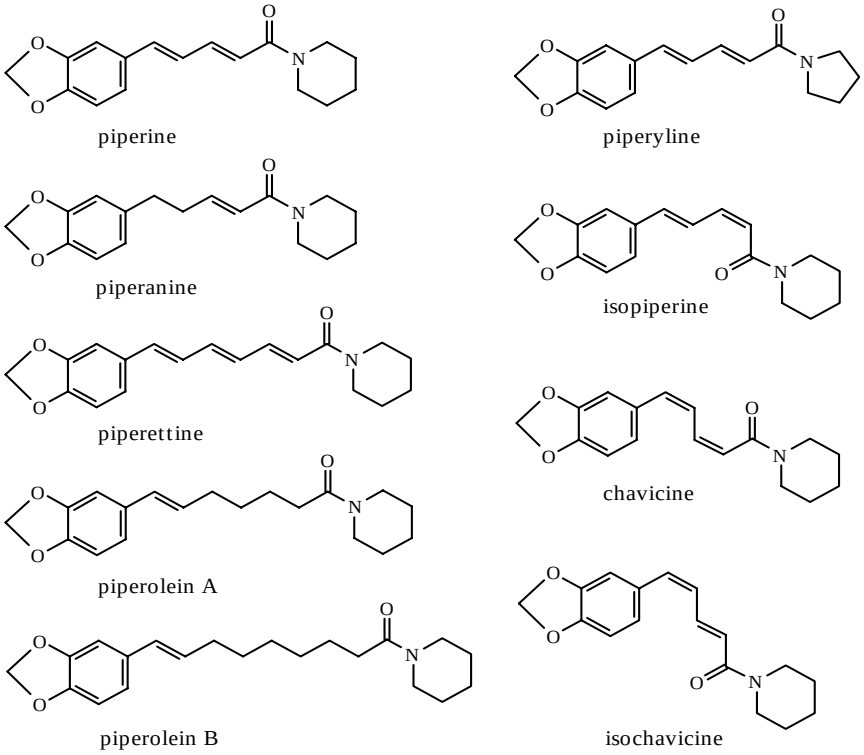

Figure 9.5

9.1.3 Pyrrolizidine alkaloids

Over 250 pyrrolizidine alkaloids have been identified to date, about half of them deemed toxic. They are principally found as complex mixtures of 10 or more alkaloids in about 6000 plants belonging to more then 10 families. The most important plants belong to the Fabaceae, Boraginaceae, and Asteraceae families. In these families, their existence is predominantly restricted to several subtribes and genera, where the dry matter content usually ranges from 0.1 to 1%. In Europe, the representatives of the Fabaceae family are insignificant and only medical plants, belonging to the families Boraginaceae and Asteraceae, are important.

The proposed biosynthetic pathway of the two predominant potato glycoalkaloids from the aglycone solanidine is schematically outlined in Figure 9.26. The individual glycosylation steps are catalyzed by specific glycosyltransferases (EC 2.4.1.-). For example, the formation of α-solanine via γ-solanine and β_2-solanine is catalyzed by UDP-galactose:solanidine galactosyltransferase, UDP-glucose:solanidine glucosyltransferase, and UDP-rhamnose:β-steroidal glycoalkaloid rhamnosyltransferase, respectively.

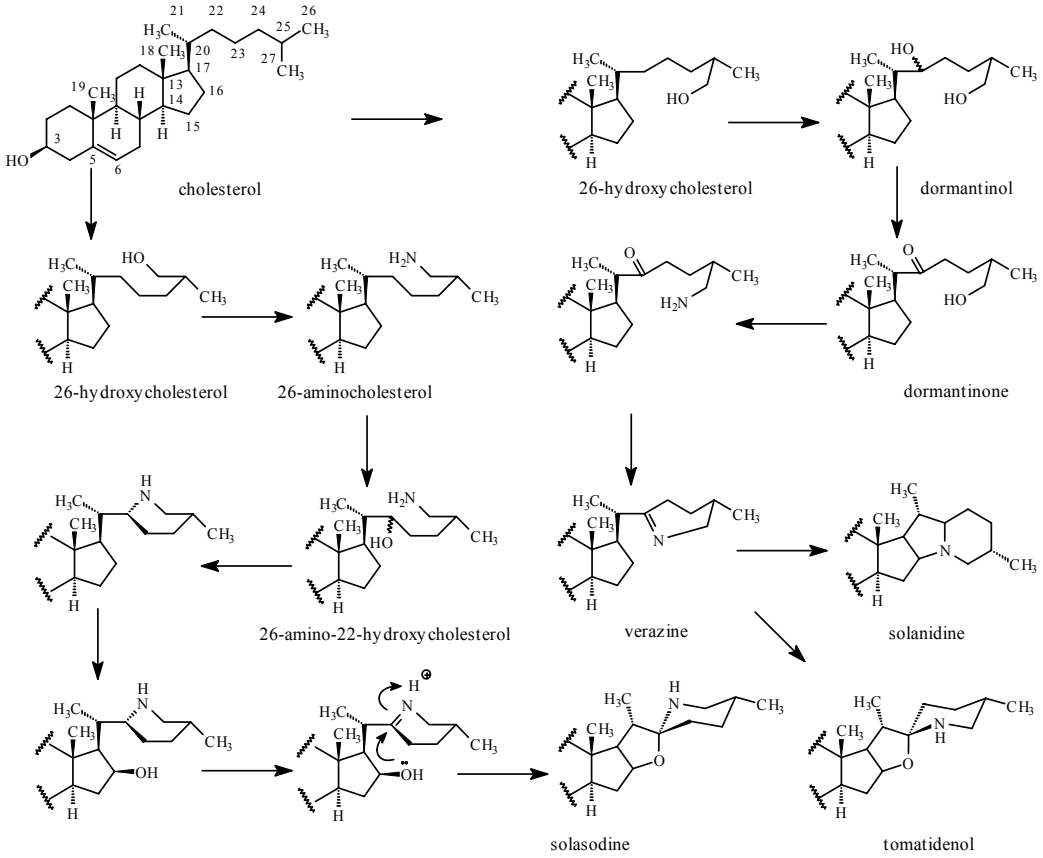

Figure 9.25

9.2 Cyanogens

The ability of living organisms to produce hydrocyanic acid (HCN) by decomposition of cyanogenic substances is a part of their chemical defense systems termed cyanogenesis. The cyanogenic substances of plants are usually cyanogenic glycosides (β-glycosides of 2-hydroxynitriles, also known as cyanohydrin glycosides), pseudocyanogenic glycosides (methylazoxymethanol glycosides or azoxyglycosides), and cyanolipids. Furthermore, many plants produce cyanide as a by-product in the biosynthesis of the plant hormone ethylene and during early seed germination (see Chapter 1.7.6.4).

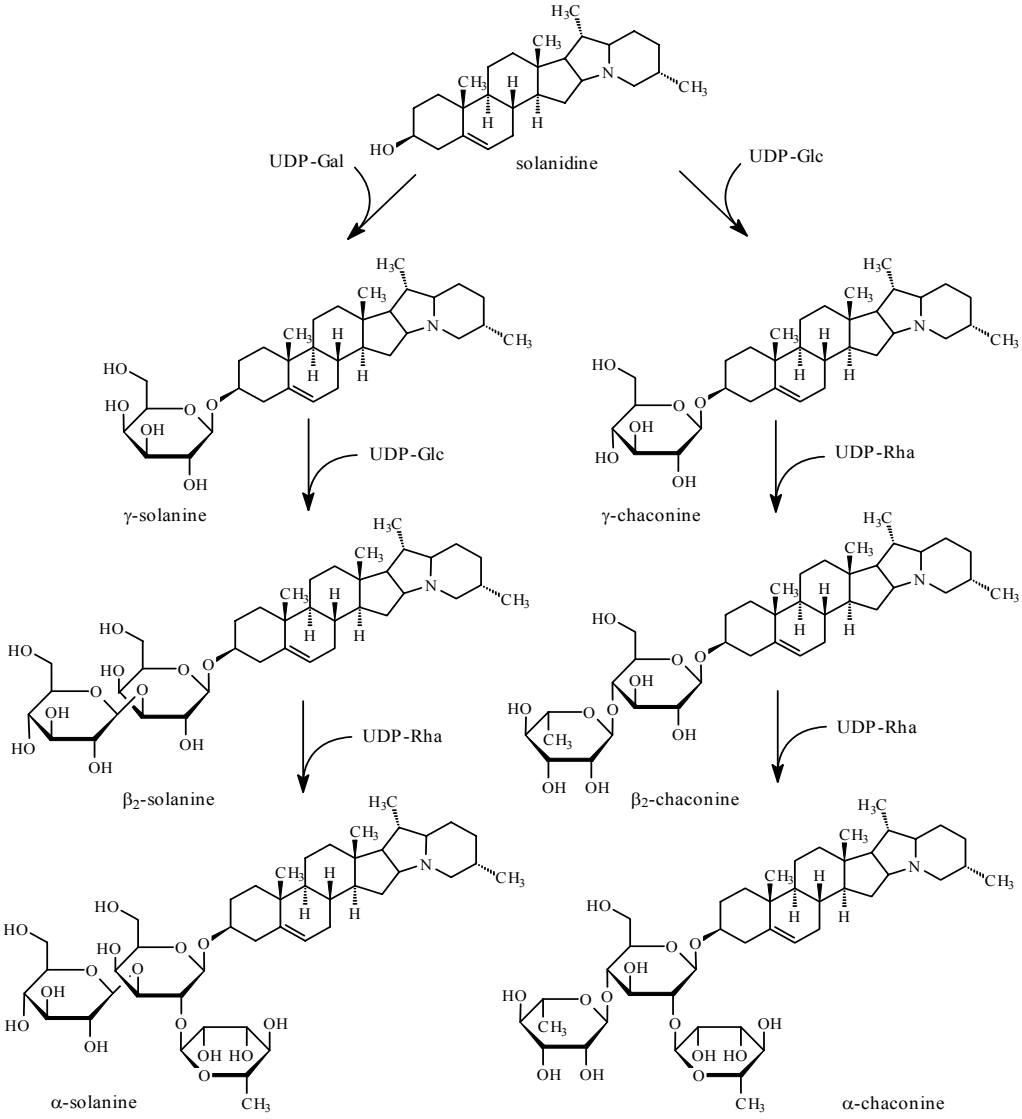

Figure 9.26

9.2.1 Cyanogenic glycosides

Cyanogenic glycosides are β-glycosides of α-hydroxynitriles (nitriles of 2-hydroxy-carboxylic acids). A total of about 25 cyanogenic glycosides involved in seed germination and plant defense against herbivores has been reported in at least 2500 higher plant species belonging to the Fabaceae, Poaceae, Araceae, Asteraceae, Euphorbiaceae, and Passifloraceae families. These plants include several economically important plants. Cassava, also known as tapioca or manioc (*Manihot esculenta*, *M. carthaginensis*; Euphorbiaceae), and sorghum (*Sorghum album*, *S. bicolor*; Poaceae) are especially important staple starchy foods containing cyanogenic glycosides.

Cyanogenic glycosides have the general formula shown in Figure 9.27. The structural variations are of three types, involving the nature of the sugar moiety, the nature of the groups R^1 and R^2 (or R^3), and the chirality of the carbinol carbon atom. Generally, a particular cyanogenic glycoside occurs only in one or two plant families, and conversely, only one or two cyanogenic glycosides are present in a given plant.

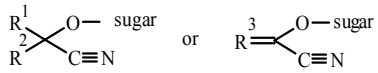

Figure 9.27

The major cyanogenic glycosides found in the edible parts of plants used for human or animal consumption are shown in Figure 9.28 and Table 9.3, respectively.

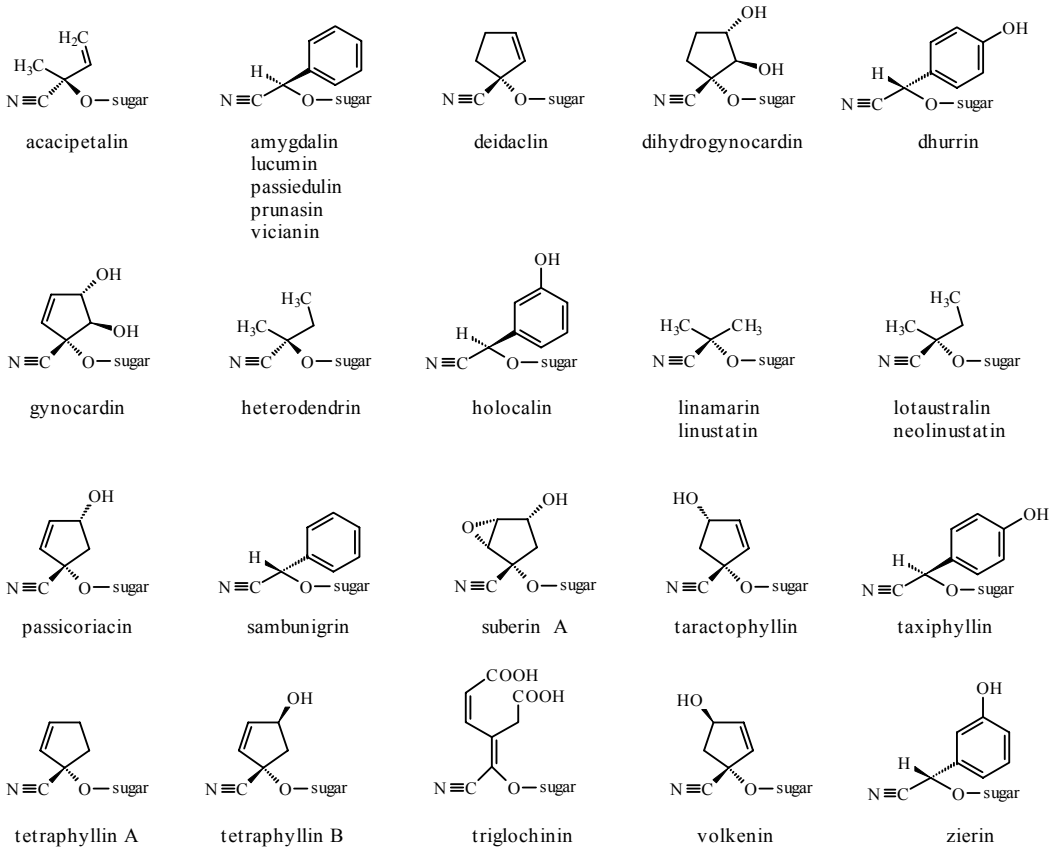

Figure 9.28 (see Table 9.3)

Linamarin and lotaustralin occur in cassava and lima (butter) beans (*Phaseolus lunatus*; Fabaceae), dhurrin comes from sorghum grains, amygdalin from bitter almonds (*Prunus amygdalus*; Rosaceae), prunasin from many fruit stones (e.g. stones of cherries, plums, and peaches; species *Prunus* and *Persica*; Rosaceae), passiedulin and its analogues from passion fruits, known as granadilla or maracuja (*Passiflora edulis*; Passifloraceae),

sambunigrin, holocalin, and zierin from unripe elderberries (*Sambucus nigra*; Adoxaceae), linustatin and neolinustatin from flax seeds (*Linum usitatissimum*; Linaceae), and taxiphyllin from bamboo shoots (*Bambusa vulgaris*; Poaceae).

Table 9.3 Structure and occurrence of some cyanogenic glycosides (see Figure 9.28)

Trivial name	Sugar [9.15)]	Isomer	Occurrence
Acacipetalin (proacacipetalin)	D-glucose	*S*	*Acacia*
Amygdalin	gentiobiose	*R*	*Prunus*
Deidaclin	D-glucose	*R*	*Deidamia*, *Passiflora*
Dhurrin	D-glucose	*S*	*Sorghum*
Dihydrogynocardin	D-glucose	1*S*,4*S*,5*R*	*Passiflora*
Gynocardin	D-glucose	1*S*,4*S*,5*R*	*Gynocardia*, *Pangium, Taractogenes, Rawsonia*
Heterodendrin (dihydroacacipetalin)	D-glucose	*S*	*Acacia*
Holocalin	D-glucose	*R*	*Sambucus*
Linamarin (phaseolunatin)	D-glucose	-	*Trifolium*, *Lotus*, *Phaseolus*, *Manihot*
Linustatin	gentiobiose	-	*Linum*
Lotaustralin (methyllinamarin)	D-glucose	*R*	*Lotus*, *Manihot*
Lucumin	primeverose	*R*	*Lucuma*
Neolinustatin	gentiobiose	*R*	*Linum*
Passicoriacin	D-glucose	*S*	*Passiflora*
Passiedulin	D-allose	*R*	*Passiflora*
Prunasin	D-glucose	*R*	*Prunus*, *Persica*, *Malus*, *Pyrus*, *Sorbus*, *Cydonia, Carica*
Sambunigrin	D-glucose	*S*	*Prunus*, *Sambucus*
Taractophyllin	D-glucose	1*R*,4*S*	*Passiflora*
Suberin A	D-glucose	1*R*,*2R*,*3R*,4*R*	*Passiflora*
Taxiphyllin	D-glucose	*R*	*Taxus*, *Triglochin*, *Bambusa*
Tetraphyllin A	D-glucose	*S*	*Tetrapathaea*, *Passiflora*
Tetraphyllin B	D-glucose	1*S*,4*S*	*Tetrapathaea*, *Passiflora*, *Mathurina, Carica*
Triglochinin	D-glucose	-	*Triglochin*, *Glyceria*, *Melica*
Vicianin	vicianose	*R*	*Vicia*
Volkenin	D-glucose	1*R*,4*R*	*Passiflora*, *Mathurina*
Zierin	D-glucose	*S*	*Sambucus*

The potential toxicity of a cyanogenic plant depends on several factors, primarily on the amount of HCN released. For example, concentration of HCN in cassava (whole tubers) ranges from 100 to 550 mg/kg. Immature bamboo shoot tips can release 8000 mg HCN/kg, immature bamboo shoots 3000 mg/kg, lima beans 100-4000 mg/kg, flex seeds 200-380 mg/kg, and stones of apricots and cherries 3200-3520 mg/kg. Overdose eating cyanogenic glycoside-containing plants by animals and humans can be lethal.

[9.15)] Gentiobiose is β-D-Glc*p*-(1→6)-D-Glc*p*, primeverose β-D-Xyl*p*-(1→6)-D-Glc*p* , and vicianose α-L-Ara*p*-(1→6)-D-Glc*p*.

Cyanogenic glycosides are mostly derived from the five hydrophobic amino acids valine (linamarin), isoleucine (lotaustralin), leucine (heterodendrin), phenylalanine (e.g. amygdalin and prunasin), and tyrosine (dhurrin, taxiphyllin, and triglochinin). Cyclopentanoid cyanogens (e.g. gynocardin) presumably originate from cyclopentenylglycine (see Chapter 1.7.3.3). The biosynthesis of cyanogenic glycosides (Figure 9.29) from amino acids proceeds by a sequence of two *N*-hydroxylations (cytochrome P450 amino acid *N*-monooxygenase, EC 1.14.13.-). The formed *N*,*N*-dihydroxyamino acid decarboxylates to (*E*)-aldoxime (aldehyde oxime). The (*Z*)-aldoxime produced by isomerization of (*E*)-aldoxime is then dehydrated to a nitrile, which is subsequently oxidized to a α-hydroxynitrile (aldehyde cyanohydrin) by another cytochrome P450 monooxygenase (EC 1.14.13.-). The stereoselectivity of the nitrile hydroxylation step varies depending on the plant, so that epimeric cyanohydrins are found in nature, though not in the same plant. The last step of adding a glucosyl moiety to the cyanohydrin is catalyzed by an UDP-glucose transferase (EC 2.4.1.-).

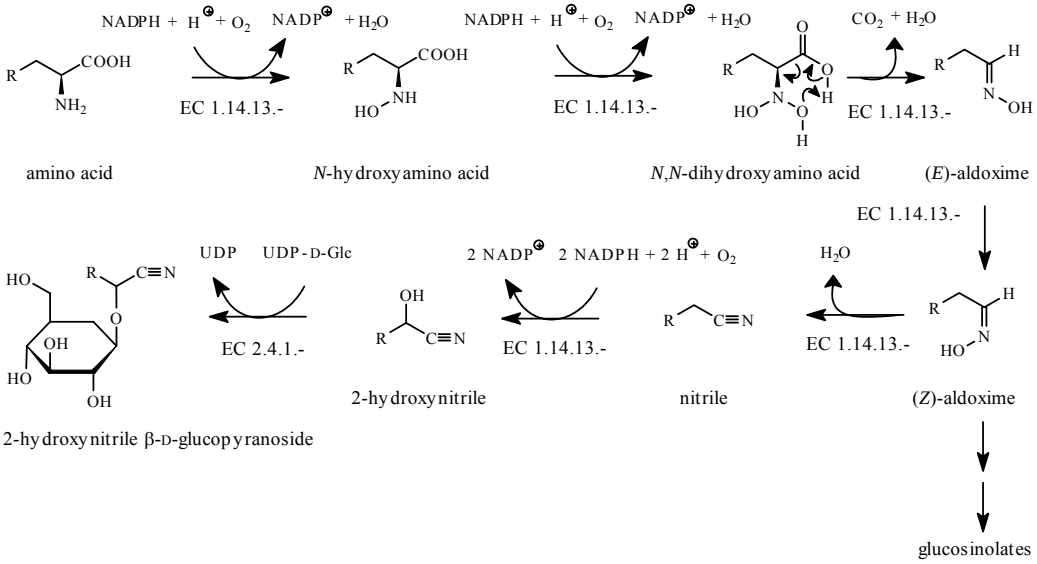

Figure 9.29

The putative biosynthetic pathway for dhurrin in sorghum (*Sorghum bicolor*) exemplifies the individual reaction sequences (Figure 9.30). The enzymes involved in the biosynthesis of dhurrin in sorghum are tyrosine *N*-monooxygenase (EC 1.14.13.81), 4-hydroxyphenylacetaldehyde oxime monooxygenase (EC 1.14.13.68), and cyanohydrin β-glucosyltransferase (EC 2.4.1.85) [9.16]. 4-Hydroxyphenylacetonitrile produced by the second cytochrome P450 monooxygenase is thought to constitute the branch point between taxiphyllin and triglochinin in seaside arrow grass (*Triglochin maritima*).

The conversion of a cyanogenic glycoside to hydrogen cyanide involves two steps (Figure 9.30). The first step is hydrolysis (deglycosylation) of cyanogenic glycosides by

[9.16] Linamarin synthase (EC 2.4.1.63) catalyzes the formation of linamarin from 2-hydroxy-2-methylpropanenitrile and UDP-glucose.

glycosidases (EC 3.2.1.-). β-Glucosidase linamarase (EC 3.2.1.21) acts on linamarin, which is transformed to glucose and acetone cyanohydrin. Amygdalin β-glucosidase (amygdalase or amygdalinase, EC 3.2.1.117) is a highly specific enzyme that acts on amygdalin, which is only hydrolyzed to glucose and prunasin. Prunasin β-glucosidase (EC 3.2.1.118) then provides the hydrolysis of prunasin yielding glucose and mandelonitrile. Vicianin β-glucosidase (EC 3.2.1.119) transforms vicianin to vicianose and mandelonitrile.

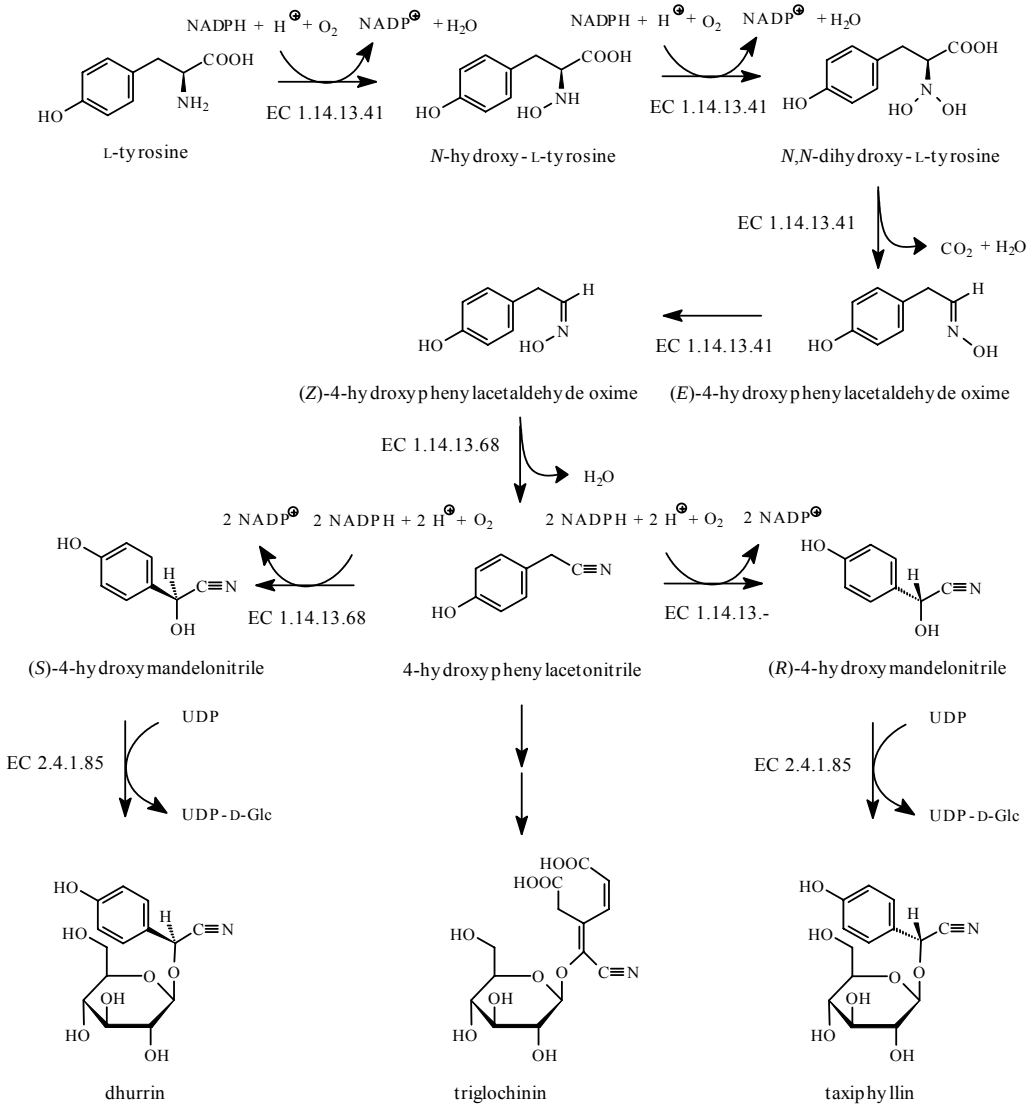

Figure 9.30

The second step is the cleavage of the released cyanohydrin by an aldehyde-lyase (EC 4.1.2.-) into hydrogen cyanide and a carbonyl compound. Such enzymes are acetone

cyanohydrin lyase (4.1.2.37), that acts on acetone cyanohydrin, and mandelonitrile lyase, also known as (*R*)-oxynitrilase (EC 4.1.2.10), that is active on a number of aromatic and aliphatic cyanohydrins. Hydroxymandelonitrile lyase (EC 4.1.2.11) catalyzes the decomposition of (*S*)-4-hydroxymandelonitrile. Decomposition of cyanohydrins may also proceed spontaneously in acid media and at elevated temperature (Figure 9.31).

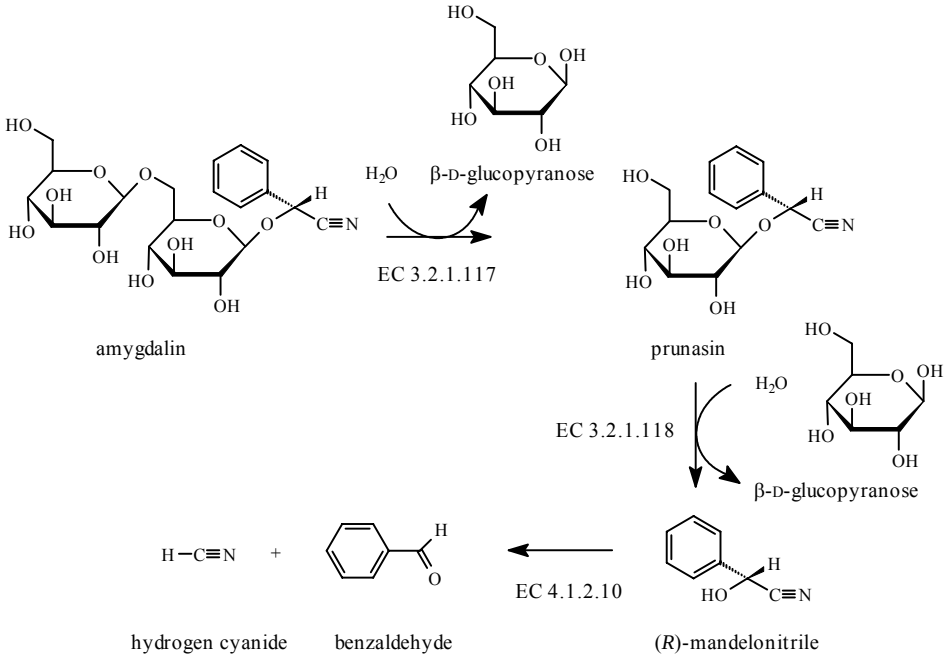

Figure 9.31

Cyanide produced in plants by decomposition of cyanogenic glycosides is detoxified to β-cyanoalanine and accumulates as asparagine (see Chapter 1.7.6.4).

9.2.2 Pseudocyanogenic glycosides

Toxic pseudocyanogenic glycosides (azoxyglycosides) accumulate in the plants of the cycad family (Cycadaceae). The way in which people can be exposed to cycad azoxyglycosides is through the consumption of starchy foods prepared from cycad seeds (the sago cycad) that contain up to 0.22 mg/kg of the azoxyglycosides depending on the plant species. The main cycad azoxyglycoside is cycasin (Figure 9.32) that forms from methylazoxymethanol under the catalysis of cycasin synthase (EC 2.4.1.171).

Cycasin is mutagenic and carcinogenic only when deglucosylated to release its principal metabolite methylazoxymethanol. Methylazoxymethanol is also the aglycone of other cycad azoxyglycosides. It is responsible for their toxic properties. Consumption of cycad seeds has been implicated in the outbreak of Parkinson's disease-like neurological disorder in various locations in the Pacific, such as Guam. In mildly alkaline media, cycasin decomposes to cyanide, glucose, formic acid, and nitrogen. In acid media, the decomposition products are glucose, formaldehyde, methanol, and nitrogen.