Biosynthesis of Food Constituents: Lipids. 1. Fatty Acids and Derived Compounds – a Review

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Abstract

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This review article gives a survey of the principal biosynthetic pathways that lead to the most important common fatty acids and their derivatives occurring in foods and feeds. Fatty acids are further subdivided to saturated fatty acids and unsaturated fatty acids. This review is focused on the less common fatty acids including geometrical and positional isomers of unsaturated fatty acids, acetylenic fatty acids, branched-chain fatty acids, alicyclic fatty acids, epoxy fatty acids, hydroxy fatty acids, and oxo fatty acids. A survey is further given on the biosynthesis of the aliphatic very-long-chain components (alkanes, primary and secondary alcohols, aldehydes, ketones, and esters) of plant cuticular wax derived from saturated fatty acids. Subdivision of the topics is predominantly via biosynthesis. There is extensive use of reaction schemes, sequences, and mechanisms with enzymes involved and detailed explanations using chemical principles and mechanisms.

Keywords: biosynthesis; fatty acids; Claisen condensation; saturated fatty acids; medium-chain fatty acids; long-chain fatty acids; very-long-chain fatty acids; ultra-long-chain fatty acids; saturated fatty acids; *cis*/*trans*-fatty acids; acetylenic fatty acids; branched-chain fatty acids; alicyclic fatty acids; epoxy fatty acids; hydroxy fatty acids; oxo fatty acids; plant wax components

Polyketides, metabolites built primarily from combinations of acetic acid (acetate) units, constitute a large group of natural substances grouped together on purely biosynthetic grounds. Their diverse structure can be explained as being derived from poly-β-keto chains, $-[CH_2\text{-}C(=O)]_n-$, formed by the coupling of C2 acetic acid units via condensation reactions. The pathways to fatty acids and aromatic polyketides branch early. For fatty acids, the carbonyl groups of the growing chain are reduced before the attachment of the next C2 group, whereas for the aromatic polyketides, the poly-β-keto chain stabilises by cyclisation reactions and partial reduction (Dewick 2002).

Fatty acids become the building blocks of a large group of natural substances called lipids (Akoh & Min 1998; Velíšek & Cejpek 2006b). In foods, they are mainly found as triacylglycerols (fats and oils) and glycerophospholipids. To some extent, fatty acids are also found in the form of partial acylglycerols (diacylglycerols and monoacylglycerols), they occur as esters with other hydroxy compounds (e.g. sterols), amides (sphingolipids), and free compounds. Certain transformation products

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of fatty acids occur in natural waxes as complex mixtures. Some fatty acids are the precursors of biologically active compounds in animals (eicosanoids) and plants (oxylipins or octadecanoids), and become the precursors of many flavour-active food constituents.

1 FATTY ACIDS

Fatty acid biosynthesis is characteristic of all living organisms[1]. Fatty acids are built primarily from acetyl coenzyme A[2] (acetyl-CoA, Figure 1, R = CH_3CO) in the so-called acetate (acetic acid) pathway with chain assembly achieved by a Claisen condensation. In animal cells and yeasts, multienzyme complexes localised in cytosol, referred to as type I fatty acid synthase (FAS I), carry out the bulk of *de novo* fatty acid synthesis. In animals, it occurs primarily in liver, adipose tissue, central nervous system, and lactating mammary gland. FAS I contains seven distinct catalytic centers and is arranged around a central acyl carrier protein (ACP, Figure 2) (Velíšek & Davídek 2000). In prokaryotes and plants, distinct soluble enzymes localised in mitochondria and plastids, referred to as type II fatty acid synthase (FAS II), carry out the reactions (Gurr *et al*. 2002; Vance & Vance 2002).

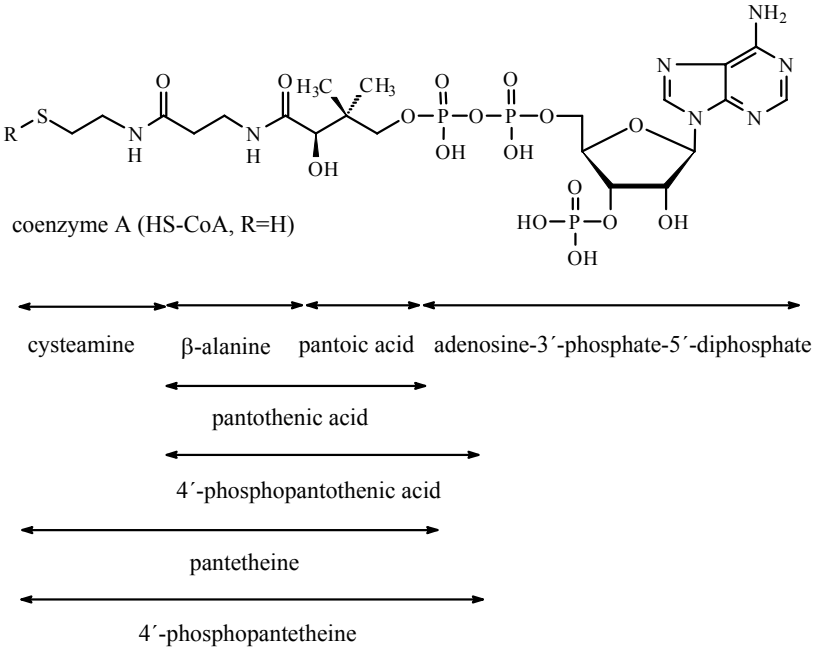

Figure 1

[1]The catabolic pathways of lipids are at least as important as the anabolic ones since many lipids are storage compounds (i.e. triacylglycerols) serving as energy reservoirs and are activated upon need (Velíšek & Cejpek 2006b). In general, fatty acids can be catabolised by α-, β-, and ω-oxidation. The fatty acid (in the form of acyl-CoA thioesters) β-oxidation proceeds in mitochondria and in peroxisomes. It is the preferred degradation that leads to fatty acid by two carbons shorter in length. Mitochondrial oxidation is predominantly responsible for the degradation of long-chain fatty acids and very-long-chain fatty acids are oxidised predominantly, if not exclusively, in peroxisomes. In seeds and leaves, the α-oxidation (probably localised in peroxisomes) generates at first α-hydroxy acid and in a further step, the terminal carboxyl group is released as formic acid which is converted to carbon dioxide. The product is a fatty acid that is reduced by one C_1 unit. The ω-oxidation of fatty acids (in the endoplasmic reticulum) at C_ω methyl group yields at first ω-hydroxy acid, which is further oxidised to α,ω-dicarboxylic acid and this, by β-oxidation, to lower fatty acid homologues.

[2]Acetyl-CoA (connecting glycolysis and the citric acid cycle) is formed from HS-CoA (Figure 1, R=H) and the glycolytic product pyruvic acid. The biosynthesis of acetyl-CoA from pyruvic acid is a five-step reaction called oxidative decarboxylation, which is catalysed by the multifunctional enzyme pyruvate dehydrogenase ($NADP^+$) (EC 1.2.1.51). Acetyl-CoA is also produced by the β-oxidation of fatty acids, reversing the process of their biosynthesis.

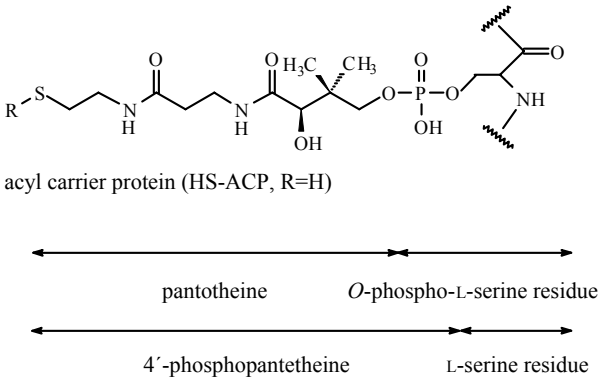

Figure 2

1.1 Saturated fatty acids

The biosynthesis of saturated fatty acids *de novo* involves the initial irreversible step, carboxylation of acetyl-CoA to malonyl-CoA, catalysed by the biotinyl-protein acetyl-CoA carboxylase (EC 6.4.1.2). The reaction involves ATP, carbon dioxide (as bicarbonate anion HCO_3^-), and biotin as the carrier of carbon dioxide (Figure 3). Biotin is a prosthetic group attached via the ε-amino group to a lysine residue. The bicarbonate ion is first activated as N^1-carboxybiotin, at the expense of ATP, before the transfer to acetyl-CoA to form malonyl-CoA (Smith 1994). Acetyl-CoA and malonyl-CoA (and also butyryl-CoA in the case of ruminants) then enter the first reaction of the sequence catalysed by FAS I.

FAS I catalyses the seven following reactions (Figure 4). In the first reaction catalysed by ACP *S*-acetyltransferase (EC 2.3.1.38), acetyl-CoA is attached to the thiol group of the L-cysteine residue in ACP under splitting off of HS-CoA. Mechanistically, this is a two-step process, in which the group is first transferred to the ACP and then to the cysteine-SH group of the enzyme domain. In the second reaction, malonyl-CoA is added to the ACP-sulfhydryl group by ACP *S*-malonyltransferase (EC 2.3.1.39) under the elimination of HS-CoA. In the third reaction catalysed by 3-oxoacyl-ACP synthase (*β*-oxoacyl-ACP synthase, EC 2.3.1.41), the Claisen reaction follows giving *β*-oxoacyl-ACP, i.e. acetoacetyl-ACP. In the fourth reaction, the oxo group is reduced stereospecifically by 3-oxoacyl-ACP reductase (EC 1.1.1.100) to the corresponding *β*-hydroxy ester, i.e. (*R*)-3-hydroxybutyryl-ACP. The elimination of water in the fifth reaction is catalysed by 3-hydroxyacyl-ACP dehydratase (EC 4.2.1.61) and yields *trans*-*α*,*β*-unsaturated ester, i.e. (*E*)-but-2-en-1-yl-ACP. In the sixth reaction, the reduction of the double bond by the NADPH-dependent enoyl-ACP reductase (EC 1.3.1.10) generates fatty acyl-ACP, i.e. butyryl-ACP. This can feed back into the system, condensing again with malonyl-ACP, and going through successive reduction, dehydration, and reduction steps, gradually increasing the chain length by two carbons for each cycle, until the required chain length is obtained. Thus, the combination of one acetic acid starter unit with seven

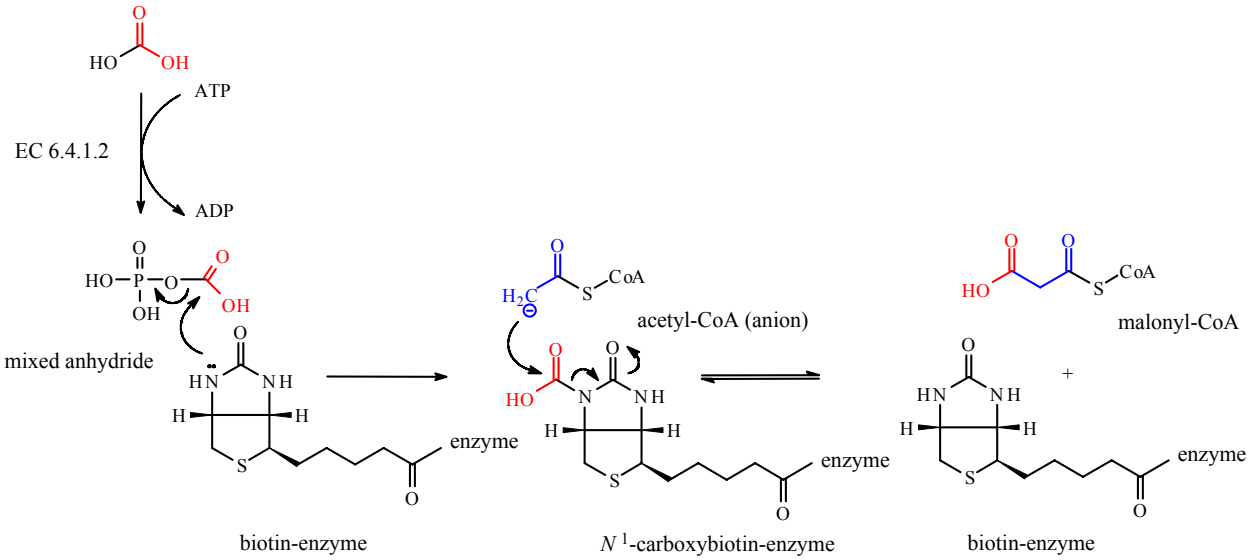

Figure 3

malonic acids (the elongation cycle repeats for a total of seven cycles) would give the typical end product of the mammalian FAS I, C16 saturated fatty acid, i.e. hexadecanoic (palmitic) acid[3]. Its synthesis requires a series of 37 sequential reactions from acetyl-CoA and malonyl-CoA. The two carbons at the methyl end of palmitic acid chain are provided by acetic acid, while the remainder are derived from malonic acid which itself is produced by carboxylation of acetic acid.

At that point, the fatty acyl chain termination and the release of the free fatty acid or the conversion to the corresponding CoA ester is achieved in the seventh reaction, i.e. by hydrolysis of the thioester by the intrinsic acyl-ACP thioesterase (EC 3.1.2.14), a component of the FAS I complex. The free fatty acid released can be also transformed to acyl-CoA thioester by acyl-CoA synthetase (EC 6.2.1.3). Acyl-CoA thioesters are then used, e.g., for the elongation, desaturation, biosynthesis of triacylglycerols in adipocytes and phospholipids in biomembranes (Hunt & Alexon 2002).

FAS I products other than the even number straight-chain fatty acids (the linear combination

[3]In mammals, two identical polypeptides (termed α-multifunctional domains), containing all the catalytic centres and ACP, form the fatty-acid synthase I (FAS I) complex (EC 2.3.1.85) (KEGG).

The yeast (*Saccharomyces cerevisiae*) FAS I (EC 2.3.1.86) is a multi-functional protein having two non-identical subunits (α and β). ACP is associated with the α-subunit that additionally sustains 3-oxoacyl-ACP synthase (EC 2.3.1.41) and 3-oxoacyl-ACP reductase (EC 1.1.1.100) activities. The β-subunit is required for ACP *S*-acetyltransferase (EC 2.3.1.38) and ACP *S*-malonyltransferase (EC 2.3.1.39), 3-hydroxyacyl-ACP dehydratase (EC 4.2.1.61), NADPH-dependent enoyl-ACP reductase (EC 1.3.1.10, it also requires FMN in addition to NADPH as a cofactor), and NADH-dependent enoyl-ACP reductase (EC 1.3.1.9). Since FAS I in yeasts is deficient of the acyl-ACP thioesterase (EC 3.1.2.14) associated with animal FAS I, the principal product of biosynthesis is palmitoyl-CoA instead of free palmitic acid. The termination of the synthesis by HS-CoA formation instead of hydrolysis results in a slower release od free fatty acids from the yeast complex compared to animal FAS I. Some fungi have at least two different FAS I multienzyme complexes, in addition to FAS I, another very similar complex being specifically required in polyketide production (KEGG).

Bacteria and plants synthesise fatty acids using a series of discrete FAS II enzymes (KEGG; Kunst & Samuels 2003). There are two soluble FAS II systems in plants, one in plastids (chloroplasts) and another in mitochondria. The FAS II of the plastids represents the major pathway of plants *de novo* fatty acid synthesis, corresponding to the mammalian FAS I and bacterial FAS II systems. Three different types of FAS complexes are required for the synthesis of fatty acids in the plastid. They differ in their condensing enzymes (3-oxoacyl-ACP synthetase, 3-ketoacyl-ACP synthetase, KAS, EC 2.3.1.41), which have strict acyl chain length specificities. The first condensation to form a four-carbon product is carried out by KAS III (EC 2.3.1.41). Intermediate (C4-C16) chain lengths are produced by KAS I (EC 2.3.1.41). Palmitoyl-ACP is then either hydrolysed to free palmitic acid (acyl-ACP thioesterase, EC 3.1.2.14) or elongated by 2 carbon atoms to C18 fatty acid (octadecanoic or stearic acid, the combination of one acetic acid starter unit with eight malonic acids) by KAS II (EC 2.3.1.41) (Shimakata & Stumpf 1982). Later, the stearoyl-ACP can follow 2 pathways. In most cases, it is desaturated to oleoyl-ACP in a reaction catalysed by the intraplastidal enzyme stearoyl-ACP desaturase (EC 1.12.99.6) and oleoyl-ACP is hydrolysed (acyl-ACP thioesterase, EC 3.1.2.14) to ACP and free oleic acid. Occassionally, stearoyl-ACP is hydrolysed (acyl-ACP thioesterase, EC 3.1.2.14) to ACP and free stearic acid. The main products from the intraplastidal *de novo* fatty acid biosynthesis in plants are oleic acid, to a lesser extent palmitic acid, and to a much lesser extent stearic acid. The free fatty acids are then exported outside the plastid and used (in their respective CoA thioester form) for the biosynthesis of storage triacylglycerols. The specificities of the acyl-ACP thioesterases thus determine to a large extent the chain lengt and unsaturation of most plant fatty acids, both in storage triacylglycerols and in membrane glycerolipids biosynthesised within the cytoplasm. Acyl-ACP thioesterases have been classified into 2 types according to their specificity: FatA and FatB. Thioesterases of the FatA type hydrolyse oleoyl-ACP preferentially, while those of the FatB type show more specificity towards saturated acyl-ACP thioesters (Jones *et al*. 1995; Hardwood 1996). In contrast, the two reductases (EC 1.1.1.100, 3-oxoacyl-ACP reductase; EC 1.3.1.9, NADH-dependent enoyl-ACP reductase) and the dehydratase (EC 4.2.1.-, 3-hydroxyacyl-ACP dehydratase) apparently have no particular acyl chain length specificity and are shared by all three plastidal elongation complexes.

Mitochondrial fatty acid synthesis in plants and bacteria is principally considered to act as a source of C8 (octanoic) acid used as a precursor in the synthesis of a universal cofactor lipoic acid.

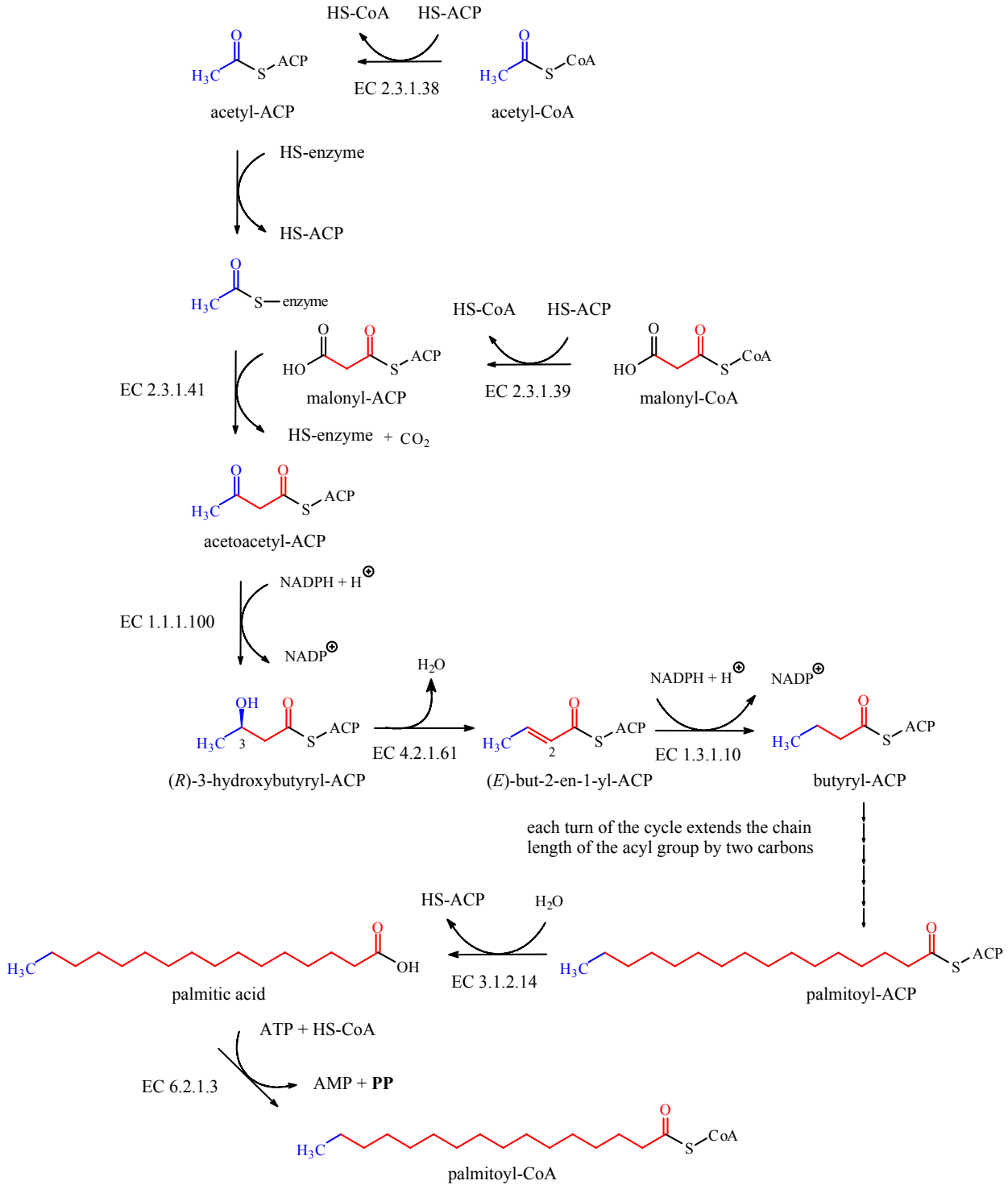

Figure 4

of acetic acid units explains why the common fatty acids are straight-chained and possess an even number of carbon atoms) are generated by means of alternative substrates. The rarer fatty acids containing an odd number of carbons typically originate from the incorporation of a different starter unit, e.g. propionic acid, using propionyl-CoA instead of acetyl-CoA (typically C15 and C17 fatty acids in milk and dairy products), or can arise by the loss of one carbon atom (via α-oxidation) from an even-numbered acid. Uniquely, in glands of special functions in mammals and birds, such as avian

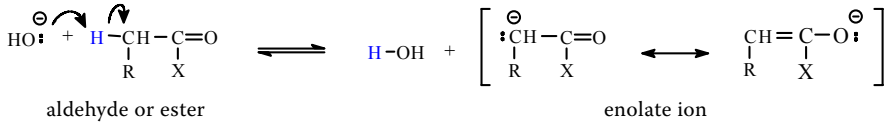

Figure 5

uropygial glands, malonyl-CoA can be replaced by methylmalonyl-CoA as a substrate, and the cycle of FAS I reactions results in the generation of methyl-branched-chain fatty acids.

1.1.1 Claisen condensation

The Claisen condensation (as well as the aldol condensation or aldol addition or aldolisation) is a general reaction of carbonyl compounds that posses an α-hydrogen atom. The C-C bond formation is achieved in a base-catalysed reaction depending on the generation of a resonance-stabilised enolate anion (Figure 5). In biochemical pathways, the enzyme catalysis obviates the need for a strong base (B:) to achieve the formation of a resonance-stabilised enolate anion (Dewick 2002).

In the next step, the enolate anion acts as a nucleophile (actually as a carbanion) and attacks the carbonyl carbon of a second molecule forming alkoxide anion. In the final step, the alkoxide anion abstracts a proton from water to form the aldolisation product. This step takes place because the alkoxide anion is a stronger base than a hydroxyl anion (Figure 6). Whether an aldol-type or Claisen-type product is formed depends on the nature of X and its potential as a leaving group. If there is no suitable leaving group (e.g. X = hydrogen atom in aldehydes and aldoses), then the aldol-type forms, the loss of the leaving group, e.g. X = RC(=O)O group in esters or X = RC(=O)S group in thioesters, yields the Claisen-type product.

Acetyl-CoA, a thioester of acetic acid, has significant advantages over oxygen esters, e.g. ethyl acetate, in that the CH_3 group hydrogens are more acidic, comparable to those in the equivalent ketone, thus increasing the likelihood of generating the enolate anion. First converting acetyl-CoA to malonyl-CoA makes Claisen condensations involving acetyl-CoA more favourable. The α-hydrogens of CH_2 group are now flanked by two carbonyl groups and have an increased acidity. An alternative rationalisation resides in that the

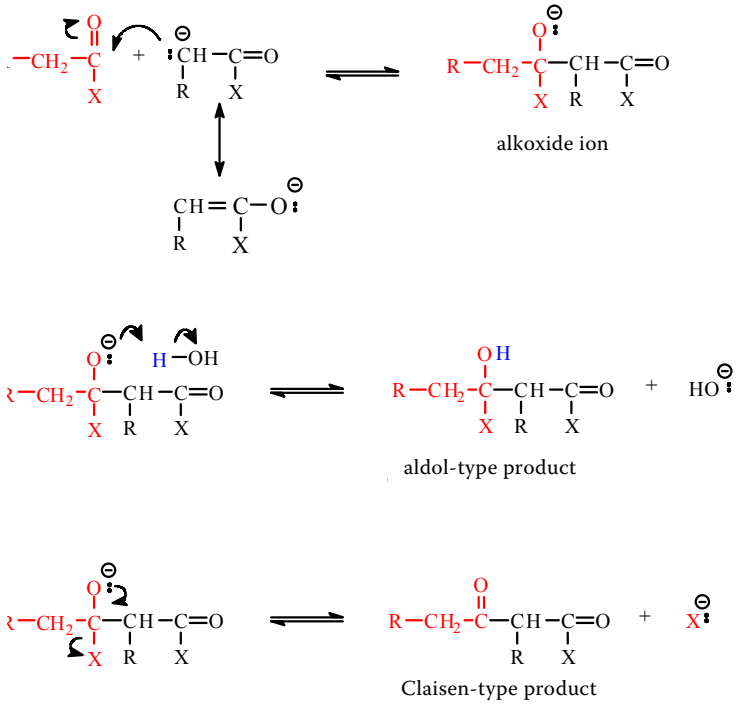

Figure 6

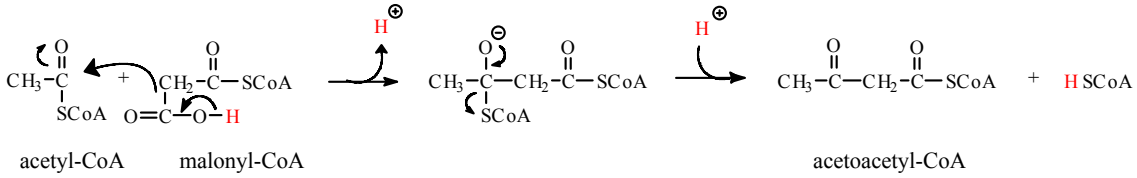

Figure 7

decarboxylation reaction of malonyl-CoA occurs to generate the enolate anion without any requirement for a strong base (Figure 7).

1.2 Medium-chain saturated fatty acids

In mammals, the chain-length of the FAS I released fatty acid is usually C16, being generally restricted to carbon chains longer than C12 (lauric acid), due to the low specificity of acyl-ACP thioesterase (EC 3.1.2.14). However, milk fats from humans, ruminants, and most other non-ruminant mammals contain high proportions of medium-chain saturated fatty acids (C8-C14). Medium-chain fatty acids are synthesised *de novo* within the mammary epithelial cell in the mammary gland during lactation as a result of a tissue-specific modification of the universal FAS I reaction (Barber *et al.* 1997; Hunt & Alexon 2002). The alteration of the specificity of the acyl-chain termination results from an interaction of FAS I with a second thioesterase that is not part of FAS I but is present in the cytosol as a discreet monomeric peptide. This thioesterase has an access to the elongating acyl chain on the 4′-phosphopantetheine prosthetic group of FAS I and can hydrolyse the thioester bond which results in the release of medium-chain fatty acids of chain length from C8 to C12.

Medium-chain fatty acids also accumulate in seeds of some plants (e.g. in palm seeds) due to the specificity of the acyl-chain termination by lauroyl-ACP thioesterase (EC 3.1.2.21) that is involved in their production. Overexpression of specific acyl-ACP thioesterases has been demonstrated to be effective for the production of bioengineered oils, e.g. oils with high levels of lauric or stearic acids (Gibson *et al.* 1994).

1.3 Long-chain saturated and unsaturated fatty acids

Fatty acids from dietary sources and the palmitic acid produced by fatty acid synthesis *de novo* are typically modified to give rise to other fatty acids. These modifications may include the chain elongation to give long-chain (C16-C18), very-long-chain (C20-C26), and ultra-long-chain (C28-C38) fatty acids, and desaturation, giving rise to unsaturated fatty acids. Unsaturated fatty acids can arise by more then one biosynthetic routes, but in most organisms the common mechanism resides in desaturation (oxidation) of the corresponding saturated fatty acid catalysed by desaturases in the endoplasmic reticulum. Fatty acid elongation and desaturase systems act jointly in the generation of long-chain and very-long-chain unsaturated fatty acids. The formation of the double bond occurs strictly stereospecifically. Most reactions result in a *cis*-linkage, however, enzymes also exist that can generate *trans*-double bonds. The unsaturated fatty acids, which dominate lipids, contain one, two or three allyl groups in their acyl residues.

1.3.1 Long-chain saturated fatty acids

In eukaryotes, the production of long-chain and higher saturated and unsaturated fatty acids takes place independently of FAS in the endoplasmic reticulum and mitochondria[4]. The predominant site of this process is in the endoplasmic reticulum membrane. This process resembles that catalysed by FAS, but the individual activities appear to be on the separate, membrane-bound elongases. Unlike ACP during the *de novo* fatty acid synthesis, HS-CoA acts as the acyl carrier molecule in fatty acid elongation. The elongation starts in the condensation of acyl-CoA and malonyl-CoA

[4]In the mitochondria, fatty acid elongation process is essentially a reversal of β-oxidation, except that one NADPH and one NADH are required (β-oxidation yields NADH and $FADH_2$). Mitochondrial fatty acid elongation acts primarily on acyl-CoA substrates shorter than 16 carbons.

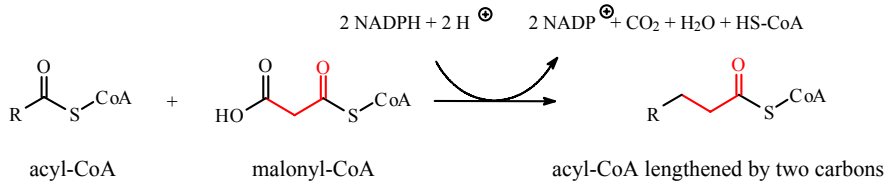

Figure 8

(3-oxoacyl-CoA synthase, EC 2.3.1.16), the latter being also required as a chain-extending unit in the subsequent cycles. The condensation is followed by 3-oxo group reduction to (*S*)-3-hydroxy group (3-oxoacyl-CoA reductase, EC 1.1.1.- in mammals, EC 1.1.1.211 in plants), dehydration of (*S*)-3-hydroxy-CoA to (*E*)-2-enoyl-CoA (3-hydroxyacyl-CoA dehydrase, EC 4.2.1.17), and reduction of 2-enoyl-CoA (enoyl-CoA reductase, EC 1.3.1.44), similarly to the sequence of reactions in FAS. The reduction reactions of elongation require NADPH as a cofactor, just as with the similar reactions catalysed by FAS. The overall reaction is given in Figure 8.

Starting from palmitoyl-CoA, the elongation of its chain by the microsomal malonyl-CoA-dependent elongase produces stearic acid (Figure 8). In plants, the extension of the ubiquitous palmitic and stearic acids to long- and ultra-long-chain fatty acids is mainly used for the production of aliphatic wax components. It is not known how many of the above putative condensing enzymes participate in the wax production, and how many different condensing enzymes are needed for the elongation of C18 to C38 fatty acid (Kunst & Samuels 2003).

1.3.2 Long-chain monounsaturated fatty acids

The insertion of a double bond at the specific position of a saturated fatty acid as catalysed by desaturases can occur in most tissues. In mammalian and fungal cells, the formation of a double bond in a fatty acid involves the endoplasmic reticulum membrane desaturases that utilise CoA esters. Plant desaturases are soluble enzymes found in the plastids that introduce double bonds into fatty acids. Subsequent desaturation yielding polyunsaturated fatty acids is mediated by membrane bound proteins. Plant desaturases utilise ACP esters and act on bound fatty acids instead of on free fatty acids (Rawlings 1998).

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 9, the names are given for the appropriate fatty acid, the structures shown represent the thioesters involved in the conversions).

A palmitoyl and stearoyl thioesters are the preferred substrates for the mammalian stearoyl-CoA desaturase[5] (EC 1.14.19.1) and plant stearoyl-ACP desaturase (EC 1.14.19.2) that generate (9Z)-hexadec-9-enoic (palmitoleic) and (9*Z*)-octadec-9-enoic (oleic) acids[6], respectively (Figure 10). Both palmitoleic and oleic acids then become the dominant storage forms of fatty acids in animal adipose tissue and are used for the synthesis of a variety of other long-chain unsaturated acids.

A number of plant species produce unusual fatty acid positional isomers. Chain elongation of

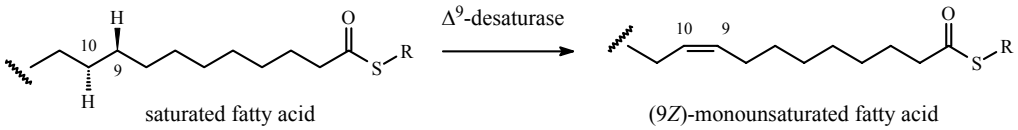

Figure 9

[5]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 (Kim & Ntambi 1999).

[6]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.

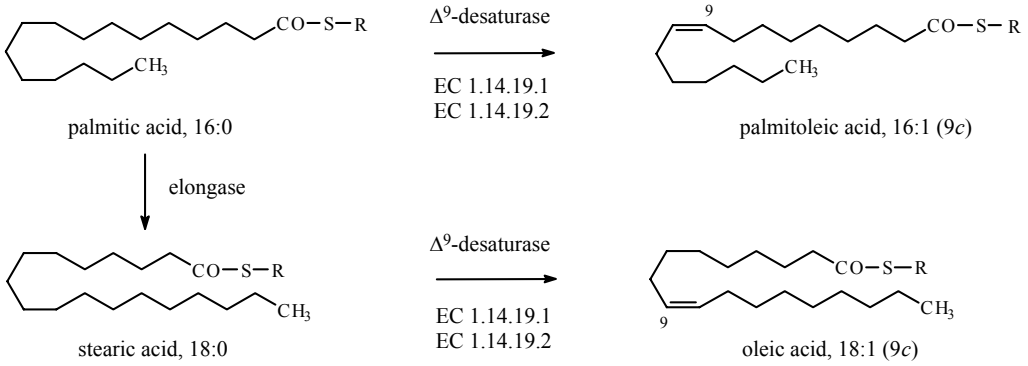

Figure 10

palmitoleic acid towards carboxyl terminus yields (11*Z*)-octadec-11-enoic (vaccenic or asclepic) acid, the minor fatty acids of many seed oils and animal fats as the constituents of their triacylglycerols (Chisholm & Hopkins 1960) (Figure 11). Seeds of the *Apiaceae* family plants commonly used as spices, such as parsley (*Petroselinum crispum*), fennel (*Foenicum vulgare*), and coriander (*Coriandrum sativum*), are known to contain high levels (about 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, cosmetics, chemistry, and pharmaceutical industries (Weber *et al*. 1997). Its formation from palmitic acid is catalysed 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 hydrolysed 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 (Rawlings 1997; Schultz & Ohlrogge 2000).

Seed oils of some plants are rich in triacylglycerols containing very-long- and ultra-long-chain monounsaturated fatty acids belonging to the *ω*-9 group that 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 12). The first product of this elongation is (11*Z*)-eicos-11-enoic (gondoic) acid which is

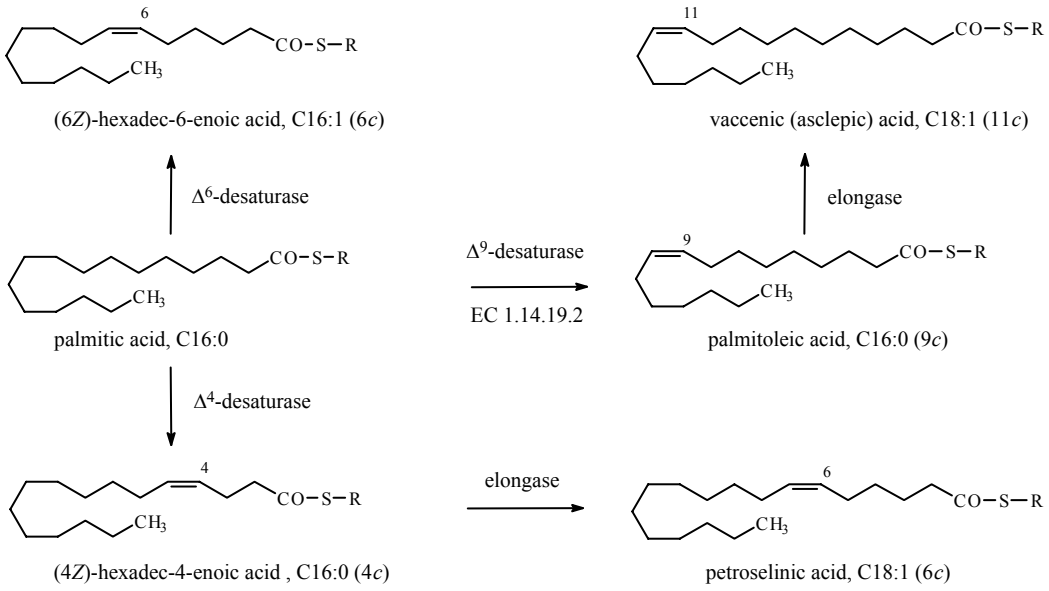

Figure 11

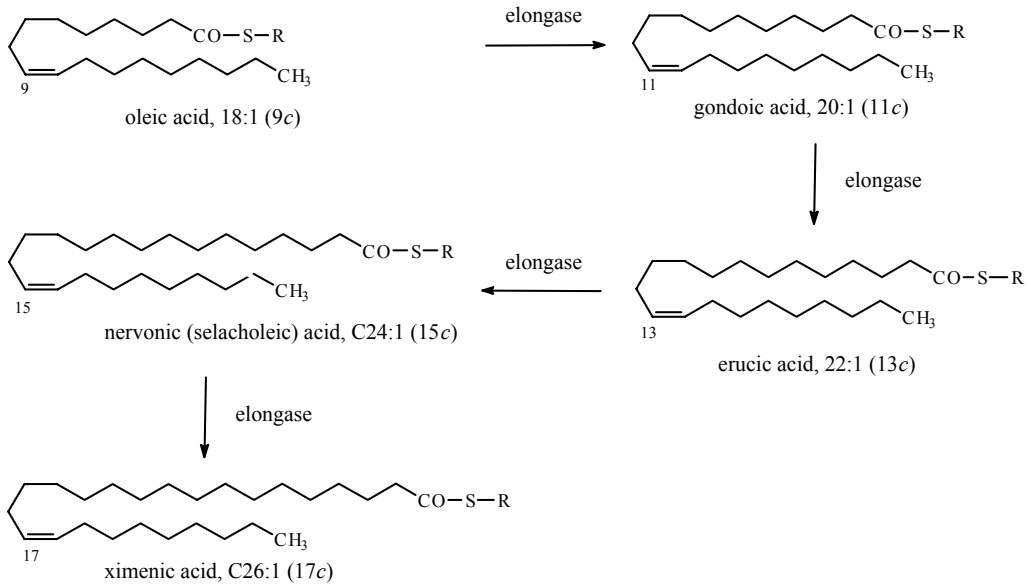

Figure 12

further elongated to (*13Z*)-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 synthesise high amounts of erucic and nervonic acids (Murphy & Mukherjee 1988). 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 the production of high-temperature lubricants and engineering polyamide fibers, similar to nylon.

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 $-(CH = CH\text{-}CH_2)_n-$ (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)[7], but never beyond C-9. 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 in plants and fungi 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*,12Z,15*Z*)-octadeca-9,12,15-trienoic acid (Δ^{15}-desaturase, plastidal linoleate desaturase, EC 1.14.99.)[8]. The inability of animal systems to desaturate closer to the methyl terminus than C-9 renders them unable to convert palmitic acid to linoleic or α-linolenic acids. Accordingly, linoleic and *α*-linolenic acids are referred to as essential fatty acids since they

[7]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 etc.) (Rawlings 1997).

[8]In the so called 16:3 plants (e.g. cocoa bean tissue, *Theobroma cacao*, *Sterculiaceae*), α-linolenic acid is also biosynthesised by desaturation of dodecanoic (lauric) acid to (3*Z*,6*Z*,9*Z*)-dodeca-3,6,9-trienoic acid followed by chain elongation (Gunstone 1984).

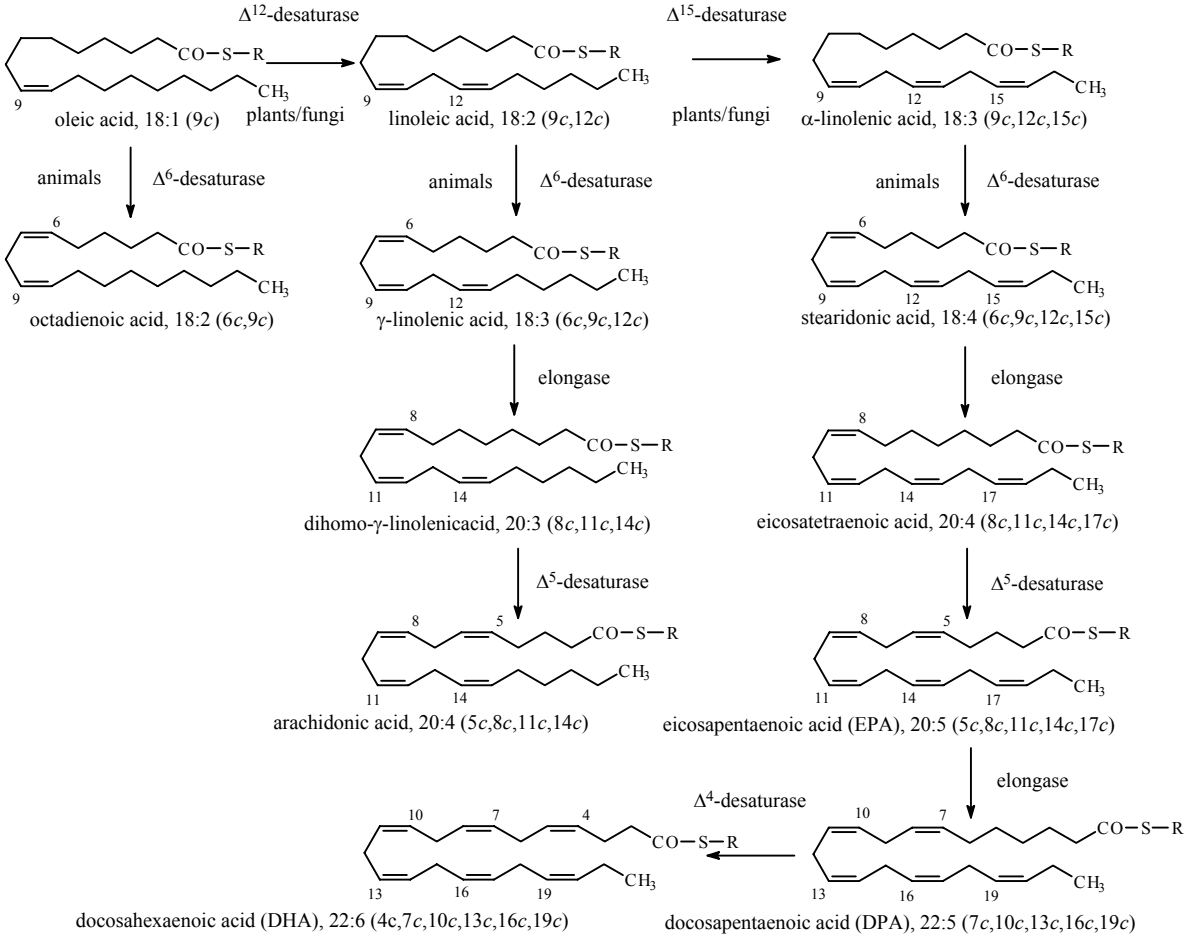

Figure 13

cannot be synthesised *de novo* and must be obtained from the plant materials in the diet (Figure 13) (Dewick 2002; Napier 2002).

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 synthesised 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[9] of the 1-series and 2-series, respectively.

α-Linolenic acid belonging to the so called *ω*-3 (or n-3) group of polyunsaturated fatty acids is similarly a precursor 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[10].

2 LESS COMMON FATTY ACIDS

Under appropriate conditions (during biohydrogenation as well as under heating and metal-catalysed hydrogenation), *cis*-double bonds in natural unsaturated fatty acids tend to isomerise to thermodynamically more stable *trans*-double

[9]Prostaglandins are a group of modified C_{20} polyunsaturated fatty acids occurring widely, but only in trace amounts, in animal tissues. They can regulate blood pressure, contractions of smooth muscle, gastric secretion, platelet aggregation, and brain development. Several other classes of biologically active prostaglandin-like compounds are produced from polyunsaturated fatty acids *in vivo* (e.g. isoprostanes, thromboxanes, and leukotrienes).

[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 presumably to assist maintaining the membrane fluidity in cold seawater. Arachidonic acid is also widespread in ferns and mosses (Rawlings 1997).

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 acids originating by the breakdown of terpenoids. Some plants produce seed oils of unusual structures, which include variations of the chain length or acetylenic, cycloprop(en)yl, cyclopent(en)yl, epoxy, hydroxy, and oxo functionality. Many of these fatty acids have a wide range of pharmacological and industrial uses (Rawlings 1997).

2.1 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 double bonds. They are formed in low concentrations on microbial hydrogenation (biohydrogenation) in the stomach of ruminants from the dietary unsaturated fatty acids,[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 isomerisation starts by the 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 allylic isomerisation giving the more stable (*E*)-configuration at C-11 (Figure 14).

The major substrates are linoleic and α-linolenic acids which are hydrogenated in the rumen[12] to the extent of 70–95% and 85–100%, respectively (Bauman *et al*. 2003). The major biochemical pathways for the biohydrogenation of these acids are illustrated in Figure 15 (Hartfoot & Hazlewood 1997). The initial step typically involves isomerisation of the (12*Z*)-double bond to the (11*E*)-double bond resulting in conjugated di- and trienoic fatty acids. The next step is the reduction of the (9*Z*)-double bond resulting in a (11*E*)-fatty acid. The final step is the 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 bacteria species is unknown.

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)[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 (Bauman *et al*. 2003). (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 (Bauman *et al.* 2001).

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

Figure 14

[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.

[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.

[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 (Bauman *et al*. 2001).

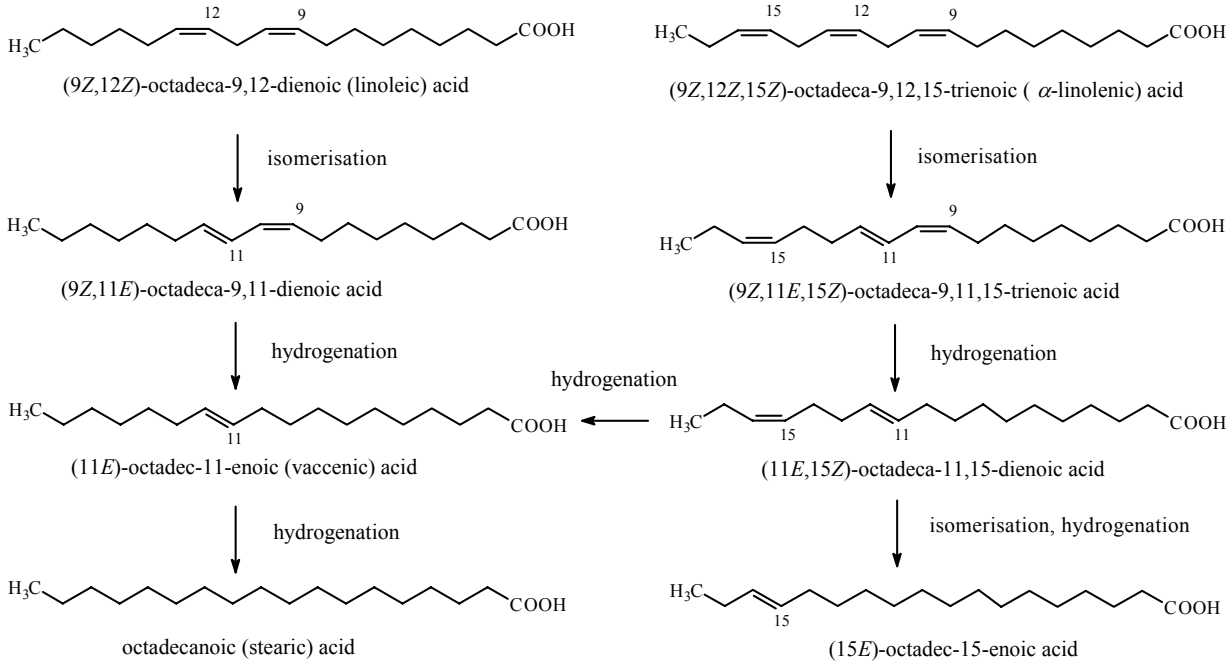

Figure 15

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 (Chin *et al.* 1992).

Many other *trans*-unsaturated fatty acids, including (9*E*)-octadec-9-enoic (elaidic) acid and its positional isomers, and isomers of conjugated linoleic acid have been found in the ruminal outflow (Bauman *et al.* 2003)[14]. 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.

2.2 Acetylenic fatty acids

Many unsaturated fatty acids found in nature contain one or more acetylenic bonds. Acetylenic (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 belonging to the *Asteraceae*, *Caesalpiniaceae*, and other families.

Acetylenic fatty acids are predominantly formed by further desaturation of olefinic systems in unsaturated fatty acid molecules. For example, the 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 16). The 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*) found, e.g., in high concentrations in oil from seeds of *Ixiolaena brevocompta* (*Asteraceae*). The toxic effect of this plant seeds has been reported in Australian sheep (Walker *et al.* 1980). 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

[14]Positional isomers of octadecenoic acid having the *trans*-double bond at carbons from C-4 to C-16, and the positional isomers of 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*)-.

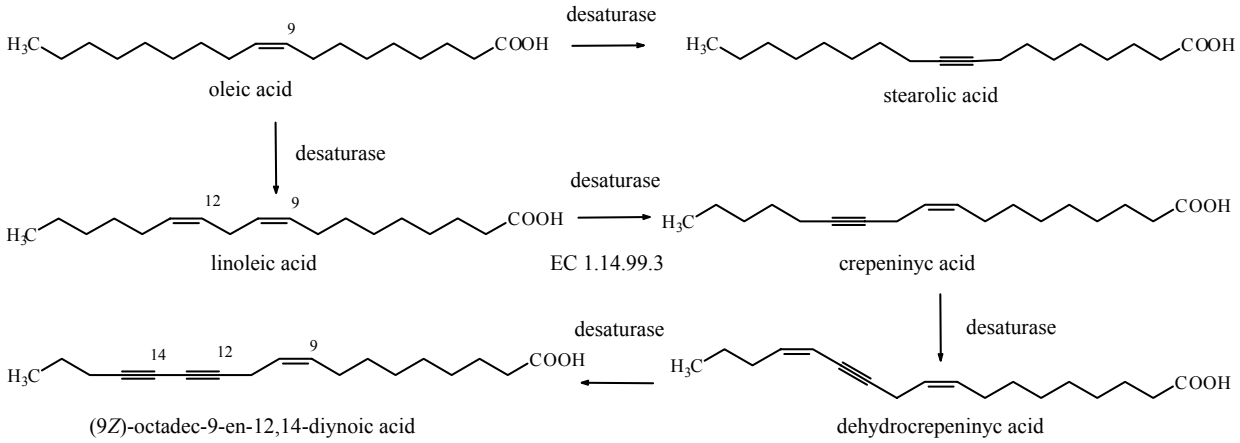

Figure 16

(9*Z*)-octadec-9-en-12,14-diynoic acid and its chain shortening by β-oxidation then yields short-chain acetylenic acids (Dewick 2002). Isomerisation 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 seed oils from several species of the genera *Exocarpus*, *Santalum, Ximenia* (*Santalaceae*), of which some are eaten as Australian bush foods (Jones *et al.* 1999). For example, ximeninic acid is the major fatty acid (40%) in the oil of *Santalum spicatum* seeds (Lie Ken Jie & Pasha 1998).

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 (Croft *et al.* 1987).

2.3 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 (Rawlings 1997). 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, catalysed 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 the acetyl-CoA starter and four methylmalonyl-CoA chain extender units (Figure 17). It occurs for example, in the preen gland wax of the goose (*Anser anser*) (Dewick 2002).

It has been shown (Kroumova *et al.* 1994) that some branched- and medium straight-chain fatty acids of tomato (*Lycopersicon esculentum*, *Solanaceae*) arise from acetyl-CoA elongation of 2-oxoacids acting as the precursors of amino acids (Velíšek & Cejpek 2006a) without the involvement of fatty acid synthase mediated reactions, suggesting the integration of amino acid and fatty acid metabolisms (Figure 18). The enzymes involved

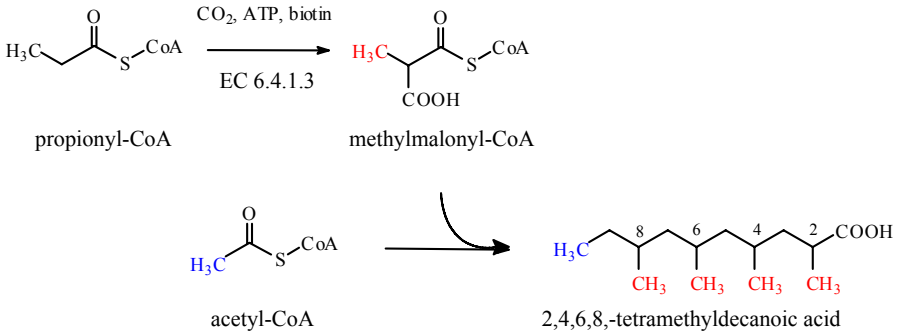

Figure 17

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.

(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, side chain (Figure 19). 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 its 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 metabolised by the β-oxidation pathway[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

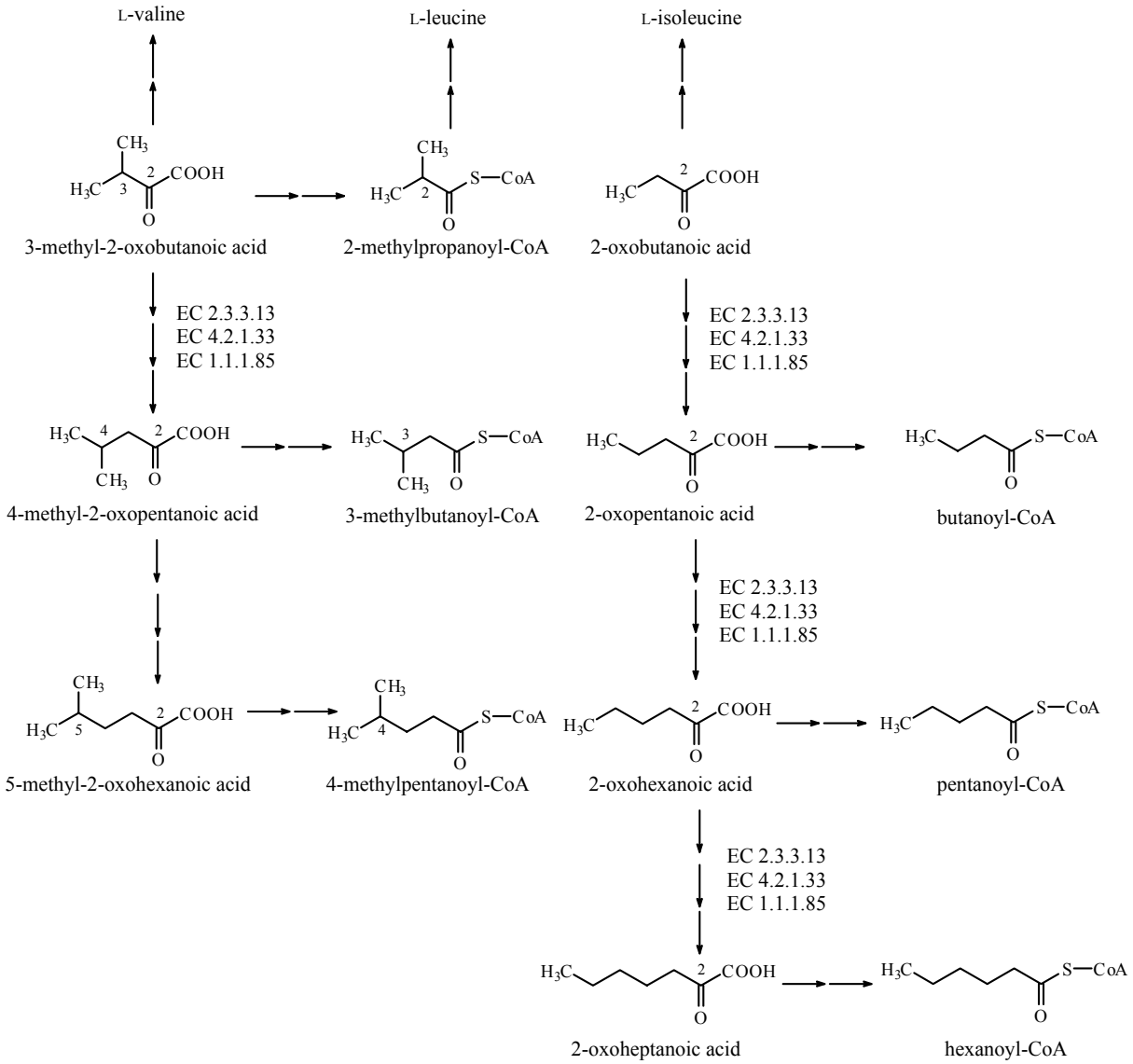

Figure 18

[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.

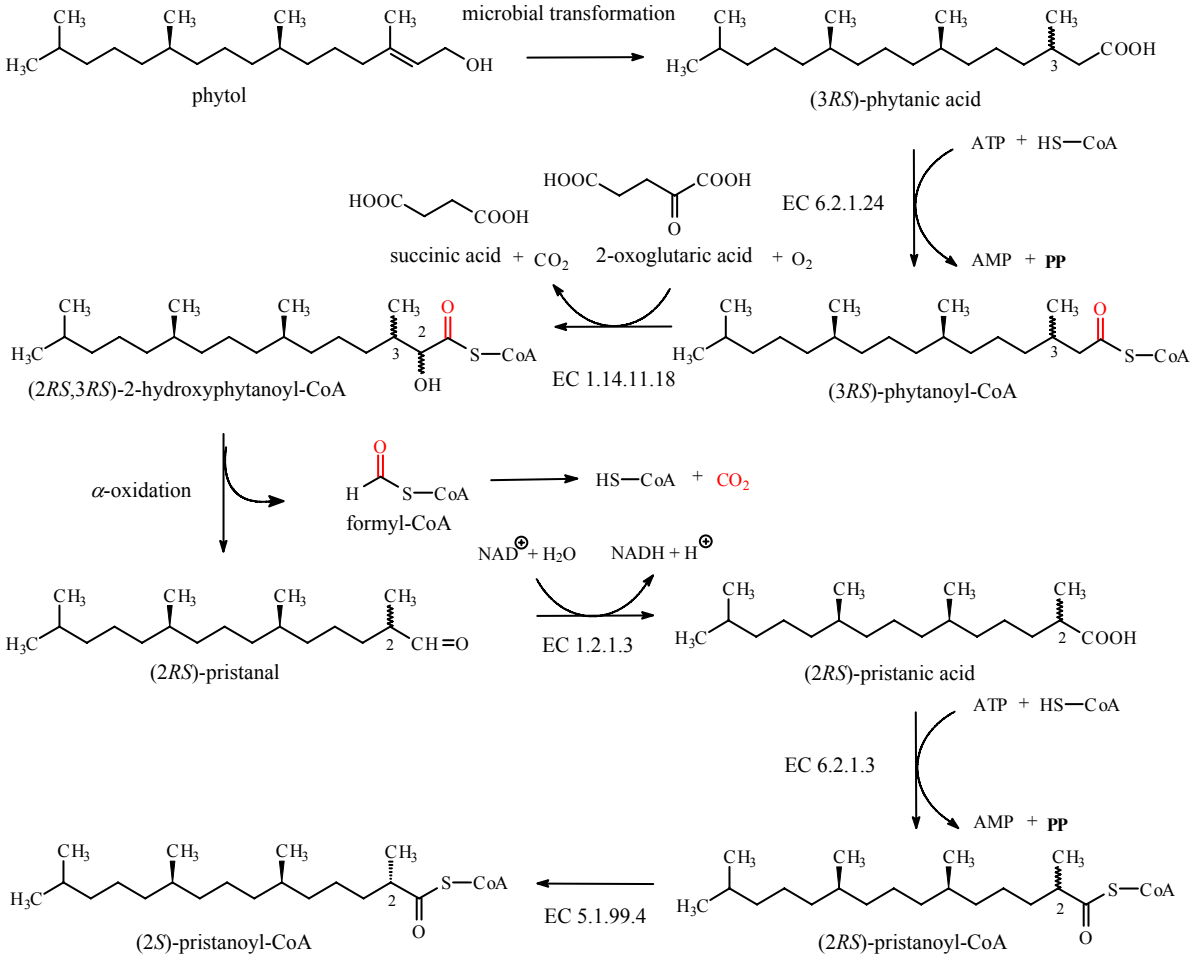

Figure 19

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 catalysed by phytanoyl-CoA hydroxylase are less well characterised. 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 hydrolyses to formic acid and HS-CoA. Both pristanal epimers, (2*RS*)-pristanal, are subsequently oxidised to (2*RS*,6*R*,10*R*)-tetramethylpentadecanoic (pristanic) acid via an aldehyde dehydrogenase (pristanal dehydrogenase, EC 1.2.1.3).

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[16] to 2-methylpropionyl-CoA, propionyl-CoA, and acetyl-CoA (Kershaw *et al.* 2001; Wanders *et al.* 2003; Mukherji *et al.* 2003).

2.4 Alicyclic fatty acids

Cyclopropane and cyclopropene fatty acids are found in the phospholipids of many plants, bacteria, and parasitic protozoa. Cyclopropene fatty acids,

[16]Peroxisomes are not only able to metabolise 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.

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 edible cottonseed oil from *Gossypium* species (*Malvaceae*). Dihydrosterculic acid was found in the seed oil (35–48%) of lychee (*Litchi sinensis*) (Gontier *et al*. 2000) and longan fruits (*Dimocarpus longan*) (Grondin *et al*. 1997) from the *Sapindaceae* family.

Cyclopropane and cyclopropene fatty acids are derived from oleic acid bound in glycerophospholipids, i.e. (3-*sn*-phosphatidyl)ethanolamine (and to lesser extent in phosphatidylglycerol or phosphatidylinositol) by *C*-methylation (electrophilic addition with *S*-adenosylmethionine, AdoMet (SAM), as the alkylating agents 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). The 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 20).

Plants lack an immune system in the sense that exists in animals, but they possess mechanisms that recognise potential pathogens and initiate defence 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, or stress and attack by pathogens. These compounds are similar in many ways to the eicosanoids derived from arachidonate in animals, which have so many varied functions, especially in the inflammatory process.

Compounds derived from cyclopentyl fatty acids, e.g. jasmonic acid and methyl jasmonate, collectively referred to as jasmonates, are important cellular regulators involved in diverse developmental processes, such as seed germination, root growth, fertility, fruit ripening, and senescence. In addition, jasmonates activate plant defense mechanisms in response to insect-driven wounding, various pathogens, and environmental stress, such as drought, low temperature, and salinity. Jasmonates are synthesised in plants via the octadecanoid pathway (Figure 21) and are similar to animal prostaglandins in structure and biogenesis. The α-linolenic acid is released from membrane lipids by phospholipase A_1 (EC 3.1.1.32) and oxidised by lipoxygenase (EC 1.13.11.12) to (9*Z*,11*E*,13*S*,15*Z*)-13-hydroperoxyoctadeca-9,11,15-trienoic acid, which is then converted by hydroperoxide dehydratase (EC 4.2.1.92) to (9*Z*,13*S*,15*Z*)-12,13-epoxyoctadeca-9,15-dienoic acid (allene oxide). Allene oxide formed by the action of hydroperoxide dehydratase is converted into cyclopentenone derivative (10*Z*,15*Z*)-12-oxophyto-10,15-dienoic acid by allene oxide cyclase (EC 5.3.99.6). Jasmonic acid is synthesised from this product through reduction by 12-oxophytodienoate reductase (EC 1.3.1.42) and three steps of β-oxidation after which it is esterified by carboxyl methylesterase. A volatile fragrant component of flowers, (*Z*)-jasmone, is formed by β-oxidation of jasmonic acid. In addition, various derivatives can be formed from jasmonic acid by hydroxylation, *O*-glycosylation, and conjugation with amino acids. In plants (e.g. *Arabidopsis thaliana*) that synthesise and contain

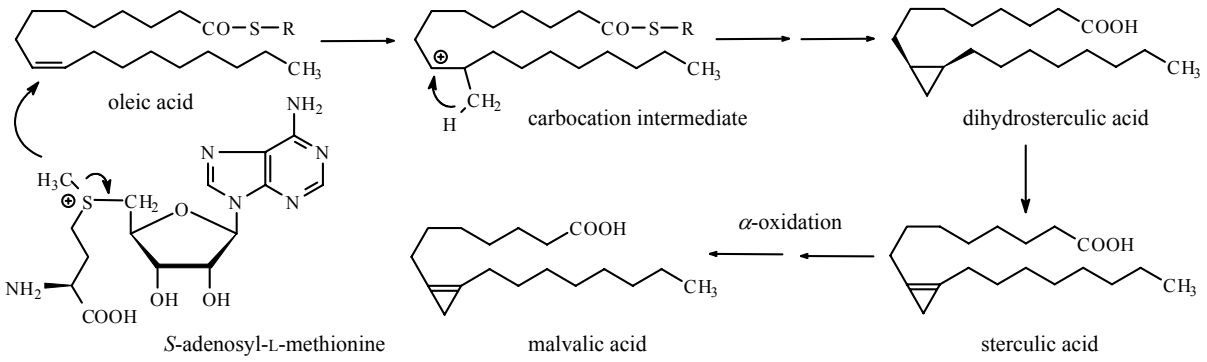

Figure 20

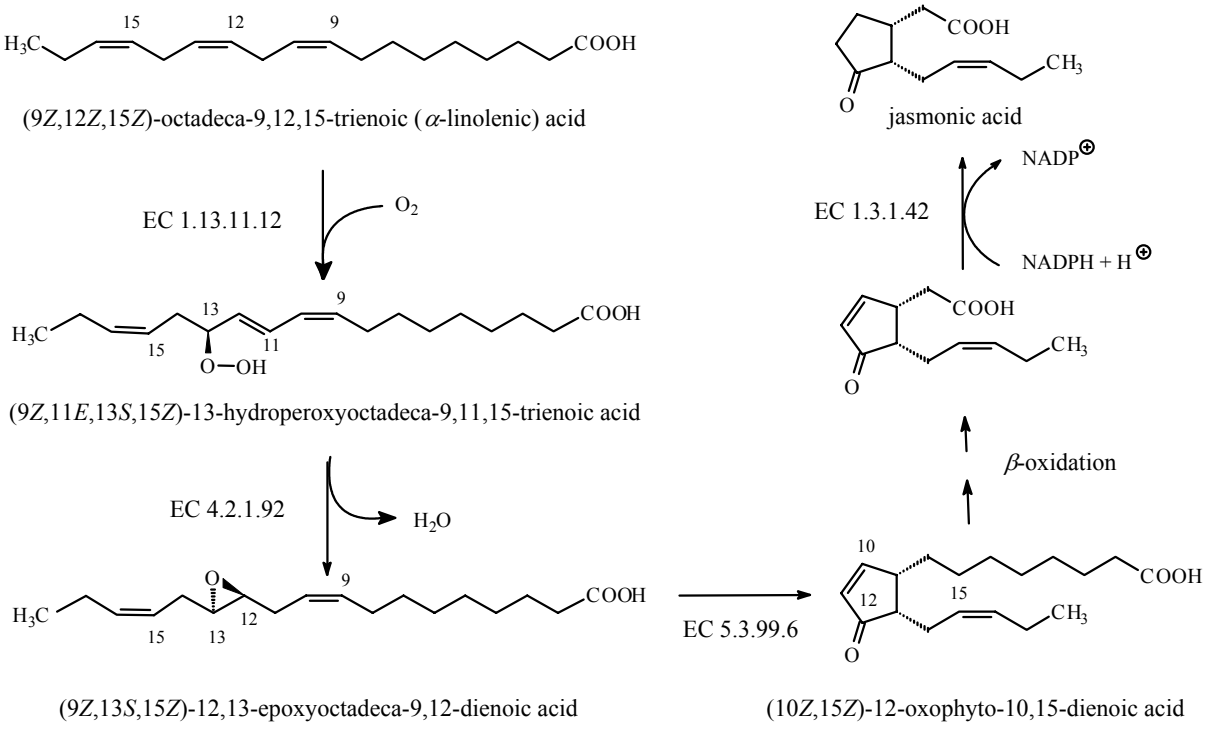

Figure 21

(7*Z*,10*Z*,13*Z*)-hexadeca-7,10,13-trienoic acid in their chloroplast galactolipids, a hexadecanoid pathway exists in parallel with octadecanoid pathway (Weber 2002; Cheong & Choi 2003).

Chaulmoogric acid, (13*R*)-13-(cyclopent-2-en-1-yl)tridecanoic acid, and its lower homologue hydnocarpic acid, (11*R*)-11-(cyclopent-2-en-1-yl)-undecanoic acid, are cyclopentenyl fatty acids found in chaulmoogra oil expressed from seeds of *Hydnocarpus wightiana* (*Flacourtiaceae*). These acids are known to arise by malonate chain extension of the coenzyme A ester of cyclopent-2-en-1-yl carboxylic acid as a starter unit alternative to acetic acid (acetyl-CoA) (Figure 22). Chaulmoogra oil provided for many years the only treatment for the relief of leprosy (Dewick 2002).

Cyclohexyl fatty acids with terminal ring structures (e.g. 11-cyclohexylundecanoic acid and 13-cyclohexyltridecanoic acids found as minor components of butter and sheep fat) are almost certainly produced by bacteria in the rumen. The mechanism involves conversion of sugars to shikimic acid and further to cyclohexanecarboxylic acid which serves as the primer for the fatty acid synthesis (Christie 2005).

2.5 Epoxy fatty acids

Epoxy fatty acids are present in a number of seed oils (in triacylglycerols, cutins) as 1,2-epoxy compounds derived from oxirane or 1,4-epoxides derived from furane. The natural species of 1,2-epoxides are C18 compounds, saturated or unsaturated. *Vernonia* plants (e.g. *V. anthelmintica* and *V. galamensis*, *Asteraceae*) are among plants identified as those containing epoxy fatty acids at high percentages (62–78%) in their seed oils (Christie 2005). The major component is vernolic or (*Z*)-12,13-epoxyoctadec-9-enoic or 12,13-epoxy oleic acid. Small quantities (about 2.5%) of 9,10-epoxy stearic acid, vernolic acid, and (*Z*)-9,10-epoxyoctadec-12-enoic (coronaric) acid were also found in peanut (*Arachis hypogaea*, *Fabaceae*) germ oil (Hammond *et al.* 1997).

Besides the oxirane-derived fatty acids, furanoid fatty acids or F-acids[17] also exist. The most frequent member of the homologous series of F-acids is known as (12*Z*,14*Z*)-12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (F6). Furan fatty acids have been found in large quantities in fish liver oils (1–6%), and in small amounts they occur in meat

[17]A common nomenclature describing these fatty acids (as F1, F2, etc.) is used. This naming originated from the elution order in gas chromatography.

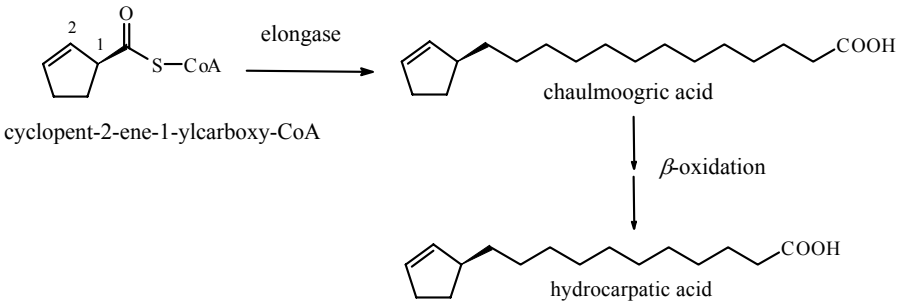

Figure 22

(located in phospholipids), butter, germ oils, and mushrooms. It appears possible that these furans serve as antioxidants in biological systems due to their ability to scavenge free radicals.

Relatively little information is available on the biogenesis of furan fatty acids. The enzyme lipoxygenase (EC 1.13.11.12, see Figure 21) is involved in the initial step of F-acid formation, as it catalyses the hydroperoxidation of linoleic and other polyunsaturated fatty acids by molecular oxygen. It has been shown that the furan methyl groups of F-acids are derived from SAM. Figure 23 shows the major steps supposed to lead from linoleic acid to (10*Z*,12*Z*)-10,13-epoxy-11,12-dimethyloctadecanoic acid or F3-acid (Batna & Spiteller 1993; Shirasaka *et al*. 1997; Spiteller 2005).

2.6 Hydroxy and oxo fatty acids

(2*R*)-2-Hydroxy fatty acids (D-2-hydroxy fatty acids of the chain-lengths from C16 to C26) are important constituents of animal and plant sphingolipids, (3*S*)-3-hydroxy fatty acids or L-3-hydroxy fatty acids (C6 to C16) are formed during *β*-oxidation of fatty acids in mammalian tissues, and increased levels of the free acids or acyl carnitines in blood and urine are indicative of disorders of fatty acid oxidation. Some 3-hydroxy fatty acids (C8 to C12) are normal components of wax secretion from the uropygial glands of certain bird species (Christie 2005). The seed oils of higher plants contain a number of hydroxy fatty acids, some of which are important agricultural commodities. The best known of these is ricinoleic acid, (12*S*,9*Z*)-12-hydroxyoctadec-9-enoic acid. Ricinoleic acid is the major fatty acid found in castor bean oil from seeds of the castor oil plant (*Ricinus communis*, *Euphorbiaceae*). It is formed by direct hydroxylation of oleic acid by the action of an oxygen- and NAD(P)H-dependent ricinoleic acid synthase (EC 1.14.13.26). Oleic acid is usually part of the phospholipid 1-acyl-2-oleoyl-*sn*-glycero-3-phosphocholine (Figure 24, the name is given for the fatty acid, the structure shown

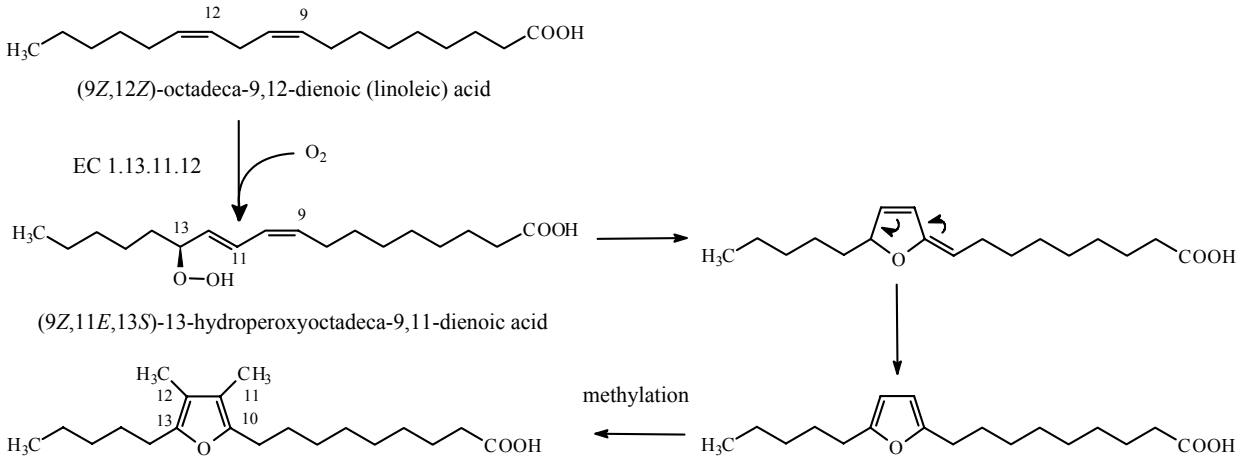

Figure 23

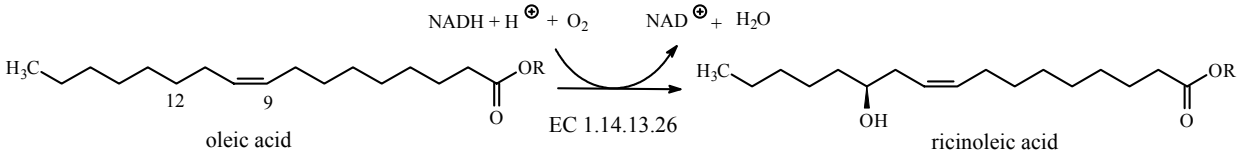

Figure 24

represents the ester involved in the biosynthesis) (Moreau & Stumpf 1981).

Oxo (or keto) fatty acids are less common than hydroxy fatty acids. For example, about 1% of milk fat consists of saturated C10-C24 oxo fatty acids, with an even number of carbon atoms, in which the carbonyl group is located on C-5 to C-13 (Belitz *et al*. 2004).

3 PLANT WAX COMPONENTS

Plant surface wax is a collective term used to describe the lipid components of the cuticle which covers the outer surface of aerial plant tissues (epicuticular wax). In addition, wax is associated with the suberin matrix in the underground and wound tissues as well as the lipids of the pollen and seed coat. In plant cuticles, wax predominantly consists of comprised very-long-chain aliphatic lipids but it also includes terpenoids and minor secondary metabolites, such as sterols and flavonoids. The focus of this review is the biosynthesis of the aliphatic very-long-chain components of plant cuticular wax. They include aldehydes, primary and secondary alcohols, alkanes, ketones, and esters, which are derived from saturated very-long- and ultra-long-chain fatty acids with the predominant chain length from 20 to 34 carbons (Hamilton 1995; Kunst & Samuels 2003).

For example, the major classes of cuticular wax components in *Arabidopsis thaliana* are alkanes (38%). Their predominant chain lengths, listed in the order of abundance, are as follows: C29 (nonacosane), C31 (untriacontane), and C27 (heptacosane); ketones (30%) of C29 (ditetradecylketone); primary alcohols (12%) of C28 (tetraenanthylalcohol), C30 (myricylalcohol or melissylalcohol), and C26 (cerylalcohol); secondary alcohols (10%) of C29, C31, and C27; aldehydes (6%) of C30 (triacontanal), and C28 (octacosanal); fatty acids (3%) of C30 (mellisic acid), and C28 (montanic acid), and wax esters (1%).

The aliphatic components of cuticular wax are synthesised in the epidermal cells from the saturated very-long- and ultra-long-chain fatty acids. It is generally believed that the cuticular wax biosynthesis takes place via multienzyme complexes composed of individual subunits, which perform the individual steps in fatty acid elongation, fatty acid reduction to aldehydes and primary alcohols, aldehyde decarbonylation to alkanes, hydroxylation of alkanes to secondary alcohols, and oxidation of secondary alcohols to ketones (Lemieux 1996). Our knowledge of the enzymes involved in the wax biosynthesis, however, remains extremely limited.

The fatty acids produced by elongases in the epidermis are used for the synthesis of the wax components. There are two principal wax biosynthetic pathways: the acyl reduction pathway, which gives rise to primary alcohols and wax esters, and the decarbonylation pathway, leading to the formation of aldehydes, alkanes, secondary alcohols, and ketones. The fatty acyl reduction of acyl-CoA esters to primary alcohols is a two-step process carried out by two separate enzymes, an NADH-dependent acyl-CoA reductase required for the reduction of fatty acids to aldehyde intermediates (the free aldehyde is not released), and an NADPH-dependent aldehyde reductase required for the further reduction of aldehydes to primary alcohols (alcohol-forming fatty acyl-CoA reductase (EC 1.1.1.-). The final step of the acyl reduction pathway is the synthesis of wax esters (esters derived from primary alcohols called cerides), a reaction catalysed by the membrane-bound wax synthase (EC 2.3.1.75). For example, the major esters of apple and leafy vegetable cuticular waxes (so called wax esters) are cerylpalmitate and cerylstearate. Hydrolysis of wax esters is catalysed by wax ester hydrolase (EC 3.1.1.50).

The decarbonylation pathway is initiated by the production of aldehydes from very-long-chain fatty acid precursors by a membrane-bound aldehyde-forming fatty acyl-CoA reductase. The generated aldehydes are then decarbonylated to odd-chain alkanes with a release of carbon monoxide. This reaction is catalysed by aldehyde decarbonylase (EC 4.1.99.5). The subsequent steps of the de-

carbonylation pathway include hydroxylation of alkanes to secondary alcohols, and oxidation of secondary alcohols to ketones. Our knowledge of the enzymes catalysing these reactions (often called secondary alcohol-forming hydroxylase and ketone-forming oxidase, respectively) is extremely limited. It was suggested that a mixed function oxidase was involved in the hydroxylation.

Hydrophobic wax components must then move through the hydrophilic cell wall matrix to reach the cuticle. Lipid transfer proteins have been suggested to mediate this movement during wax biosynthesis.

Alkanes, free and esterified alcohols, and fatty acids having branched carbon backbones are common, however, minor components of cuticular waxes. Branched structures are usually iso compounds (with an isopropyl terminal group) or anteiso compounds (a secondary butyl terminal group). It is known that branched-chain amino acids (valine, leucine, isoleucine) can serve as precursors to iso- and anteiso-branched components of cuticular waxes. Keto acid deamination products of these amino acids are thought to serve as primers which are elongated by fatty acid synthase. Elongation of propionyl-CoA could yield linear fatty acids with odd carbon atoms (Kroumova & Wagner 1999).

4 LIPOIC ACID

(*R*)-Lipoic acid also known as α-lipoic acid has a systematic name (*R*)-5-(1,2-dithiolan-3-yl)pentanoic acid. It is a sulfur-containing coenzyme that is synthesised in a wide number of organisms including bacteria, fungi, plants, and animals. Lipoic acid is essential for the activity of a variety of enzyme complexes that catalyse oxidative decarboxylations.

In the cell, very little lipoic acid exists as the free acid; almost all is bound to the ε-amino group of lysine residue of target complexes. Lipoic acid is synthesised as an offshoot of fatty acid biosynthesis pathway (Marquet *et al.* 2001; Booker 2004). The exact details of this pathway have not been completely illuminated. The enzyme of lipoic acid biosynthesis, lipoate-protein ligase (EC 2.3.1.-), can

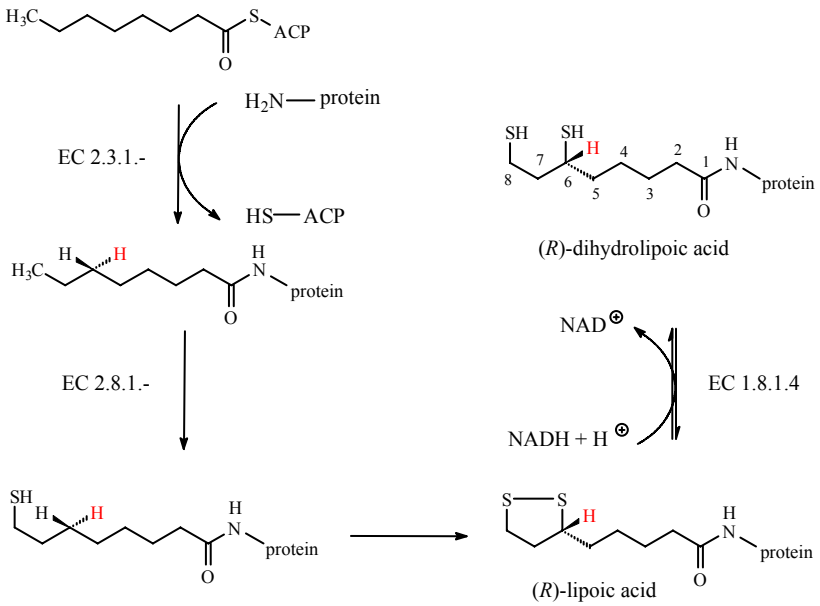

Figure 25

[18]Lipoate synthase is a member of a recently recognised class of metalloenzymes called the radical SAM superfamily. These proteins use SAM as a source 5´-deoxyadenosyl 5´-radical, which is a requisite intermediate in each enzyme's mechanism of catalysis. This radical is generated via a reductive cleavage of SAM, yielding methionine. The reaction requires the input of one electron, which is supplied by a 4Fe-4S cluster that is bound to the protein via cysteine ligands. It is speculated that the 5´-deoxyadenosyl 5´-radical abstracts one hydrogen atom from both C-8 and C-6 of a protein-derived octanoyl group, allowing insertion of sulfur atoms at each of these positions (Booker 2004).

transfer the octanoyl group (e.g. in the bacterial FAS II) from octanoyl-ACP thioester to lipoyl-accepting protein domain. An iron-sulfur enzyme[18] lipoate synthase (lipoic acid synthetase, EC 2.8.1.-) then catalyzes sulfur insertion into the octanoyl group, forming the lipoyl appendage (Figure 25).

Besides its activity as a coenzyme, lipoic acid is considered as an efficient antioxidant since with its reduced form, dihydrolipoic acid (6,8-disulfanyloctanoic acid), it constitutes a redox couple via modulation of NADH/NAD^+ ratio. This reversible reaction is catalysed by lipoic acid dehydrogenase (diaphorase, EC 1.8.1.4). Dihydrolipoic acid can scavenge hydroxyl and peroxyl radical but also chelates transition metals (e.g. Fe and Cu).

EC (Enzyme Commission) numbers and some common abbreviations

EC (Enzyme Commission) numbers, assigned by IUPAC-IUBMB, were taken from KEGG. In many structures, the unionised forms are depicted to simplify the structures, to eliminate the need for counterions, and to avoid the mechanistic confusion.

AdoHcy	*S*-adenosyl-L-homocysteine (SAH)
AdoMet	*S*-adenosyl-L-methionine (SAM)
ACP	acyl carrier protein as a part of a thioester
ADP	adenosine 5´-diphosphate
AMP	adenosine 5´-monophosphate
ATP	adenosine 5´-triphosphate
CLA	conjugated linoleic acid
CoA	coenzyme A as a part of a thioester
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FAS	fatty acid synthase
$FADH_2$	flavin adenine dinucleotide, reduced form
Fat	fatty acid thioesterase
FMN	flavin mononucleotide
KAS	3-ketoacyl-ACP synthase
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
PP	diphosphoric acid

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