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Biosynthesis of Food Constituents: Natural Pigments. Part 2 – a Review

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Abstract

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This review article is a part of the survey of the generally accepted biosynthetic pathways that lead to the most important natural pigments in organisms closely related to foods and feeds. The biosynthetic pathways leading to xanthones, flavonoids, carotenoids, and some minor pigments are described including the enzymes involved and reaction schemes with detailed mechanisms.

Keywords: biosynthesis; xanthones; flavonoids; isoflavonoids; neoflavonoids; flavonols; (epi)catechins; flavandiols; leucoanthocyanidins; flavanones; dihydroflavones; flavanonoles; dihydroflavonols; flavones; flavonols; anthocyanidins; anthocyanins; chalcones; dihydrochalcones; quinochalcones; aurones; isochromenes; curcuminoids; carotenoids; carotenes; xanthophylls; apocarotenoids; iridoids

The biosynthetic pathways leading to the tetrapyrrole pigments (hemes and chlorophylls), melanins (eumelanins, pheomelanins, and allomelanins), betalains (betacyanins and betaxanthins), and quinones (benzoquinones, naphthoquinones, and anthraquinones) were described in the first part of this review (Velíšek & Cejpek 2007b). This part deals with the biosynthesis of other prominent natural food colorants, xanthones, flavonoids, curcuminoids, carotenoids, isochromenes, and iridoids.

5 XANTHONES

Xanthones, represented as the C_6-C_1-C_6 system (Figure 30), are a group of about 70 yellow pigments restricted in occurrence to only a few families of higher plants and some fungi and lichens. The majority of xanthones has been found in basically four families of higher plants, the *Clusiaceae* (syn. *Guttiferae*), *Gentianaceae*, *Moraceae*, and *Polygalaceae* (Peres *et al.* 2000). Xanthones can be classified based on their oxygenation, prenylation and glucosylation pattern. The majority of xanthones isolated so far are of the tetraoxygenated-type. Xanthones posses a number of remarkable pharmacological activities (Peres *et al.* 2000) and have been used as, e.g. cardiovascular protective agents (Jiang *et al.* 2004) and antitumor promotors (Ito *et al.* 1998). The root of Yellow Gentian (*Gentiana lutea*, *Gentianaceae*) contains several yellow xanthones, exemplified by gentisin

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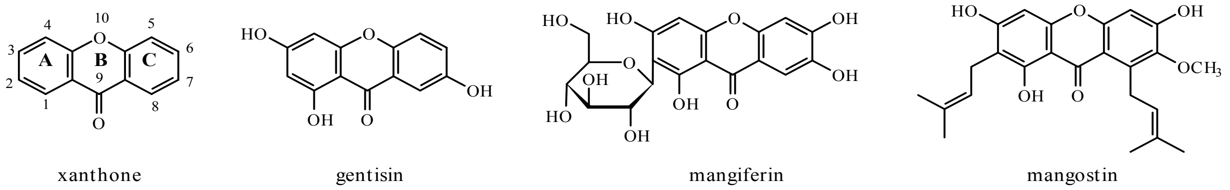


xanthone gentisin mangiferin mangostin

Figure 30

(1,3,7-trihydroxyxanthone), that are also found in the Common Centaury (*Centaurium erythraea, Gentianaceae*) (Figure 31). The Gentiana root is bitter and becomes the principal vegetable bitter employed in medicine. Before the introduction of hops, Gentian, with many other bitter herbs, was used occasionally in brewing. It is a principal ingredient in angostura bitters. 1,3,6,7-Tetrahydroxyxanthone, the aglycone of *C*-glucoside mangiferin occurs in mango leaves (*Mangifera indica, Anacardiaceae*) and has been used as a yellow textile dye. It is also known to exhibit anti-inflammatory, antioxidant, and antiviral effects. Mangostin is the major xanthone constituent of the mangosteen tree (*Garcinia mangostana, Clusiaceae*) (Bennet *et al.* 1990).

The biosynthesis of xanthones (Figure 31) originates mainly from a mixed shikimate-acetate pathway; however xanthones entirely derived from acetate have also been reported in lower plants (Peres *et al.* 2000). The main steps in the xanthone biosynthesis involve the condensation of shikimate and acetate moieties, which constitute a benzophenone intermediate followed by a regioselective, oxidative mediated intramolecular coupling to form the xanthone ring (Peters *et al.* 1998). The shikimate derivatives used as entry compounds for the tetrahydroxyxanthone biosynthesis differ in the individual plants.

In the Common Centaury, the biosynthesis of tetrahydroxyxanthone is obtained via 3-hydroxybenzoic acid (that appears to originate directly from the shikimate pathway) by the stepwise condensation of 3-hydroxybenzoyl-CoA (formed under catalysis of 3-hydroxybenzoate-CoA ligase, EC 6.2.1.-) with three malonyl-CoA to form an intermediate 2,3',4,6-tetrahydroxybenzophenone (benzophenone synthase, EC 2.3.1.151). In *Hypericum androsaemum* (*Clusiaceae*), the biosynthesis of this tetrahydroxyxanthone proceeds via benzoic acid and benzoyl-CoA (benzoate-CoA ligase, EC 6.2.1.25). The new intermediate, 2,4,6-trihydroxybenzophenone is further hydroxylated by benzophenone 3'-hydroxylase and converted to the 2,3',4,6-tetrahydroxyphenone intermediate, common to both pathways. The crucial step in this biosynthesis is the cyclisation of the benzophenone to a xanthone, catalysed by xanthone synthases. While in *C. erythraea* the xanthone synthase converts this tetrahydroxybenzophenone intermediate regioselective to 1,3,5-trihydroxyxanthone, the xanthone synthase of *H. androsaemum* converts it to the 1,3,7-trihydroxyxanthone. The last step in the biosynthesis of the tetrahydroxyxanthones is the introduction of a hydroxyl group to the C-ring of the xanthone skeleton. This reaction is carried out by the plant-specific cytochrome P450-dependend monooxygenase xanthone 6-hydroxylase to form 1,3,5,6-tetrahydroxyxanthone in *C. erythraea* and 1,3,6,7-tetrahydroxyxanthone in *H. androsaemum.* Another representative of the *Gentianaceae*, chirayta (*Swertia chirata*), hydroxylates 1,3,5-trihydroxyxanthone in the C-8 position of the C-ring resulting in the major xanthone of this species 1,3,5,8-tetrahydroxyxanthone (Wang *et al.* 2003a; BIOCYC 2007).

6 FLAVONOIDS

Flavonoids comprise a widespread group of more than 4000 higher plant secondary metabolites. Many flavonoids are easily recognised as water-soluble flower pigments in most angiosperm families (flowering plants), which themselves provide the major source of food plants (fruits, vegetables, and grains). Molecules of flavonoids posses fifteen carbon atoms and contain two benzene rings (rings A and C, respectively) connected with a chain built up of three carbon atoms that becomes a part of *O*-heterocyclic (pyrane) cycle (ring B). The skeleton of flavonoids can be thus represented as the C_6-C_3-C_6 system (1,3-diarylpropanoids). Many flavonoids are hypothetically derived from flavan (IUPAC 2006) (Figure 32).

Various subgroups of flavonoids are classified according to their substitution pattern of the ring B.

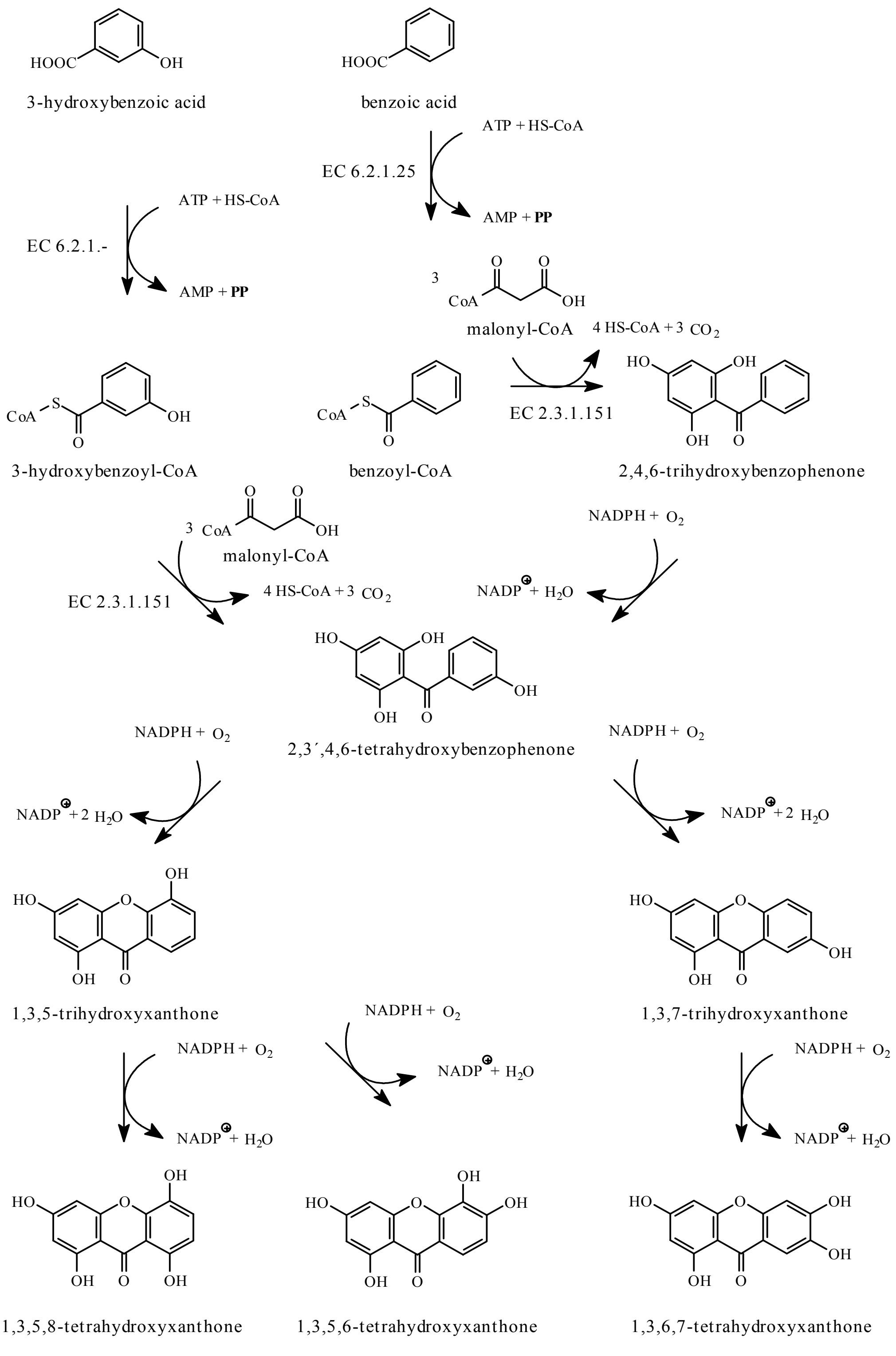
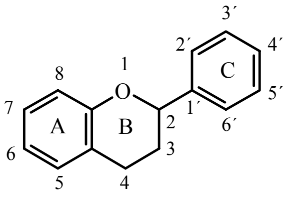


Figure 31

Both the oxidation state of the heterocyclic ring and the position of the ring B substituents are important in the classification. Derivations include oxidation of the 2(3)-carbon-carbon double bond in flavan, hydroxylation at various positions of the three rings and oxidation of the C-4 hydroxyl group. Examples of the seven major subgroups of the most important structural types are given in Figure 33. The compounds are listed according to their oxidation degree that concerns the three

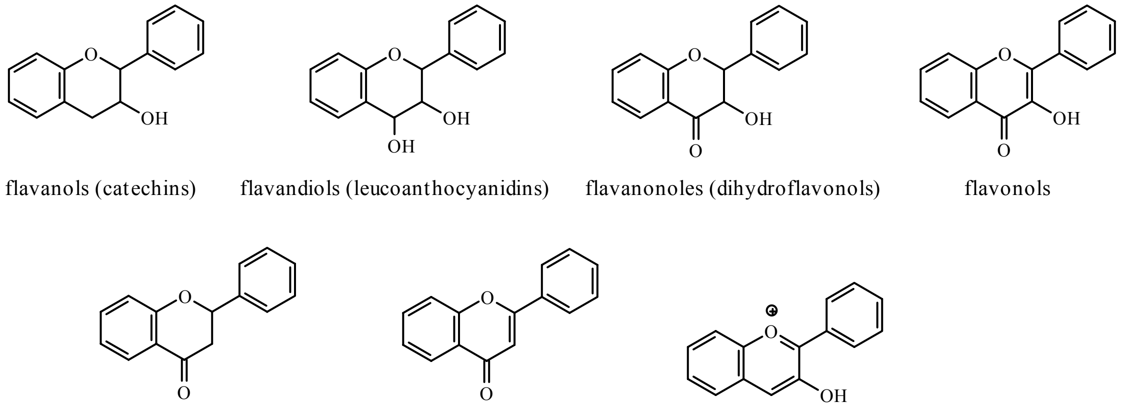


flavan

Figure 32

central carbon atoms (ring B). In the rows, the oxidation degree increases from the left to the right while in the columns the oxidation degree is equal. In the same direction their coloration increases, too. Catechins and leucoanthocyanidins are uncoloured compounds, flavanones are from uncoloured to light yellow like flavanonoles, flavones and flavonols are yellow. Due to the system of conjugated double bonds (flavilium or 2-phenylbenzopyrylium or 2-phenylchromenylium), anthocyanidins became the most prominent group of water-soluble plant pigments as they are responsible for blue, purple, violet, magenta, pink, and red coloration.[17]

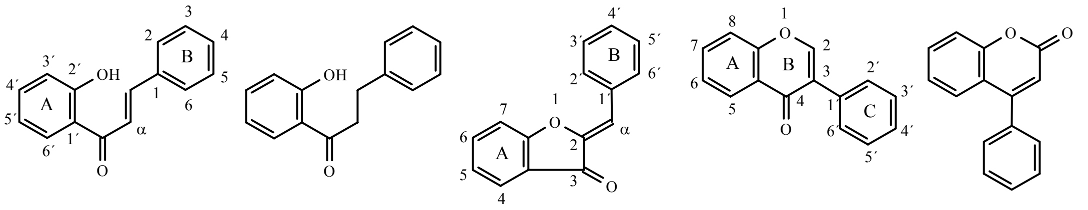
In a few cases, the six-membered heterocyclic ring B occurs in an isomeric open form (chalcones and dihydrochalcones) or is replaced by a five-membered ring (aurones). Chalcones and aurones belong to the prominent flower pigments that are responsible for their golden and yellow coloration. Isoflavonoids or 3-phenylchromen-4-ones (1,2-diarylpropanes, colourless to light yellow compounds derived from isoflavan) and neoflavonoids or 4-phenylcoumarins (1,1-diarylpropanes derived from neoflavan) are less common flavonoids having the ring C attached to the ring B at C-3 and C-4, respectively (Figure 34). Isoflavonoids and neoflavonoids can be regarded as abnormal



flavanols (catechins) flavandiols (leucoanthocyanidins) flavanonoles (dihydroflavonols) flavonols

flavanones (dihydroflavones) flavones anthocyanidins

Figure 33



chalcones dihydrochalcones aurones isoflavonoids neoflavonoids

Figure 34

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[17] According to their colour, flavonoid pigments have been classified into two large groups, the red to blue anthocyanins and the yellow anthoxanthins. Chalcones and aurones are categorised into a class of flavonoids called anthochlors or anthochlor pigments.

flavonoids[18] and are of marginal importance as plant pigments.

Most of the naturally occurring flavonoids contain multiple hydroxyl or methoxyl substituents at various positions of the rings A, B, and C. Their basic structures may be further modified by conjugation reactions like glycosylation, acylation, and polymerisation. The major proportion of flavonoids occur naturally as glycosides (Harborne 1980; Hendry & Houghton 1992; Velíšek 2002).

6.1 Chalcones and flavanones

Flavonoids (and stilbenes that are represented as the C_6-C_2-C_6 system) are products from the shikimate pathway (Dewick 2002; IUBMB 2007) (Figure 35). The cinnamoyl-CoA starter is produced from L-phenylalanine (phenylalanine deaminase, EC 4.3.1.5) and hydroxylated (cinnamate 4-hydroxylase, EC 1.14.13.11) to yield 4-hydroxycinnamic (4-coumaric, *p*-coumaric) acid, which is

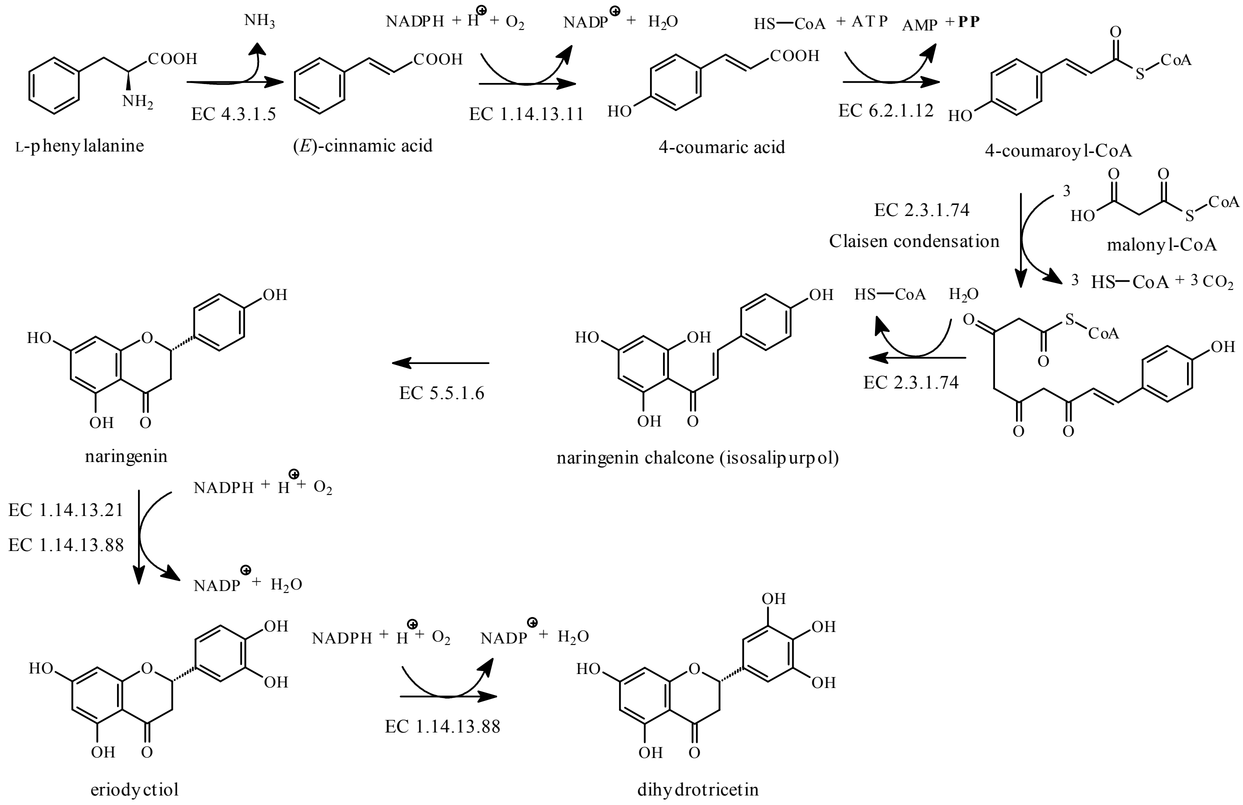

Figure 35

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[18] The colourless catechins undergo enzymatic browning reaction becoming the substrates of various oxidoreductases. Fermentation of tea leaves (*Camellia sinensis*), for example, leads to black tea pigments called oxytheotannins. They are classified as theaflavins (orange to red dimeric structures in which oxidation reactions lead to the formation of seven-membered tropolone rings) and thearubigins (heterogenous yellow, red, and brown high molecular weight pigments). Furthermore, catechins form oligomers (the condensed tannins) that contribute astringency to many fruits, foods, and drinks of plant origin. Some flavanone-7-glycosides, e.g. naringin (naringenin-7-neohesperidoside) occurring in grapefruit (*Citrus paradisi, Rutaceae*) and neohesperidin (hesperetin-7-neohesperidoside) from bitter orange (*C. aurantium*), are intensely bitter compounds. The conversion of these flavanones into dihydrochalcones produces intensely sweet compounds used as sweetening agents. Isoflavonoids are regarded as phytoestrogens as they show estrogenic activity. Some flavonoids (flavanones and flavonols) have been also commonly referred to as bioflavonoids and also referred to as vitamin P. These compounds have been appreciated as they influence the blood capillaries permeability, prevent their fragility and show activity as antioxidants and scavengers of free radicals.

subsequently transformed to 4-coumaroyl-CoA (4-coumaroyl-CoA synthetase, EC 6.2.1.12). The chain extension is provided using 3 molecules of malonyl-CoA (chalcone synthetase, EC 2.3.1.74). This initially gives a polyketide, which, depending on the enzyme available, can be folded in two different ways. These allow either aldol condensation yielding finally stilbenes or Claisen-like condensation generating chalcones that act as precursors for a vast range of flavonoids.

Flavanones are formed from the primary reaction products chalcones (chalcone-flavanone isomerase, EC 5.5.1.6) by Michael-type nucleophilic attack of a phenol group on the unsaturated ketone.[19] The key flavanone naringenin (5,7,4'-trihydroxyflavanone) may be oxidised (hydroxylated) at C-3' of the ring C by flavonoid 3'-hydroxylase (flavonoid 3'-monooxygenase, EC 1.14.13.21) or flavonoid 3',5'-hydroxylase (EC 1.14.13.88) to eriodictyol (5,7,3',4'-tetrahydroxyflavanone). The enzyme flavonoid 3',5'-hydroxylase (EC 1.14.13.88) may further oxidise eriodictyol to dihydrotricetin (5,7,3',4',5'-pentahydroxyflavanone).

Many flavonoid structures loose one of the hydroxyl groups, so that the acetate-derived aromatic ring (ring A) has a resorcinol oxygenation pattern rather than the phloroglucinol system (Dewick 2002). This modification has been tracked down to the action of a reductase enzyme 6'-deoxychalcone synthase (EC 2.3.1.70), and thus a chalcone isoliquiritigenin (2',4,4'-trihydroxychalcone) is produced rather than naringenin chalcone (2',4,4',6'-tetrahydroxychalcone) (IUBMB 2007) and yields liquiritigenin (4',7-dihydroxyflavanone), the precursor of isoflavones (Figure 36).

6.1.1 Quinochalcones

The petals of safflower (*Carthamus tinctorius*) 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 vegetable oil used quite commonly as an alternative to sunflower seed oil. Its is supposed that these pigments are biosynthesised, 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 37). Precarthamin forms from safflor yellow B by oxidation of the open-chain D-glucose residue. Oxidative decarboxylation of precarthamin catalysed by precarthamin decarboxylase yields carthamin (Cho & Hahn 2000).

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 (flavanones 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),

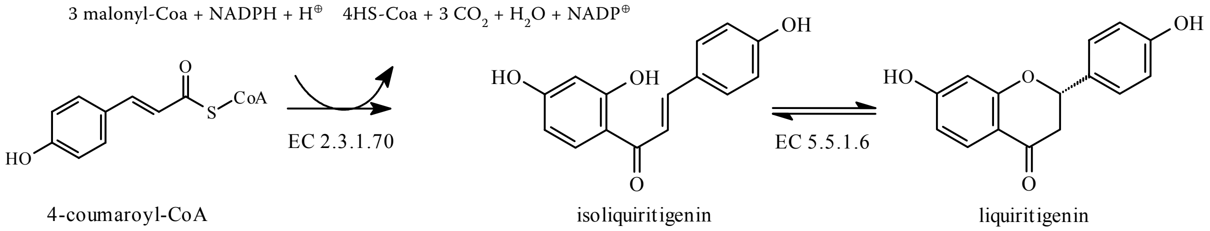

Figure 36

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[19] This isomerisation can occur non-enzymatically under acid conditions that favour the flavanone whereas basic conditions favour the chalcone. The enzyme-catalysed reaction is stereospecific and results in the formation of a single flavanone enantiomer, (2*S*)-flavanone.

and epicatechins[20] (anthocyanidin reductase, EC 1.3.1.77) (Figure 38).

Modifications to the hydroxylation patterns in the two aromatic rings may occur, generally at the flavanone, flavanonole, or flavone stage, and methylation, *O*- glycosylation, *C*-glycosylation, and dimethylallylation are also common, increasing the range of compounds enormously. Figure 39 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 40. 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.-) (IUBMB 2007; BIOCYC 2007).

6.3 Aurones

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

The pivotal precursors of aurones are chalcones. The minor 4,4',6-trihydroxyaurone can form from naringenin chalcone by a not yet characterised oxygenase enzyme. The enzyme aureusidin synthase (a copper-containing glycoprotein and a homologue of plant polyphenol oxidase, EC 1.21.3.6) catalyses the 3-hydroxylation of the ring B and the oxidative cyclisation (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

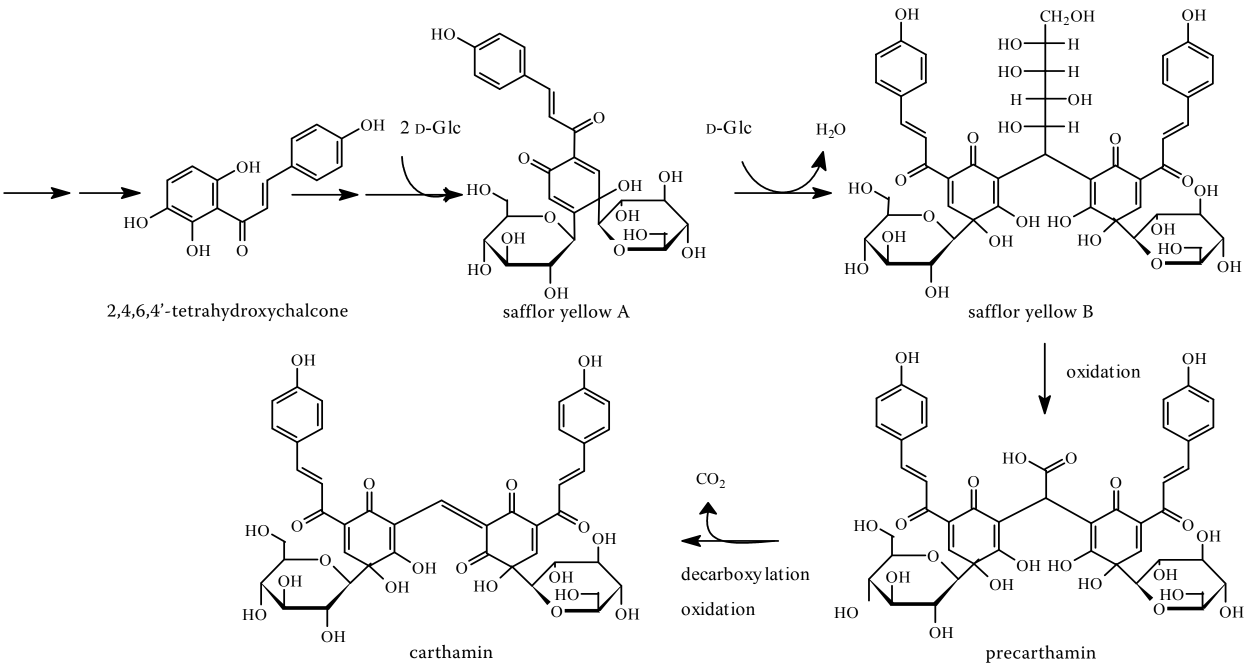

Figure 37

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

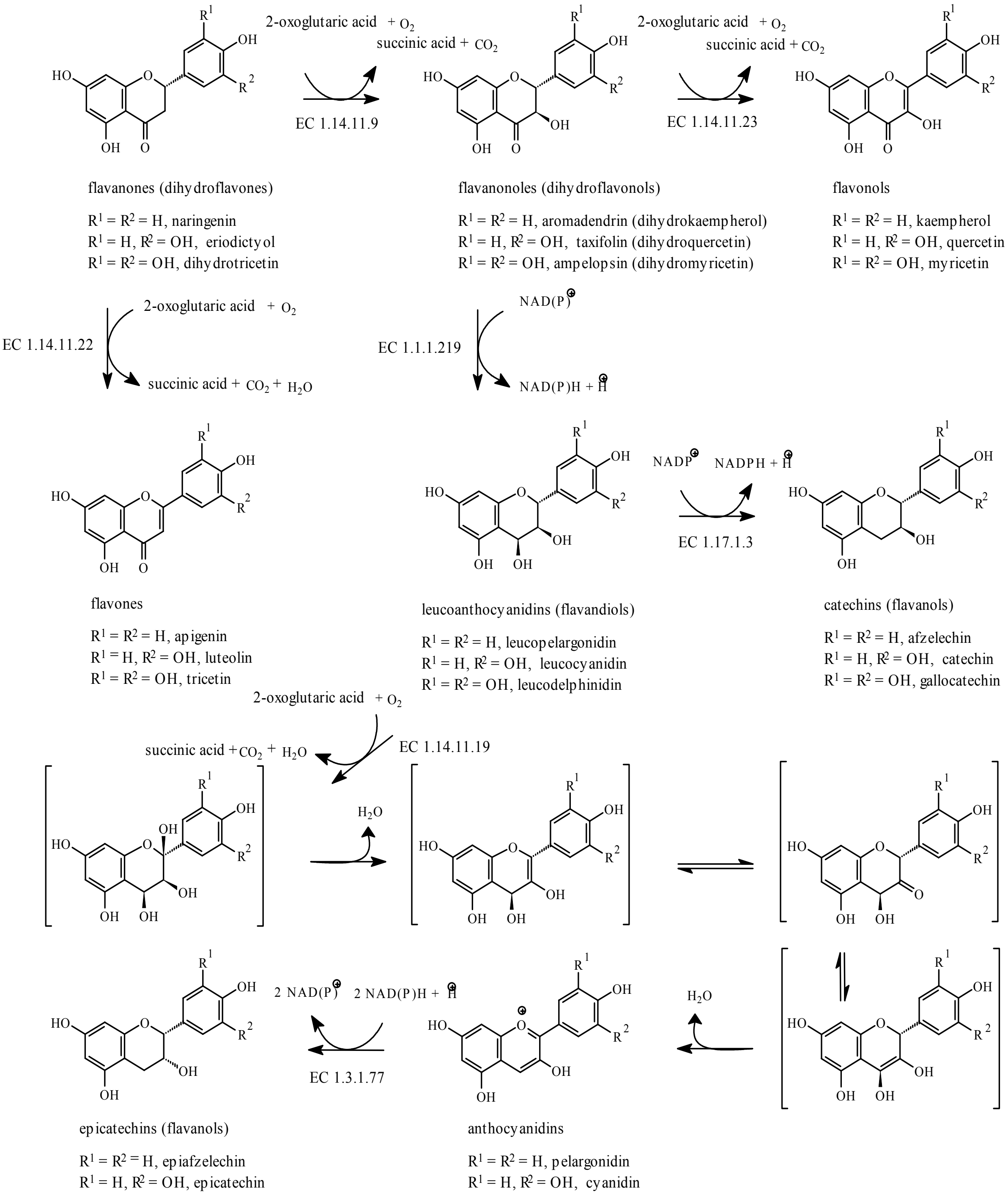

Figure 38

a 3,4-dihydroxy B-ring is not necessarily accompanied by B-ring oxygenation (Sato *et al.* 2001; Nahayama *et al.* 2001; Nahayama 2002). Thus, aureusidin may be formed from naringenin chalcone or 2',3,4,4',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 2',3,4,4',6'-pentahydroxychalcone becomes the substrate of aureusidin synthase, analogous reactions yield a mixture of aureusidin and bracteatin (Figure 41). Aurones with no or only one hydroxyl group in the ring B are produced by alternative mechanisms.

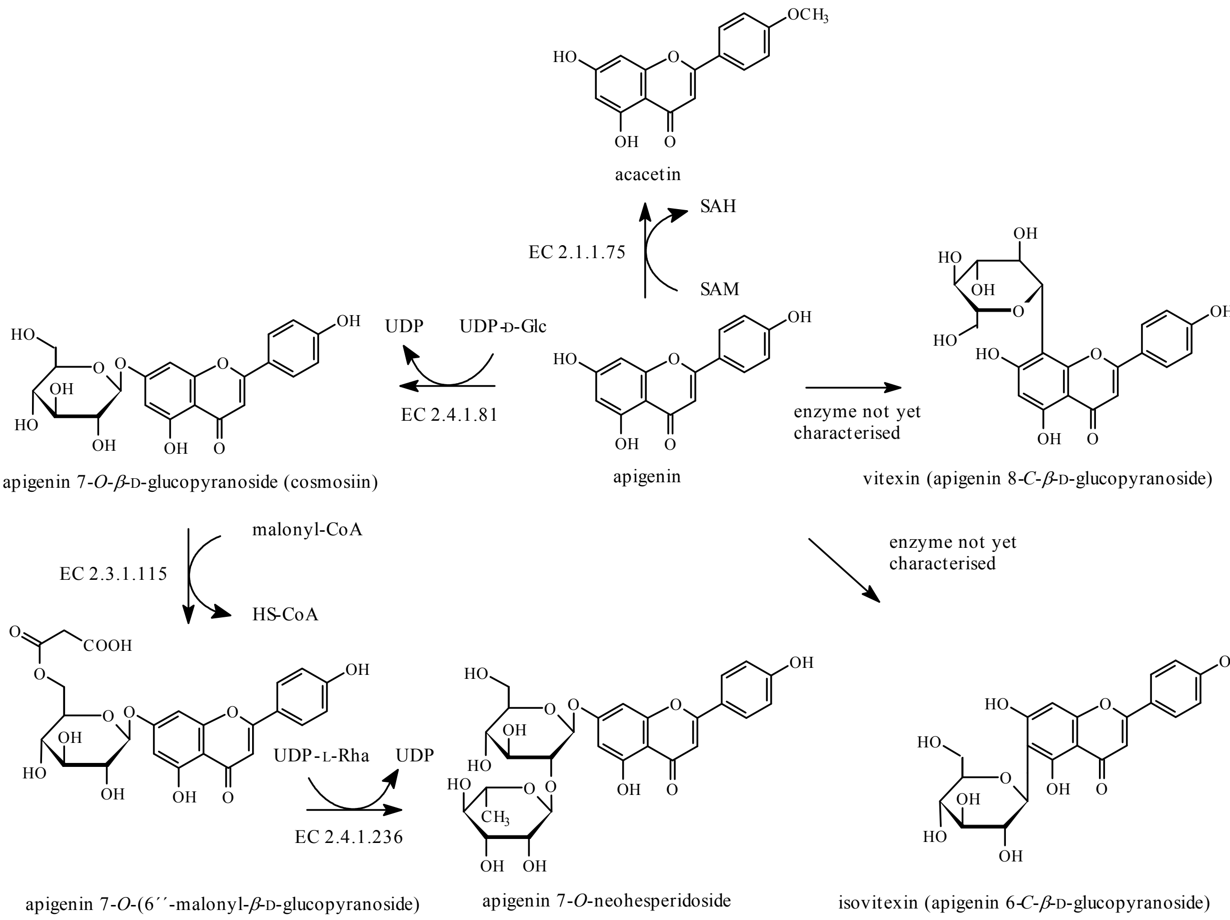

Figure 39

6.4 Miscellaneous flavonoids

Red sandalwood, called also red sanderswood, sanders or saunders, and rubywood, is the dark red heartwood of the leguminous tree *Pterocarpus santalinus* (*Fabaceae*) of India. Sandalwood red is a pigment obtained by extracting red sandalwood with water. It was used as a wool colouring and also a food colouring in medieval and renaissance food (jellies, custards) and has been used in Ayurvedic medicine as an anti-septic wound healing agent and in anti-acne treatment. Its major red components are lipophilic substances santalin A and santalin B. Some other structurally similar compounds (e.g. santalin AC and other similar compounds) were identified (Kinjo *et al.* 1995).

It is supposed that the first phase of santalins A and B biosynthetic mechanism starts with isoflavilium salts as potential electrophiles and an open chain C_{15} unit as a nucleophile (Kinjo *et al.* 1995). After the initial coupling of the isoflavilium ion and this open chain unit, a carbonium ion at C-4' could generate an intermediate able to cyclise intramolecularly, which is followed by aromatisation to yield santalin A. Its methylation at C-13' hydroxyl gives santalin B. Hydroxylation of the isoflavilium salt followed by oxidation yields santalin AC (Figure 42).

7 ISOCHROMENES

Red yeast rice, red koji rice, or *ang-khak* is a bright purplish-red fermented rice, which acquires its colour and a subtle but pleasant taste by cultivation with the mold *Monascus purpureus* (syn. *M. albidus*, *M. anka*, *M. araneosus*, *M. major*, *M. rubiginosus*, and *M. vini*). It was commonly used for red food coloring in East Asian and Chinese cuisine prior to the discovery of chemical food colours and is still used to colour a wide variety of food products including pickled tofu, red rice vinegar, Peking duck or chinese pastries that require red food coloring. It is used traditionally in the production of several types of Chinese wine, Japanese sake (*akaisake*), and Korean rice wine

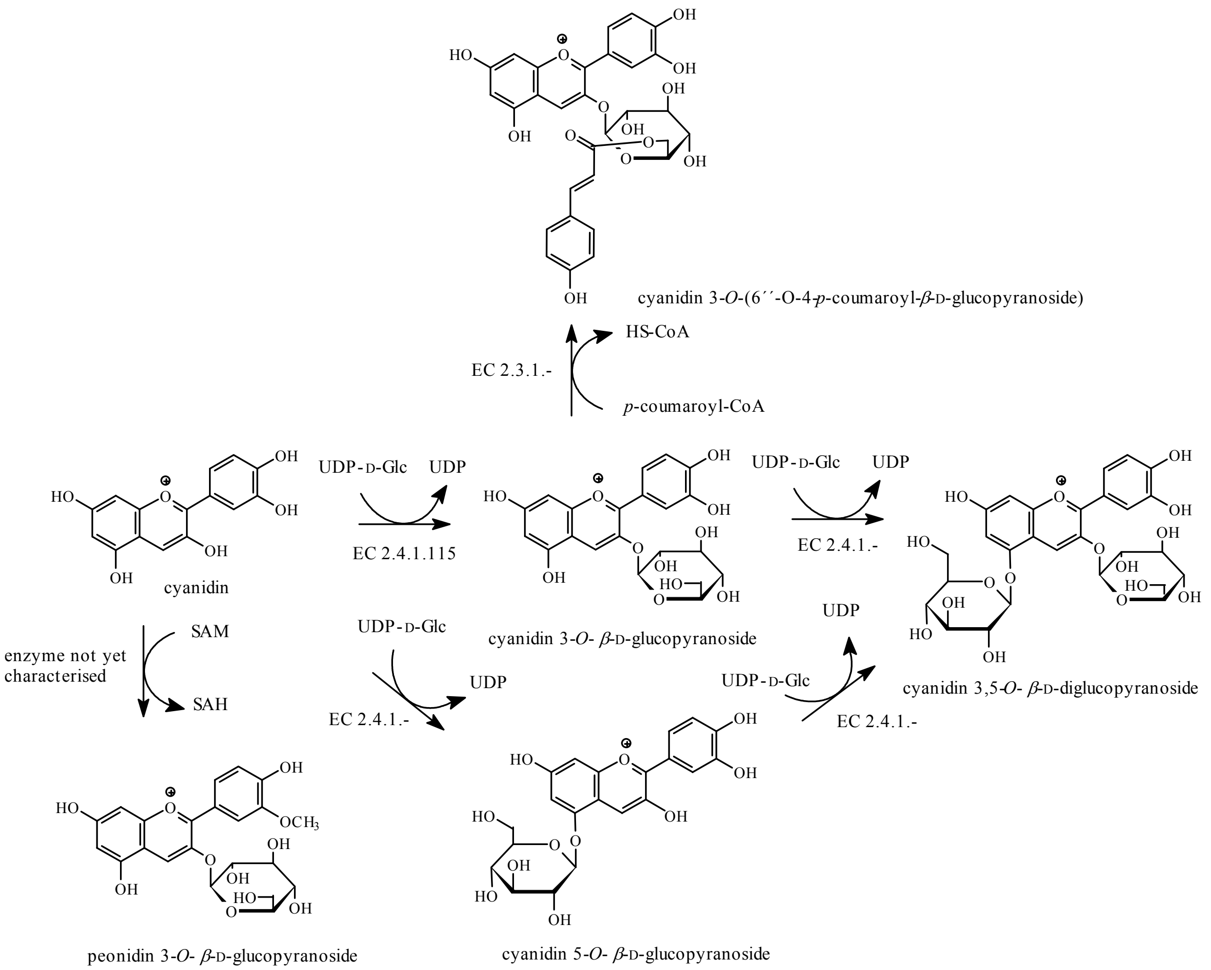

Figure 40

(*hongju*) giving them a reddish colour (Ma *et al.* 2000; Wild *et al.* 2002). It has also been used in Chinese herbal medicine.[21]

The major *Monascus* pigments (called monascus red or just monascus) are a mixture of lipophilic azaphilones. They comprise yellow pigments monascin and ankaflavin, their oxidised forms (orange pigments) monascorubin and rubropunctatin, and their nitrogen analogues (red pigments) rubropunctamine and monascorubramine) which are derived from 1*H*-isochromene (Figure 43).

The main polyketide[22] chain of the azaphilone pigments is assembled from acetic acid (the starter unit) and five malonic acids molecules (the chain extender unit) in a conventional way. The hexaketide chromophore is possibly synthesised via monascusone A. The side chain of these pigments forming a fused γ-lactone system is derived by condensation of 2-oxohexanoic acid and 2-oxooctanoic acids synthesised via fatty acid synthetic pathway (Figure 44). Nitrogen analogues of azaphilones (Figure 45) form by their reactions with amino acids (Ogihara *et al.* 2000; Jongrungruangchok *et al.* 2004).

8 CURCUMINOIDS

Curcuminoids comprise a group of polyphenols related to lignanes. Molecules of curcuminoids contain two benzene rings connected with a chain built up of seven carbon atoms (diarylheptanoids), which can be thus represented as the C_6-C_7-C_6

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[21] Recent discoveries of cholesterol-lowering statins (a class of hypolipidemic agents used to lower cholesterol levels in people with or at risk for cardiovascular disease) produced by the mold and other fungi has prompted research into the red yeast rice possible medical uses (Furberg 1999).

[22] In filamentous fungi, the polyketide pathway is the major route for the biosynthesis of secondary metabolites, including various mycotoxins.

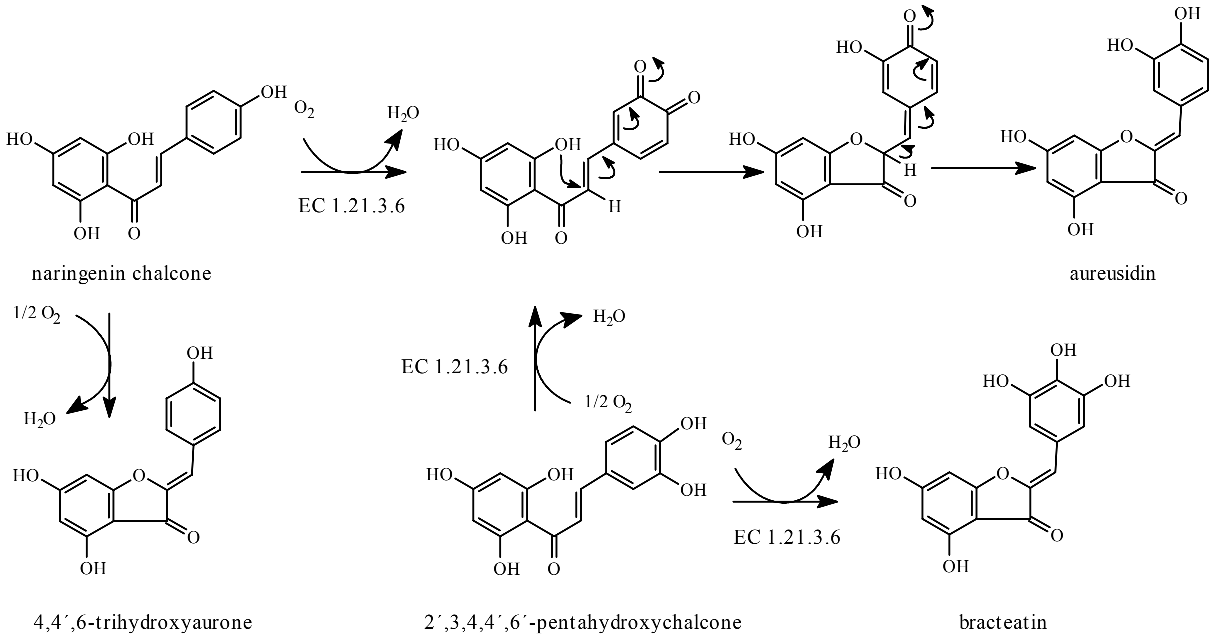

Figure 41

system. Curcuminoids are responsible for the yellow colour of turmeric (*Curcuma longa*), a member of the ginger family (*Zingiberaceae*). Its dried roots are ground into a deep yellow spice[23] that is a significant ingredient in most commercial curry powders and has found application in canned beverages, dairy products, ice creams, yoghurts, biscuits, sweets, cereals etc. Curcumin, i.e. (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione, is the principal lipophilic pigment of turmeric (less then 2% dry matter). Curcumin is accompanied by two other minor pigments (about 0.6% dry matter), demethoxycurcumin and bisdemethoxycurcumin.

Similarly to flavonoids, curcuminoids are derived from the intermediates in the phenylpropanoid pathway such as 4-coumaryl-CoA that condenses with malonyl-CoA (perhaps involving a diketide intermediate) yielding bisdemethoxycurcumin (Ramirez-Ahumada *et al.* 2006). Enzymes similar to polyketide synthase (called curcuminoid synthase) are most likely responsible for the formation of the basic backbone structure of curcuminoids (Figure 46). Bisdemethoxycurcumin is then hydroxylated (cytochrome P450 hydroxylase) and methylated (using SAM-dependent *O*-methyltransferase) to yield demethoxycurcumin, which on further hydroxylation and methylation yields curcumin (the enzymes were not yet characterised). Curcumin can exist in at least two tautomeric forms, keto and enol. The enol form is more stable in solutions.

It is speculated that demethoxycurcumin can alternatively form from 4-coumaroyl-CoA and caffeoyl-CoA instead of two molecules of 4-coumaroyl-CoA by the direct action of polyketide synthase. Analogously, curcumin might be formed from two molecules of feruoyl-CoA (Ramirez-Ahumada *et al.* 2006). The enzymes involved in the biosynthesis of these phenolic acid derivatives from 4-coumaroyl-CoA are shikimate *O*-hydroxycinnamoyltransferase (EC 2.3.1.133), *p*-coumaroyl-5-*O*-shikimate 3'-hydroxylase (EC 1.14.13.36) and caffeoyl-CoA *O*-methyltransferase (EC 2.1.1.104) (IUBMB 2007). The biosynthesis of shikimic acid envolved in these reactions was already reviewed (Velíšek & Cejpek 2006).

9 CAROTENOIDS

Carotenoids are a group of more than 700 very common yellow, orange and red (exceptionally

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[23] Turmeric is also thought to have many medicinal activities such as antiseptic and antibacterial. It is currently being investigated for possible benefits in Alzheimer's disease, cancer, and liver disorders. Colourless tetrahydrocurcuminoids from turmeric may have antioxidant, skin lightening properties, and may be used to treat skin inflammations, making these compounds useful in cosmetic formulations.

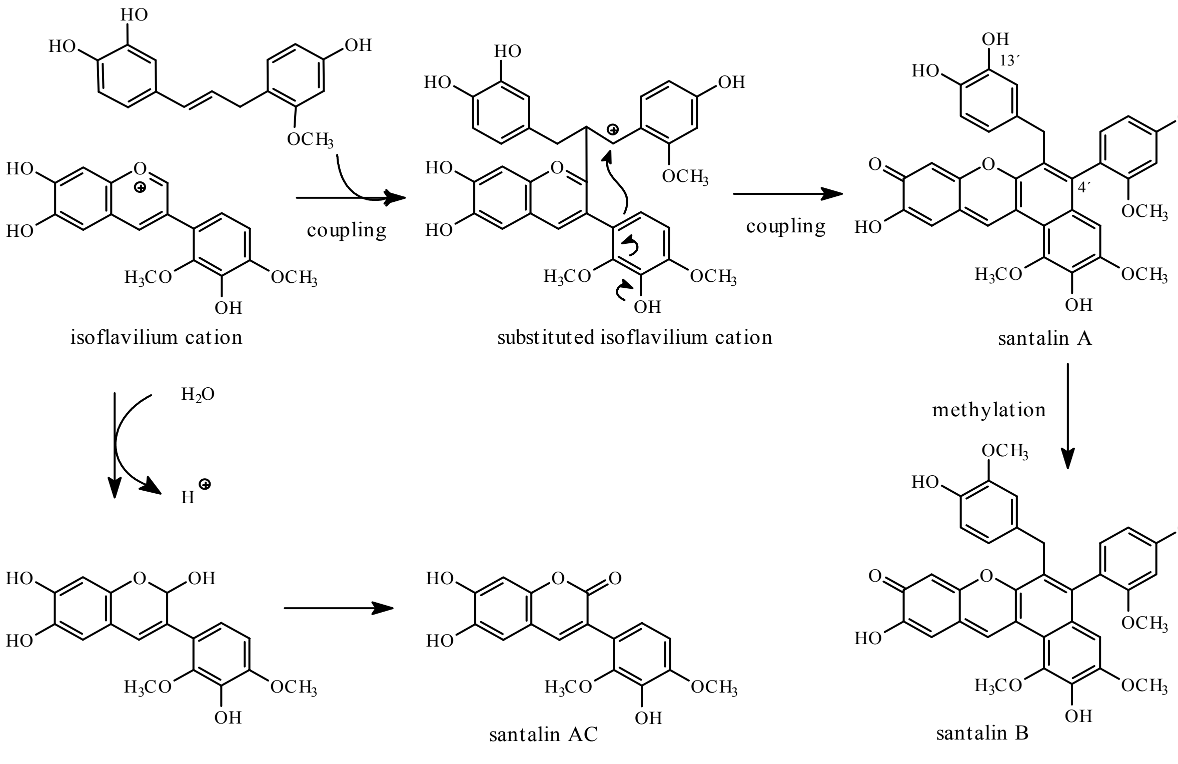
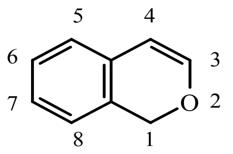


Figure 42

also yellow-green) lipophilic pigments that occur in all organisms capable of photosynthesis including green algae, higher plants, and also some bacteria. The coloration of many flowers, fruits, vegetables, and grains is caused by carotenoid-containing chromoplasts that are usually devoid of chlorophylls. Unlike chlorophylls and many other plant pigments, they are also found in various animals and animal products (milk, butter, egg yolk, salmon, shrimp) though they are always of the plant origin (Sandmann 1994).

All carotenoids are terpenoids (tetraterpenoids) as they contain 40 carbon atoms in eight C_5 isoprene units linked in a regular head to tail manner, except in the centre of the molecule where the order is inverted tail to tail, so that molecule is symmetrical. The two methyl groups near the centre are separated by six carbon atoms and the other methyl groups by five carbon atoms. Almost all other carotenoids are formally derived from this basic non-cyclic $C_{40}H_{56}$ hydrocarbon by subsequent hydrogenation, oxidation, ring formation (cyclisation), shifting of double bonds and/or methyl groups, chain shortening, and combinations of these reactions.



1*H*-isochromene

Figure 43

Carotenoids can be classified into carotenes (hydrocarbons without additional *O*-containing groups) and xanthophylls (carotenoids containing oxygen functions). The most frequently found *O*-containing functions are hydroxyl groups (monols, diols, and polyols), epoxy-(5,6-epoxides and 5,8-epoxides), methoxy-, formyl-, oxo-, carboxy-, and ester groups. Some xanthophylls can be bound to proteins and sugars.

9.1 Carotenes

The biosynthesis of carotenoids involves a series of steps, i.e. the formation of the key building stone mevalonic acid (a product of acetic acid metabolism) which gives rise to isopentenyl diphosphate, the common C_5 precursor of all natural isoprenoids, the formation of geranylgeranyl diphosphate, the formation of acyclic phytoene, cyclisation to form alicyclic carotenes, and the formation of xanthophylls.

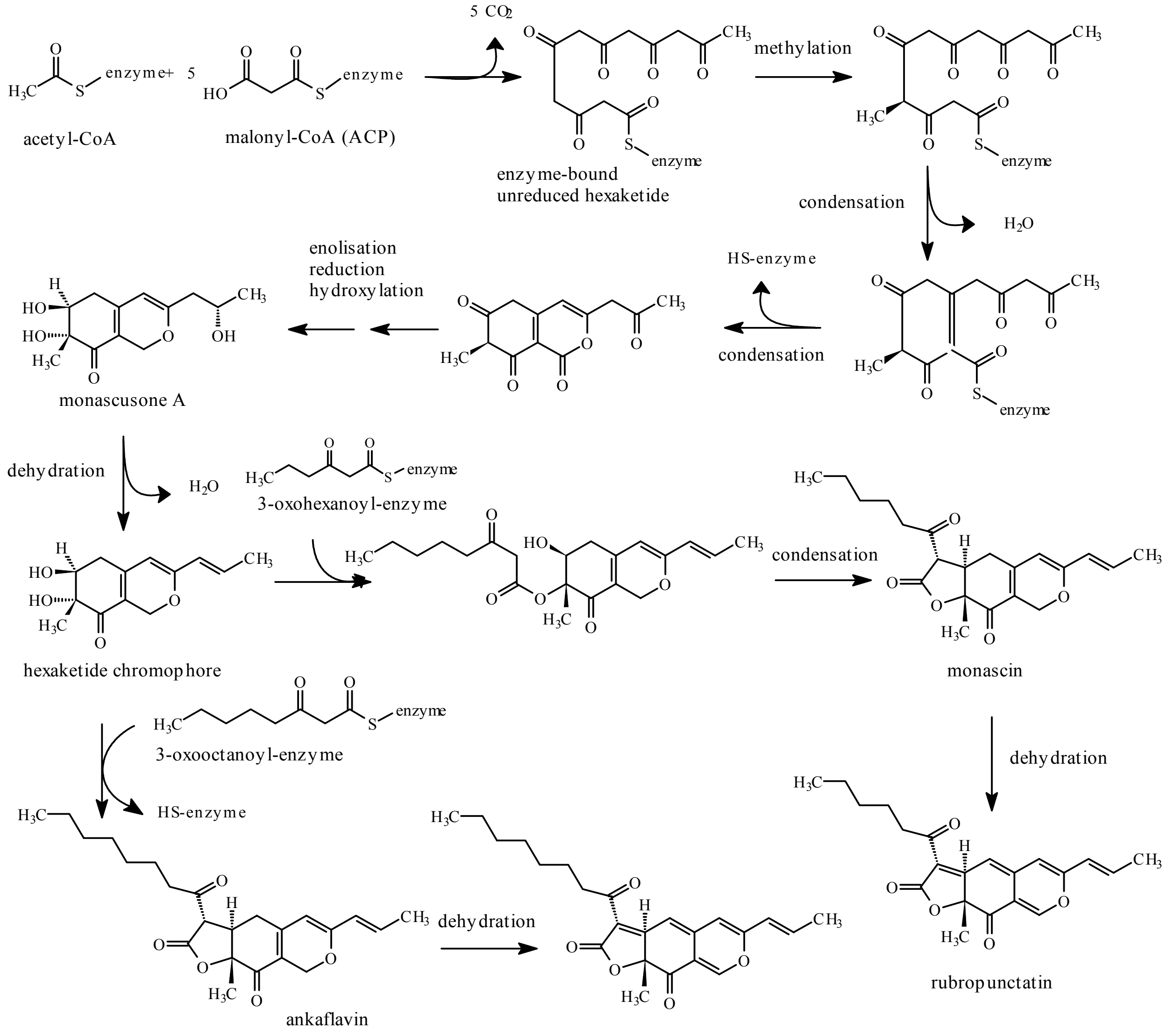
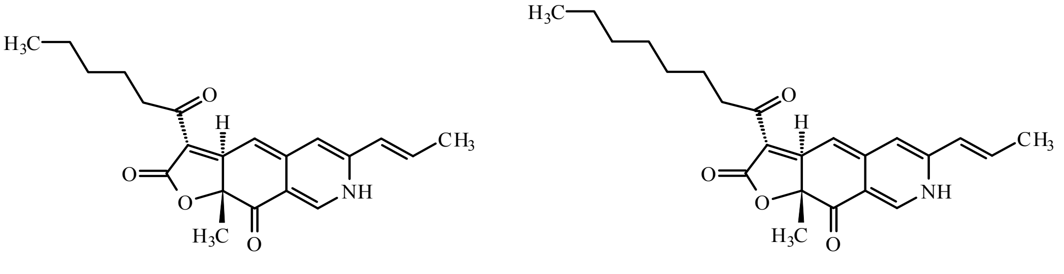


Figure 44

Through the condensation of two molecules of geranylgeranyl diphosphate (electrophilic addition giving tertiary cation), catalysed by phytoene synthase (EC 2.5.1.32), phytoene (15-*cis*-7,8,11,12,7',8',11',12'-octahydro-ψ,ψ-carotene), the first carotene, is formed via a cyclopropyl compound prephytoene diphosphate (Figure 47) (IUBMB 2007).

For the phytoene formation, a proton is lost from C-15 generating a new double bond in the centre of the molecule, and thus a short conjugated chain is developed. In fungi and higher plants, 15-*cis*-phy-



rubropunctamine

monascorubramine

Figure 45

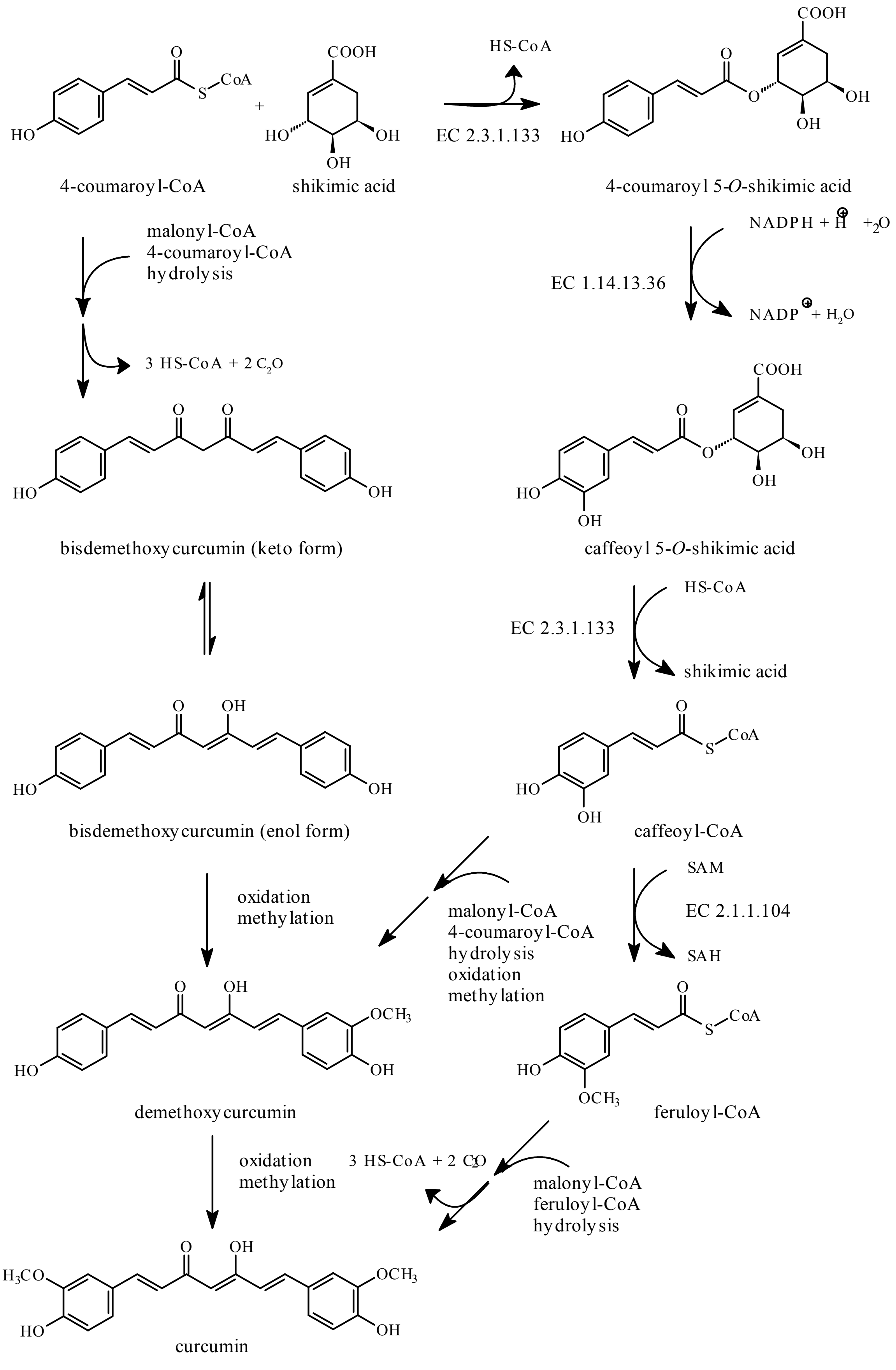

4-coumaroyl-CoA
shikimic acid
HS-CoA
EC 2.3.1.133
4-coumaroyl 5-O-shikimic acid
malonyl-CoA
4-coumaroyl-CoA
hydrolysis
3 HS-CoA + 2 C2O
NADPH + H⁺ + 2O
EC 1.14.13.36
NADP⁺ + H2O
bisdemethoxycurcumin (keto form)
caffeoyl 5-O-shikimic acid
HS-CoA
EC 2.3.1.133
shikimic acid
bisdemethoxycurcumin (enol form)
caffeoyl-CoA
oxidation
methylation
malonyl-CoA
4-coumaroyl-CoA
hydrolysis
oxidation
methylation
SAM
EC 2.1.1.104
SAH
demethoxycurcumin
feruloyl-CoA
oxidation
methylation
3 HS-CoA + 2 C2O
malonyl-CoA
feruloyl-CoA
hydrolysis
curcumin

Figure 46

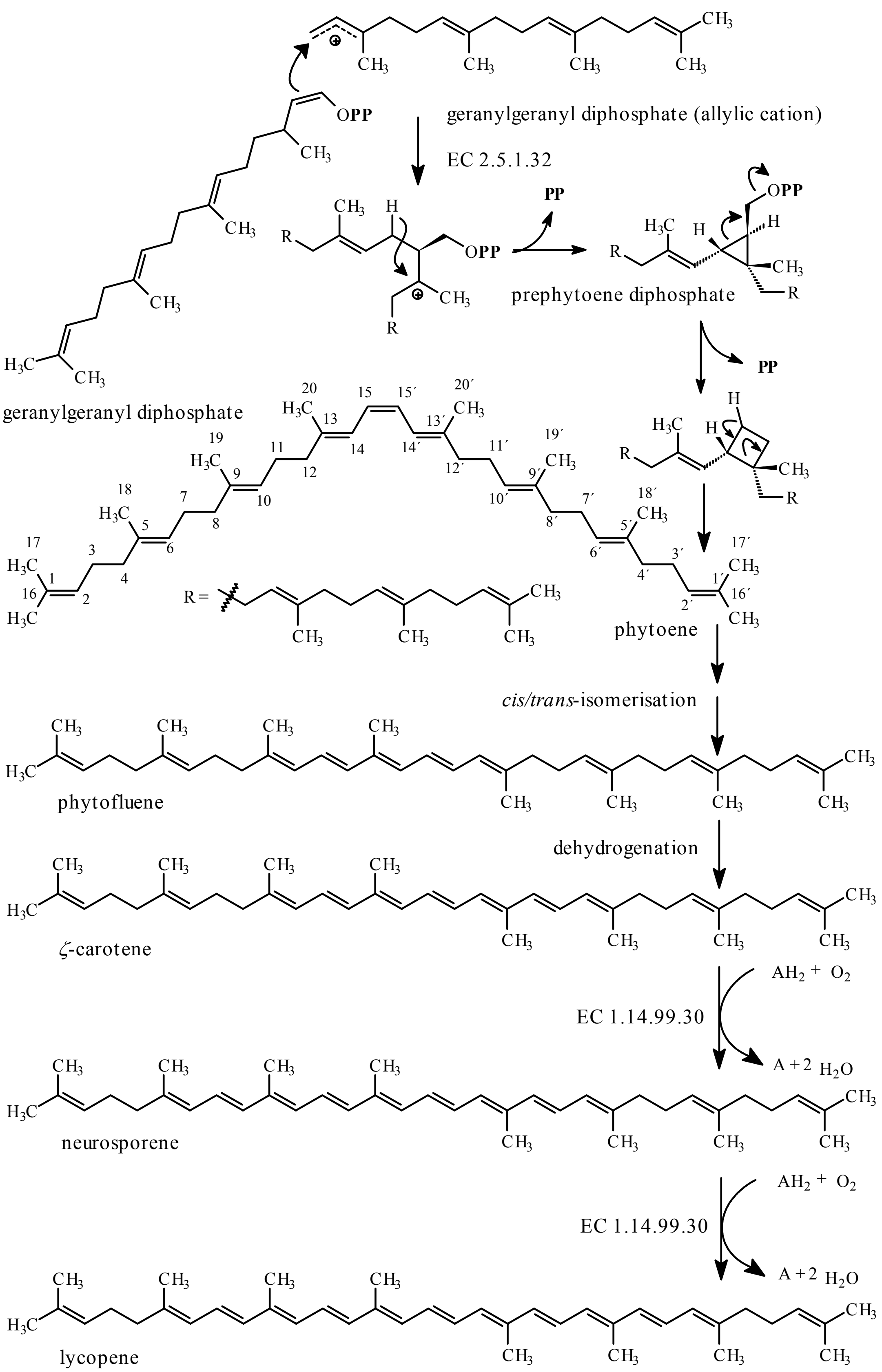

geranylgeranyl diphosphate (allylic cation)
EC 2.5.1.32
OPP
PP
prephytoene diphosphate
PP
geranylgeranyl diphosphate
R =
phytoene
cis/trans-isomerisation
phytofluene
dehydrogenation
ζ-carotene
EC 1.14.99.30
AH2 + O2
A + 2 H2O
neurosporene
EC 1.14.99.30
AH2 + O2
A + 2 H2O
lycopene

Figure 47

toene is generally found, the 15-*trans*-isomer occurring in some bacteria. Their occurrence depends on the stereochemistry of the hydrogen removal from C-15 (Gross 1991). Then, through the dehydrogenation sequence, the chromophore is extended by two double bonds alternately from either side so that phytoene is converted into lycopene (thirteen double bonds, eleven of which are conjugated) through stepwise dehydrogenation via phytofluene, ζ-carotene, and neurosporene (carotene 7,8-desaturase, EC 1.14.99.30) (IUBMB 2007). Since all the carotenes have all-*trans* configuration, a *cis/trans*-isomerisation of the central double has to be involved in fungi and plants, probably at the phytoene level. As a result, plants accumulate small amounts of the *cis*-carotenoids.

Acyclic carotenes, such as phytoene, phytofluene, ζ-carotene, and neurosporene, generally occur in small amounts. In nature, they accompany the major carotenes and xanthophylls, e.g. in the oil obtained from palm *Elaeis guineensis*, *Arecaceae* (Tai & Choo 2000). Lycopene, however, is the major red pigment of e.g. ripe tomatoes (*Solanum lycopersicum*, syn. *Lycopersicon esculentum*, *Solanaceae*). The majority of carotenes (and xanthophylls) found in nature are cyclic[24]

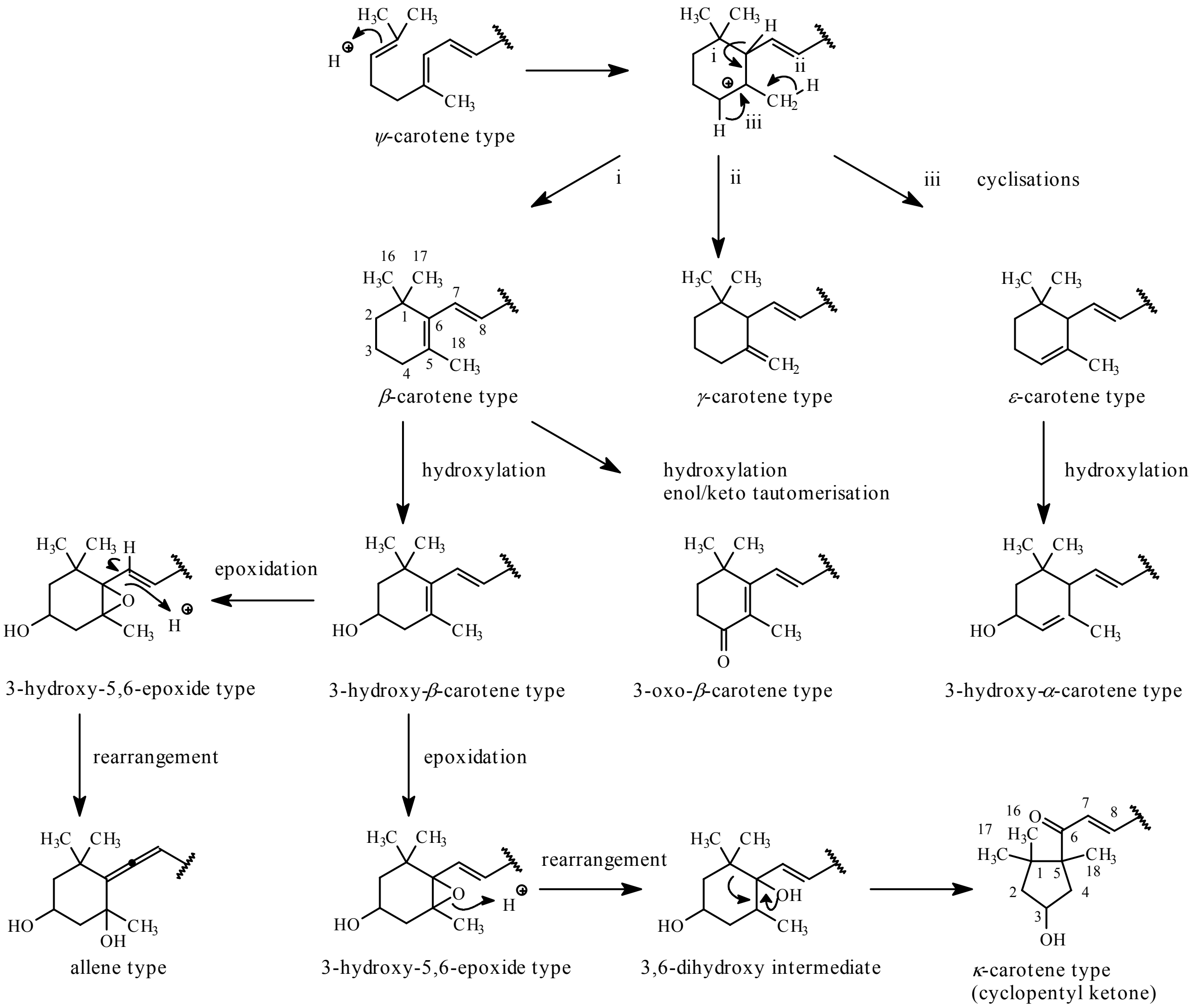


Figure 48

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[24] Carotenoids with cyclic ends are integral constituents of plants, algae, and cyanobacteria photosynthetic reaction centers. Higher plant chloroplasts typically accumulate lutein, β-carotene, violaxanthin, and neoxanthin in the thylakoid membrane-bound photosystems. β-Carotene is generally found in the reaction center where it plays a critical photoprotective role by quenching triplet chlorophyll and singlet oxygen, and can undergo a rapid degradation during photooxidation. In the chromoplasts of ripening fruits, flower petals, and the chloroplasts of senescing leaves, carotenoids are found in membranes or in oil bodies or other structures within the stroma.

at their termini and loose, as a consequence, the terminal double bond(s). The cyclisation of one or both end groups of the linear all-*trans*-lycopene (*ψ*-carotene type) into carotenes with *β*-rings (*β*-carotene type), *ε*-rings (*ε*-carotene type) or not very common *γ*-rings (*γ*-carotene type) is an important branch-point in the biosynthesis of carotenoids. The cyclisation reaction are catalysed by lycopene *β*-, *ε*- or *γ*-cyclases (EC 5.5.1.-) that are not yet well characterised[25] (Figure 48). The reaction sequences and the enzymes involved are schematically shown in Figure 49.

The best-known carotene produced from lycopene (*ψ*,*ψ*-carotene) is *β*,*β*-carotene (known as *β*-carotene), the major pigment of carrot (*Daucus carota*, *Apiaceae*), which has two *β*-rings (Figure 50). If this ring is formed at just one terminus, then the result is *β*,*ψ*-carotene (*γ*-carotene); if the *ε*-ring is formed at just one terminus of the molecule, then *ε*,*ψ*-carotene (*δ*-carotene) forms. If at one terminus the *β*-ring and at the second terminus the *ε*-ring are formed, then the product is *β*,*ε*-carotene (known as *α*-carotene). Carotene with two *ε*-rings (*ε*-carotene) is usually found in trace amounts.

9.2 Xanthophylls

Carotenes are oxygenated in various ways (hydroxylation, epoxydation, etc.) as a late step in the biosynthesis to form xanthophylls which represent most of the carotenoid pigments in plants. The reaction mechanisms are given in Figure 48

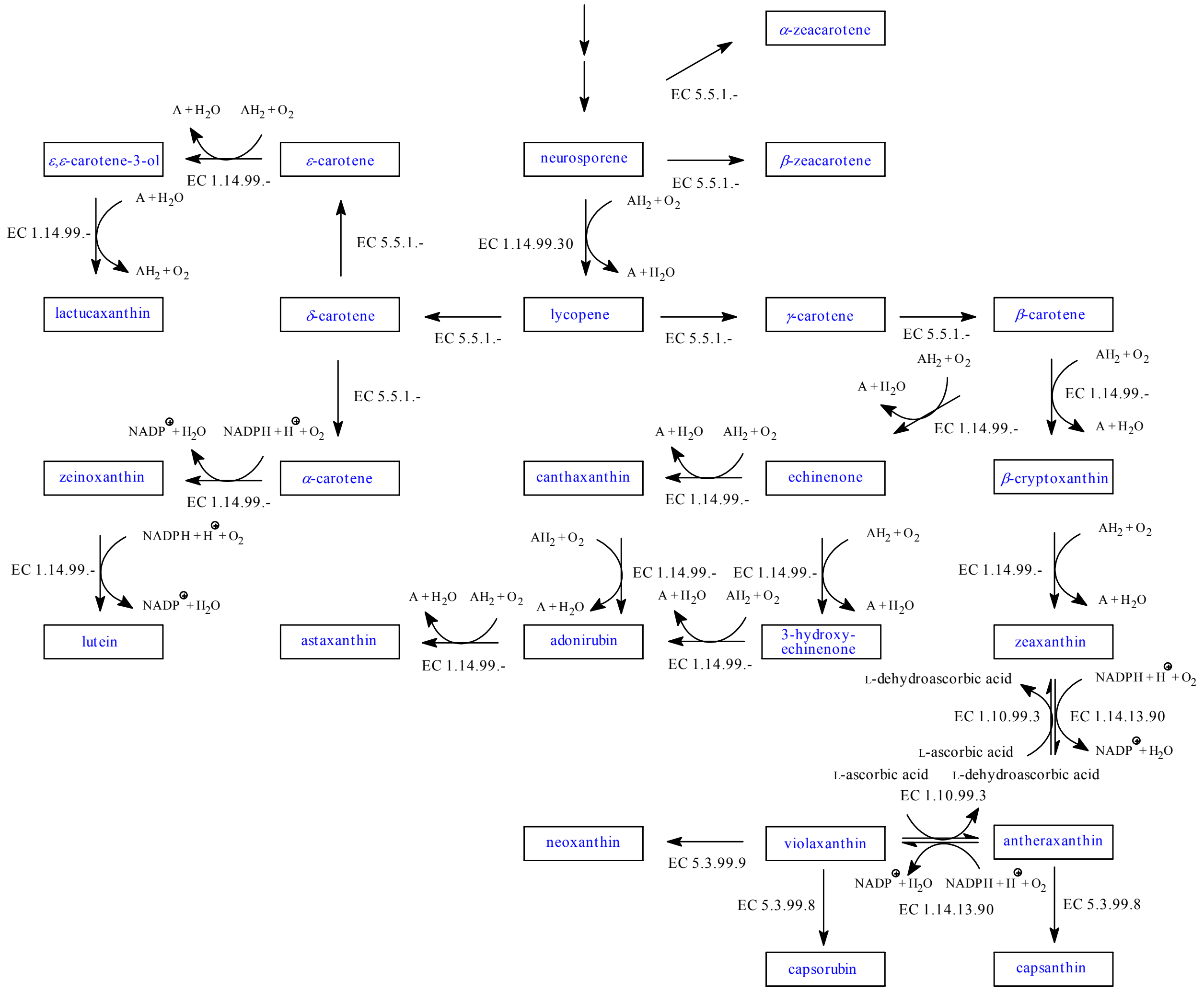

Figure 49

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[25] Lycopene *ε*-cyclase (EC 5.5.1.-) also catalyses the formation of *α*-zeacarotene (one *ε*-ring) from neurosporene, while under the catalysis of lycopene *β*-cyclase (EC 5.5.1.-) *β*-zeacarotene (one *β*-ring) is formed (Figures 51 and 52). Both zeacarotenes occur e.g. in palm oil as the minor pigments (Tai & Choo 2000).

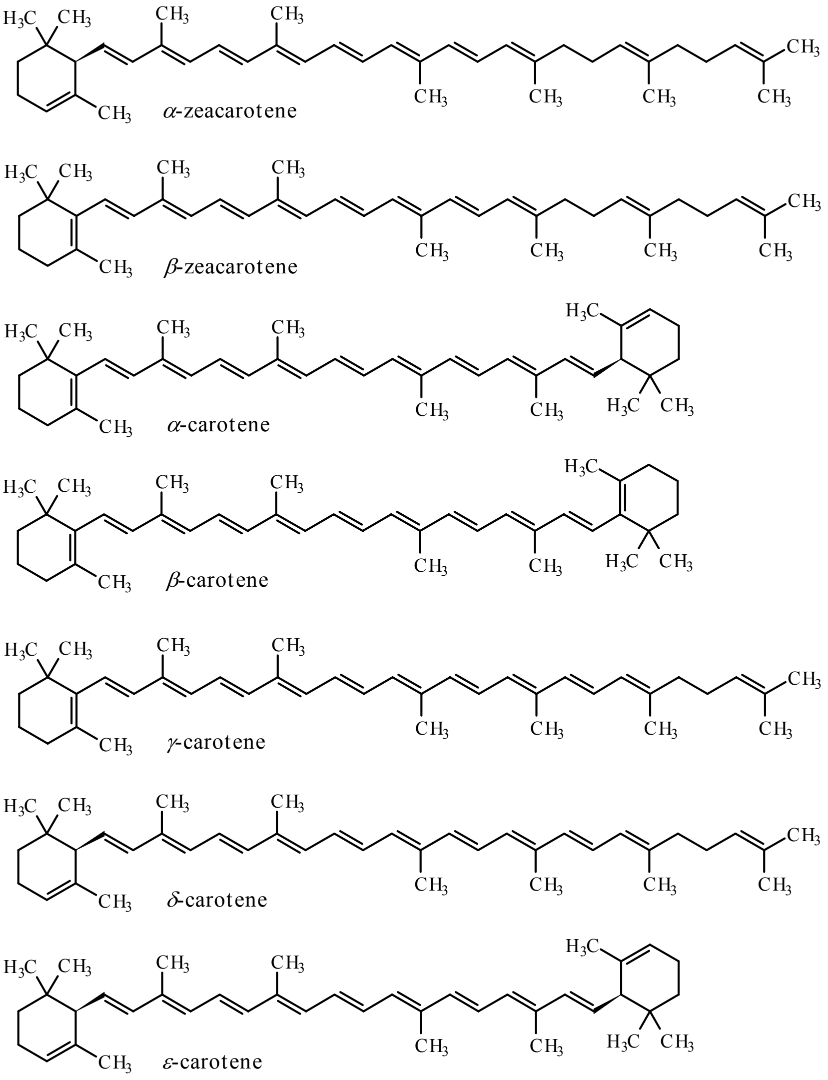

Figure 50

and the sequences leading to the most prominent xanthophylls are schematically shown in Figure 49. The enzymes envolved include NADPH-dependent β-ring hydroxylase (EC 1.14.99.-), NADPH-dependent ε-ring hydroxylase (EC 1.14.99.-), β-ring carotene 3-hydroxylase using reduced substrates AH_2 (EC 1.14.99.-), β-ring carotenoid 4-ketolase (EC 1.14.99.-) using reduced substrates AH_2, violaxanthin de-epoxidase (EC 1.10.99.3), zeaxanthin epoxidase (antheraxanthin epoxidase, EC 1.14.13.90), capsanthin-capsorubin synthase (EC 5.3.99.8), and neoxanthin synthase (EC 5.3.99.9). Structures of the most important xanthophylls are given in Figure 51.

Hydroxylation plays an important role in the oxygenation of carotenes and is catalysed by hydroxylases acting specifically onto the individual rings (e.g. on β-rings or ε-rings etc.) (Figure 48). Zeinoxanthin (β,ε-carotene-3-ol), for example, is formed from α-carotene by hydroxylation of the β-ring under the action the NADPH-dependent β-ring hydroxylase (EC 1.14.99.-). Lutein (β,ε-carotene-3,3'-diol) is a product of zeinoxanthin hydroxylation using NADPH-dependent ε-ring hydroxylase (EC 1.14.99.-). β-Cryptoxanthin (β,β-carotene-3-ol) forms from β-carotene by hydroxylation under the catalysis of β-carotene 3-hydroxylase (EC 1.14.99.). Zeaxanthin (β,β-carotene-3,3'-diol is a dihydroxyderivative of β-carotene formed from β-cryptoxanthin by the action of the same enzyme. Echinenone (β,β-carotene-4-one) is formed from β-carotene under the catalysis of β-ring carotenoid 4-ketolase (EC 1.14.99.-).

Lettuce (*Lactuca sativa*, *Asteraceae*) is a rare example of plants known to accumulate substantial amounts of lactucaxanthin. Zeaxanthin is the yellow pigment of corn (*Zea mays*, *Poaceae*) and many other plant materials, astaxanthin (*β*,*β*-carotene-3,3'-dihydroxy-4,4'-dione) or (3*S*,3*S*')-astaxanthin is commonly found in marine animals and is responsible for the pink to red coloration of crustaceans, shellfish, and some fish (e.g. salmon). These animals are unable to synthesise carotenoids and astaxanthin is produced by the modification of *β*-carotene obtained from the diet (Velíšek 2002). Crustaceans (e.g. shrimps, prawns, and crabs) have astaxanthin covalently bound to proteins (carotenoproteins) forming dark blue-red and green-red pigments. Denaturation and hydrolysis of proteins on cooking releases free astaxanthin.[26]

Antheraxanthin and violaxanthin are the most important representatives of epoxides derived from *β*-carotene. The light-dependent interconversion of these xanthophylls is due to the violaxanthin cycle (xanthophyll cycle), which involves deepoxidation of violaxanthin in the light and reepoxidation of the zeaxanthin formed in the dark, via antheraxanthin. Violaxanthin further becomes the precursor of the allene xanthophyl neoxanthin. In photosynthetic tissues, carotenoids generally occur in their stable *trans*-configuration. For neoxanthin, however, it would appear that the 9'-*cis* isomer – 9'-*cis*-neoxanthin, is favored in photosynthesising tissues, while *trans*-neoxanthin preferentially accumulates in non-photosynthetic plant tissues or plants grown in the dark or under low light conditions.

The biosynthesis of cyclopentyl ketones with the *κ*-ring (ketoxanthophylls) involves a pinacolic rearrangement of the 3-hydroxy-5,6-epoxy end-group. Thus antheraxanthin is the precursor of capsanthin, the major red pigment of the fruit of the genus *Capsicum* (*Solanaceae*), both the sweet bell peper (*C. annuum*) and the hot type chili (*C. frutescens*).[27]

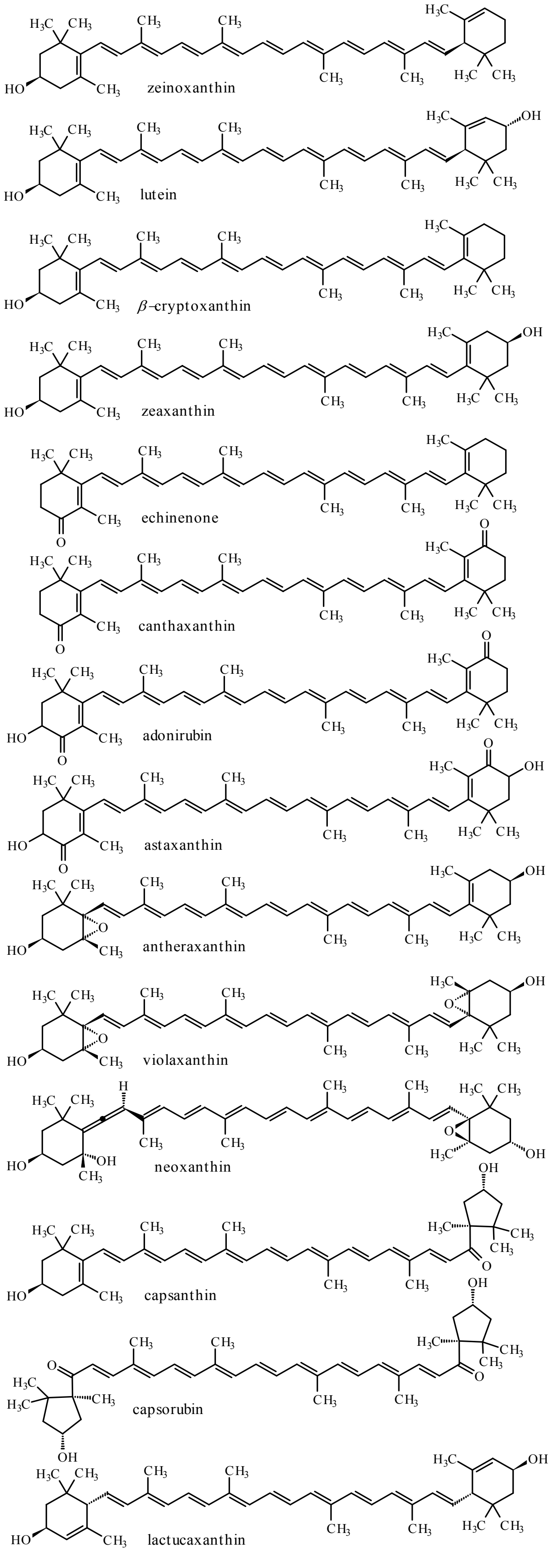

Figure 51

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[26] The crab *Homarus gammarus* contains, e.g. a blue carotenoprotein *α*-crustacyanin. The protein (320 kDa) composes of 16 subunits each of which contains one molecule of astaxanthin (Zagalaski 1994).

[27] The fruit of *Capsicum* plants have a variety of names depending on the place and type. They are commonly called chili pepper, red or green pepper, or just pepper in Britain and the US; the large mild form is called bell pepper in the US, capsicum in Australian English and Indian English, and paprika in some other countries (although paprika can also refer to the powdered spice made from various capsicum fruit).

Violaxanthin is the precursor of the minor pigment capsorubin and β-cryptoxanthin-5,6-epoxide becomes the precursor of cryptocapsin.

9.1.3 Apocarotenoids

Cleavage of carotenes and xanthophylls leads to various fragments clasified 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) (Velíšek & Cejpek 2007a). Among the important catabolic products of carotenoids, called diapocarotenoids, is the food colour 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 colour[28] for a variety of foods (margarines, cheeses, desserts, breakfast cereals, liquers, etc.). It is also still used in the cosmetic industry for the body care

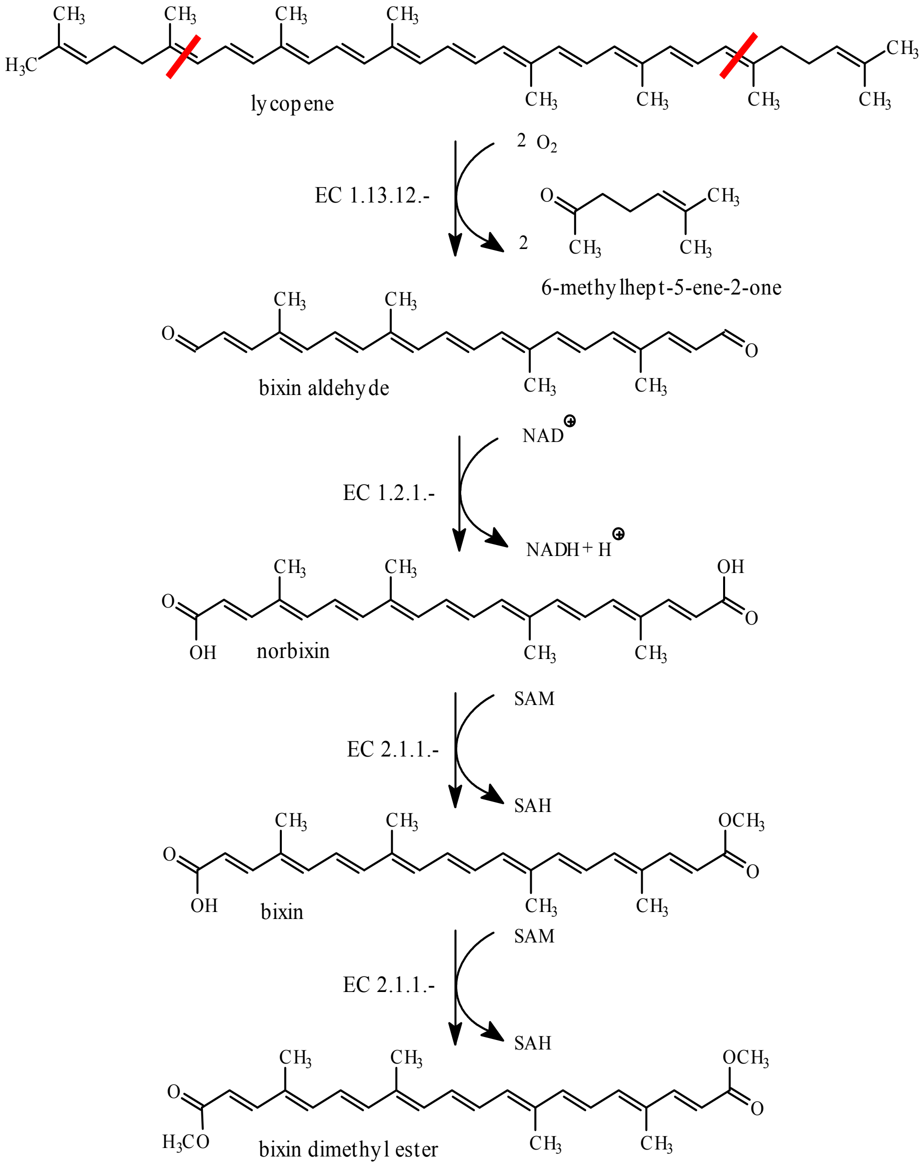

Figure 52

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[28] 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% (Tennant & O'Callaghan 2005).

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 *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 bixin (9'-*cis*-bixin) (Jako *et al.* 2002; BIOCYC 2007).

The biosynthesis of bixin (Bouvier *et al.* 2003a, b; BIOCYC 2007) starts with all-*trans*-lycopene which is split by lycopene cleavage dioxygenase (EC 1.13.12.-) to two apocarotenoids, methyl-(4-

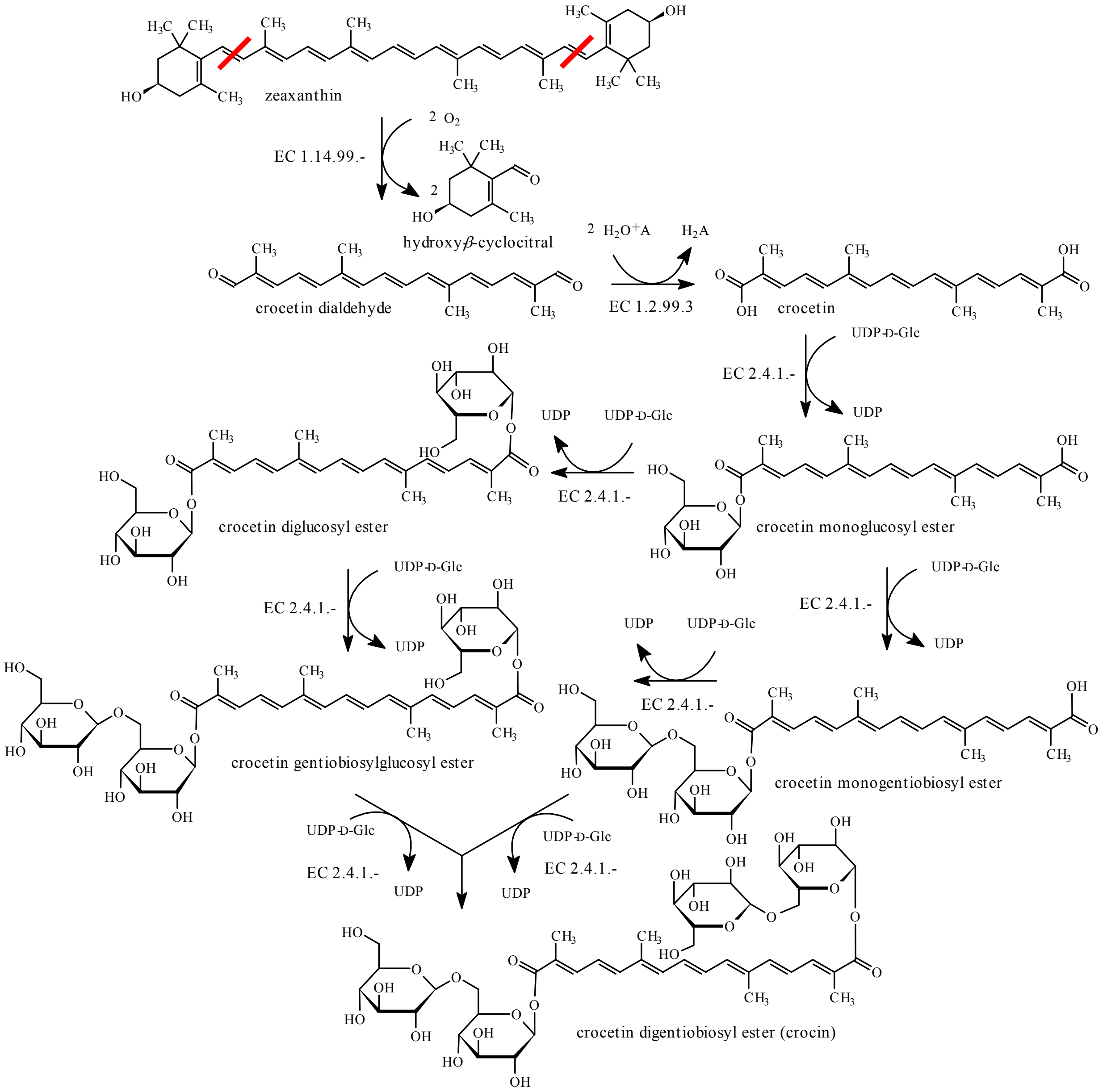

Figure 53

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[29] 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 saffron aroma (Carmona *et al.* 2006).

methylpent-3-en-1-yl)ketone (6-methylhept-5-ene-2-one) and the residual C_{32} aldehyde called bixin aldehyde (Figure 52). Bixin aldehyde dehydrogenase (EC 1.2.1.-) oxidises 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 colour as well as the bitter taste and fragrance in stigmas of saffron (*Crocus sativus*, *Iridaceae*)[29], this extremely expensive spice, are due in large part to the products of degradation of the xanthophyll zeaxanthin. The colour 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 53), 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 oxidised 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 ester (crocetin monogentiobiosyl ester glucosyltransferase, EC 2.4.1.-). Crocetin diglucosyl ester gives the same glycoside in a reaction catalysed by glucosyltransferase (EC 2.4.1.-). Final glucosylation of crocetin gentiobiosylglucosyl ester yields crocin (crocetin digentianobiosyl ester) (Côté *et al.* 2001; Moraga *et al.* 2004).

9.2 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.[30]

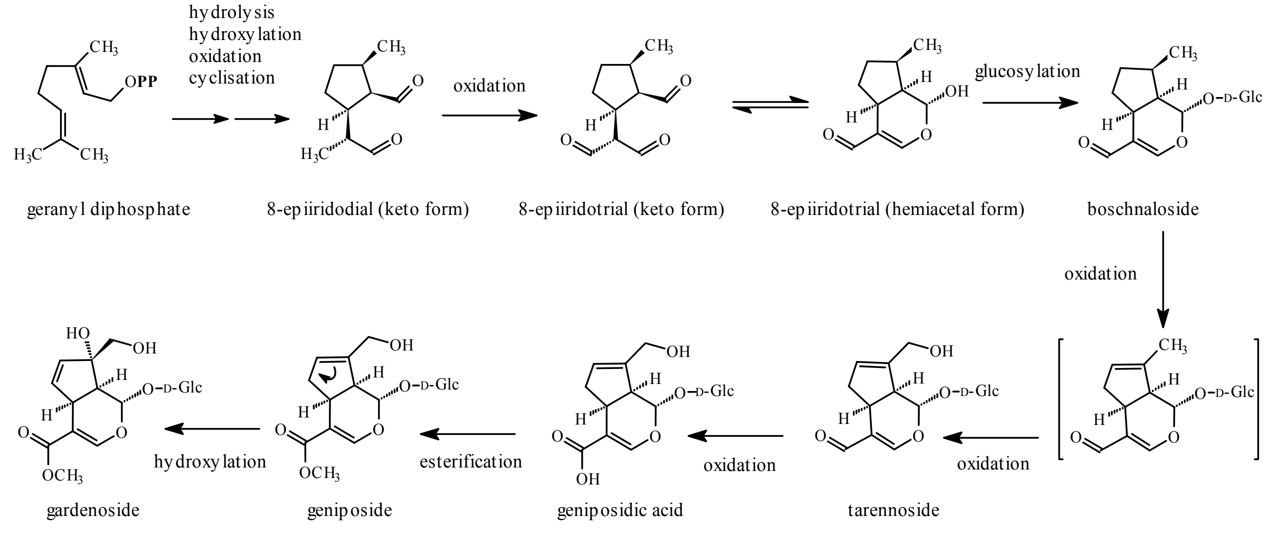

Figure 54

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[30] 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 (Hendry& Houghton 1992).

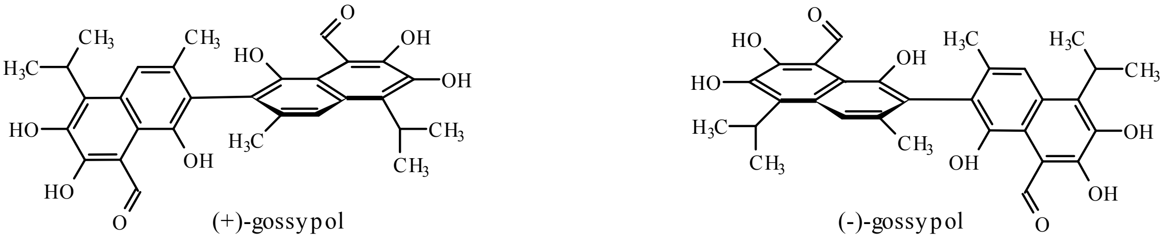

Figure 55

The major gardenia pigments, gardenoside and geniposide, are biosynthesised from the iridodial isomer (1*R*,2*S*,5*R*,8*S*)-iridodial known as 8-epi-iridodial. The biosynthesis proceeds via 8-epiiridotrial, and 8-epi-7-deoxyloganic acid and leads to boschnaloside, tarennoside, geniposidic acid, geniposide, and gardenoside (Figure 54). 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 (Sampaio-Santos & Kaplan 2001; Collu *et al.* 2001).

9.3 Miscellaneous terpenoids

Gossypol or 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 fibre cotton that grows around the seeds, for the cottonseed oil, and other products. Cottonseed oil is used in salad and cooking oils and, after hydrogenation, in shortenings and margarine. The cake, or meal, remaining after the oil extraction is used in poultry and livestock feeds. Gossypol occurs naturally in the plant in two forms referred to as (+)-gossypol and (–)-gossypol (Figure 55) (Dewick 2002) in the ratio of 3:2 (Liu *et al.* 2005; Benedict *et al.* 2006). (–)-Gossypol can be toxic to nonruminant animals (when cottonseed serves as feed) and even to human beings.[31]

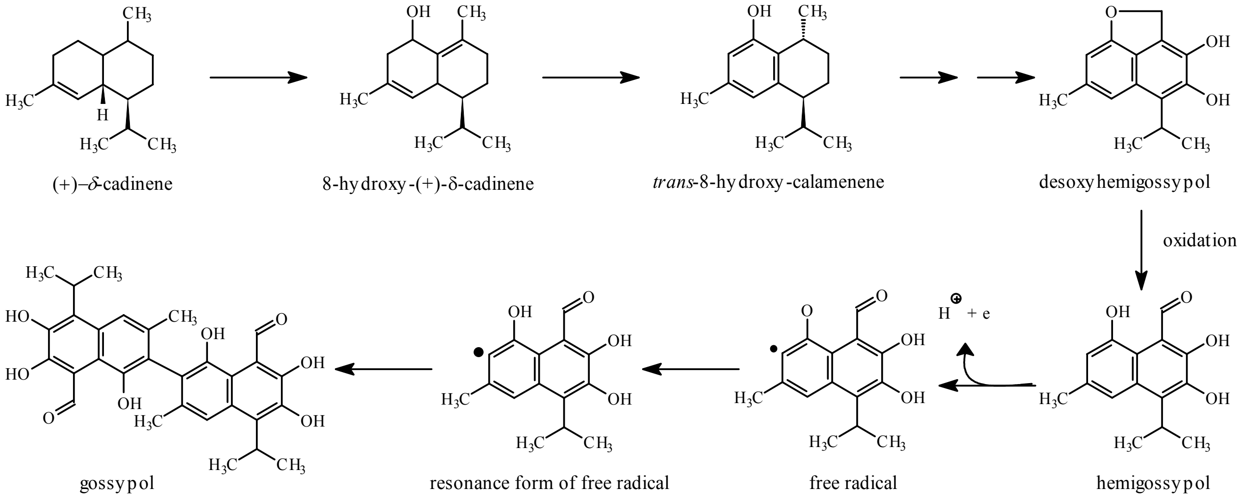

Figure 56

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[31] The toxic symptoms are associated with the (–)-isomer, which permeates cells and is active as a male contraceptive, altering sperm maturation, spermatozoid motility, and inactivation of sperm enzymes. In addition to its contraceptive properties, gossypol has also long been known to possess anti-malarial properties. Other researchers are investigating the anti-cancer properties of gossypol (Shidaifat *et al.* 1996; Coutinho 2002).

Being a dimeric sesquiterpenoid in which the loss of hydrogen has led to an aromatic system, gossypol is biosynthesised by the mevalonic acid pathway via isopentenyl diphosphate and dimethylallyl diphosphate, geranyl diphosphate, and (*E*,*E*)-farnesyl diphosphate. Farnesyl diphosphate eliminates diphosphate and is transformed to a macrocyclic intermediate, by a 1,3-hydride shift to a macrocyclic bicyclic intermediate, and further to cadinene cation and δ-cadinene, i.e. (1*S*,8a*R*)-1-isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene. The latter reaction is catalysed by (+)-δ-cadinene synthase (EC 4.2.3.13). By various oxidative processes, δ-cadinene yields (+)-8-hydroxycadinene, *trans*-8-hydroxycalamenene, desoxyhemigossypol, and hemigossypol (Wang *et al.* 2003b). One electron oxidation of hemigossypol, catalysed by a peroxidase(s), produces a free radical and a bimolecular coupling (*ortho* to the phenol group) of this radical (Benedict *et al.* 2006) yields two optically active enantiomers, i.e. (+)-gossypol and (–)-gossypol (Figure 56).

EC (Enzyme Commission) numbers and some common abbreviation

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 counter-ions, and to avoid the mechanistic confusion.

ACP	acyl carrier protein
ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
CDP	cytidine 5'-diphosphate
CMP	cytidine 5'-monophosphate
CoA	coenzyme A as a part of a thioester
CTP	cytidine 5'-triphosphate
Glc	glucose
HS-CoA	coenzyme A
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
P	phosphoric acid
PP	diphosphoric acid
Rha	rhamnose
SAH	*S*-adenosyl-L-homocysteine (AdoHcy)
SAM	*S*-adenosyl-L-methionine (AdoMet)
UDP	uridine 5'-diphosphate

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