Biosynthesis of Food Constituents: Natural Pigments. Part 1 – a Review

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Abstract

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This review article gives a 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 hemes, chlorophylls, melanins, betalains, and quinones are described using the enzymes involved and the reaction schemes with detailed mechanisms.

Keywords: biosynthesis; tetrapyrroles; hemes; chlorophylls; eumelanins; pheomelanins; allomelanins; betalains; betaxanthins; betacyanins; benzoquinones; naphthoquinones; anthraquinones

Natural pigments are coloured substances synthesised, accumulated in or excreted from living or dying cells. The pigments occurring in food materials become part of food, some other pigments have been widely used in the preparation of foods and beverages as colorants for centuries. Many foods also owe their colours to pigments that form in food materials and foods during storage and processing as a result of reactions between food constituents, notably the non-enzymatic browning reaction and the Maillard reaction. Furthermore, many artificial colorants have been used to improve the colour of foods.

Almost all biological pigment structures can be reduced to no more than a few major classes. The most important chromophores of biological pigments are *N*-heterocyclic compounds, *O*-heterocyclic compounds, quinones, and tetraterpe-noids. Despite their varied structures, all of them are synthesised by only a few biochemical pathways. There are also groups of pigments that defy simple classification and pigments that are rare or limited in occurrence.

1 TETRAPYRROLES

Tetrapyrroles (tetrapyrrole pigments) represent a relatively small group of pigments that contribute the greatest range of colours and are, at the same time, the most abundant globally. All are based on the same structure in which the pyrrole-derived rings A, B, C, and D (Figure 1) are joined into tetrapyrrole by one-carbon unit links (methine bridges) between the pyrrole rings α position providing a chromophore with a system of conjugated double bonds. Tetrapyrroles occur either in their

basic structure of bilins   basic structure of porphyrins   basic structure of chlorophylls
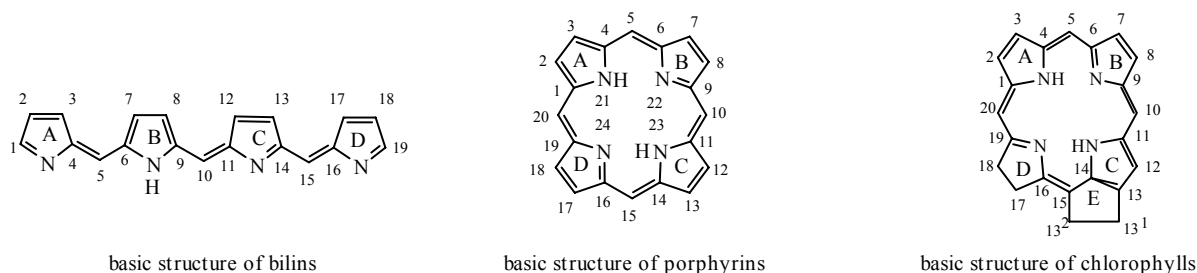
Figure 1

linear form (sometimes bound to sugars), which are known as bile pigments or bilins or in the cyclic forms based on a macrocyclic tetrapyrrole, which is known as porphyrin[1] (IUPAC 2007).

The tetrapyrroles are mostly classified according to their occurrence in nature. The linear bilins comprise two groups of pigments, phycobilins and bile pigments. The first group comprises the pigments of some algae, the second group are the typical pigments of animal urine and faeces (formed by the breakdown of heme pigments). The porphyrin based pigments constitute the chromophore of two most important metalloproteins, i.e. red heme pigments (hemes) chelated with iron, and green chlorophyll pigments (chlorophylls) chelated with magnesium[2]. The basic structure (IUPAC 2007) of the majority of chlorophylls represents the cyclic tetrapyrrole, i.e. 17,18-dihydroporphyrin, with partially reduced ring D and the fifth ring (ring E) formed by cyclisation of one of the propionic acid side chains attached at C-13 (Figure 1). The colour of the particular cyclic tetrapyrrole is determined largely by the structure and substitutions

of the tetrapyrrole molecule itself and relatively little by the metal (Gross 1987, 1991; Hendry & Houghton 1992).

1.1 Porphyrins

The biosynthesis of porphyrins requires the availability of a suitable amino acids and its constituent nitrogen to yield the building block of all tetrapyrroles in nature. This building block, 5-aminolevulinic (δ-aminolevulinic or 5-amino-4-oxopentanoic) acid is sinthesised via a different pathway in animal cells, lower plants, and bacteria that in green plants.

In animal cells, 5-aminolevulinic acid biosynthesis involves two steps. The first step is the biosynthesis of succinyl-CoA from succinic acid and HS-CoA under the catalysis of succinyl coenzyme A synthetase (ADP forming, also known as succinate thiokinase, EC 6.2.1.5). The second step is the condensation of glycine with succinyl-CoA to L-2-amino-3-oxoadipic acid by the pyridoxal phosphate-containing enzyme 5-aminolevulinate

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[1]The 1–24 numbering system upon which the numbering system for corrinoids is based was adopted for the porphyrin nucleus shown in Figure 1. The 2,3,7,8,12,13,17, and 18 positions have commonly been referred to generically as β-positions (i.e. of the pyrrole rings). Similarly, positions at 1,4,6,9,11,14,16, and 19 have been referred to generically as α-positions, while those at 5,10,15, and 20 are referred to generically as *meso*-positions (IUPAC 2007).

[2]Aside from its importance as the prosthetic group of hemoglobin (the respiratory pigment in red blood cells of vertebrates) and myoglobin (the pigment in muscle fibers), hemes are also the prosthetic groups of a small number of enzymes, such as detoxifying cytochromes and cytochromes (Voet & Voet 1990).

Chlorophylls are found in most plants, algae, and cyanobacteria. Their molecules in *Eucaryota* are specifically arranged in and around pigment protein complexes called photosystems, which are embedded in the thylakoid membranes of chloroplasts. Thylakoid membranes are a phospholipid bilayer membrane-bound compartment internal to chloroplasts, and represent the majority of its internal structure. Chloroplasts like all other plastids (including chromoplasts and leucoplasts), are organelles found in the photosynthetic eukaryotic species. Chromoplasts are responsible for the pigment synthesis and storage, they mainly store carotenoids but also other pigments. In chloroplasts, some carotenoids are also used as accessory pigments in photosynthesis. Leucoplasts are non-pigmented and predictably located in roots and non-photosynthetic tissues of plants. They may become specialised for bulk storage of starch, lipid or protein and are then known as amyloplasts, elaioplasts, or proteinoplasts, respectively (Gross 1987, 1991; Hendry & Houghton 1992).
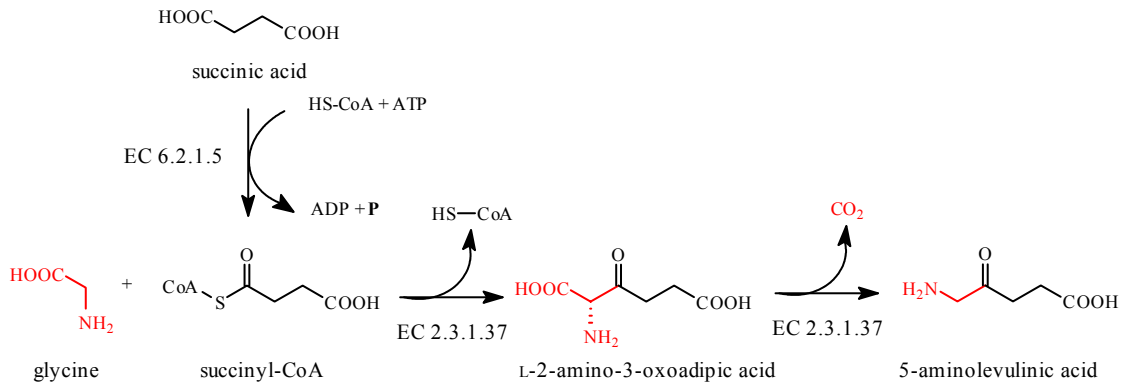
Figure 2

synthase (EC 2.3.1.37) (Figure 2). ʟ-2-Amino-3-oxo-adipic acid eliminates carbon dioxide and yields 5-aminolevulinic acid, which is then exported to cytoplasm for further metabolism (IUBMB 2007; Bergfeld *et al.* 2003).

In green plants, 5-aminolevulinic acid is formed from ʟ-glutamic acid in three reactions (Figure 3). In the first reaction, glutamate-tRNA ligase (EC 6.1.1.17) catalyses the ligation of glutamic acid to transfer RNA (tRNA^{Glu}). In the second reaction, the glutamyl-tRNA complex is converted to a glutamic acid 1-semialdehyde by glutamate-tRNA reductase (EC 1.2.1.70). Using pyridoxamine 5'-phosphate as a cofactor, glutamate 1-semialde-hyde 2,1-aminomutase (glutamate semialdehyde aminotransferase, EC 5.4.3.8) converts glutamic acid 1-semialdehyde to 5-aminolevulinic acid via 4,5-diaminopentanoic acid (IUBMB 2007).
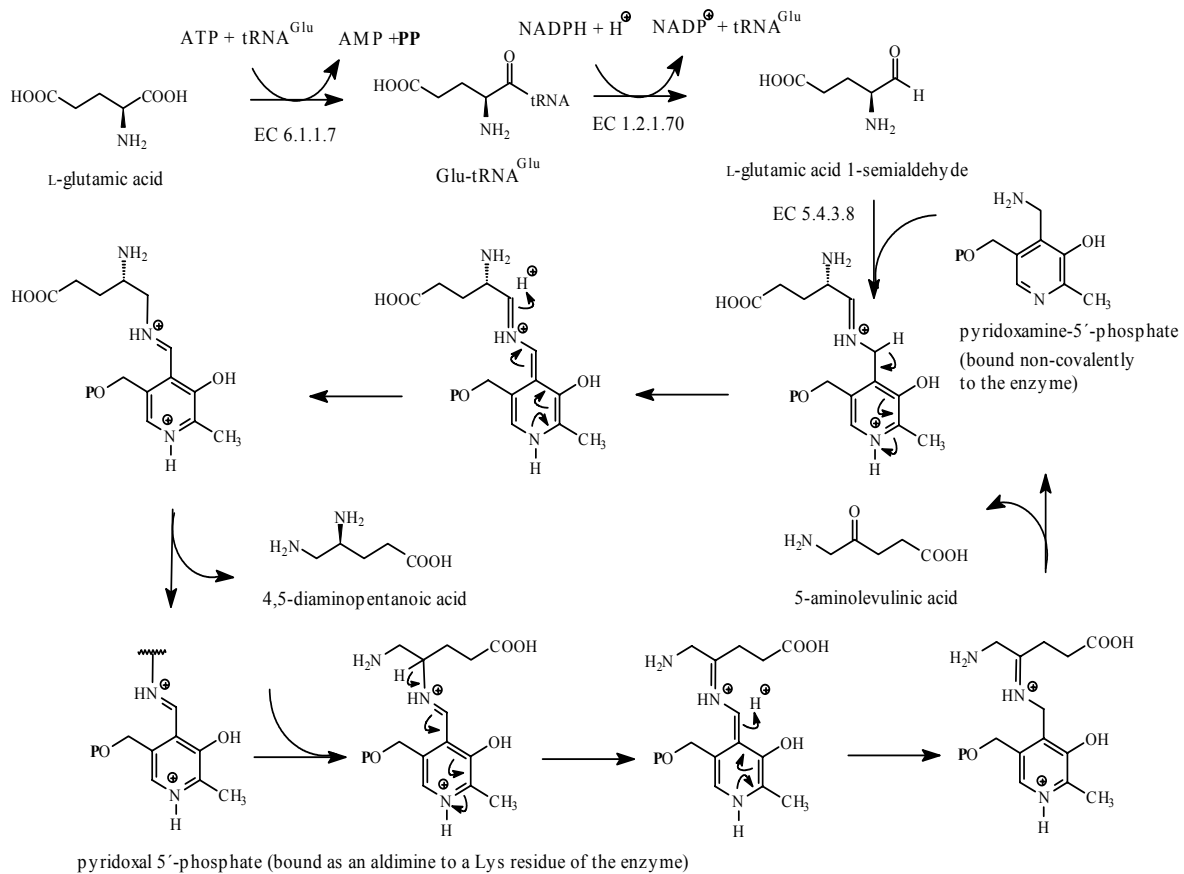

Figure 3

The next three steps of porphyrin biosynthesis follow those of vitamin B_{12} coenzyme (adenosylcobalamin) pathway. These steps (Figure 4) start with the condensation of two 5-aminolevulinic acids to form porphobilinogen (aminolevulinate dehydratase or porphobilinogen synthase, EC 4.2.1.24), four porphobilinogens are converted to hydroxymethylbilane (hydroxymethylbilane synthase, EC 2.5.1.61), which is subsequently transformed to uroporphyrinogen III also known as urogen III (uroporphyrinogen-III synthase, EC 4.2.1.75), the common intermediate in the biosynthesis of corrinoids and porphyrins (KEGG 2007; VELíšEK & CEJPEK 2007b).

In the pathway leading to porphyrins, uroporphyrinogen decarboxylase (uroporphyrinogen decarboxylase, EC 4.1.1.37) catalyses decarboxylation of the acetic acid residues attached to C-2, C-7, C-12, and C-18 of uroporphyrinogen III to yield coproporphyrinogen III also known as coprogen III (Figure 4). The action of coproporhyrinogen oxidase (EC 1.3.3.3)[3] then transforms the two 2-carboxyethyl groups (propionic acid residues) at C-3 and C-8 to vinyl groups (by oxidative decarboxylation) forming the colourless product protoporphyrinogen IX. Protoporphyrinogen oxidase (EC 1.3.3.4) then modifies the one-carbon units (methylene bridges) to methine bridges to yield
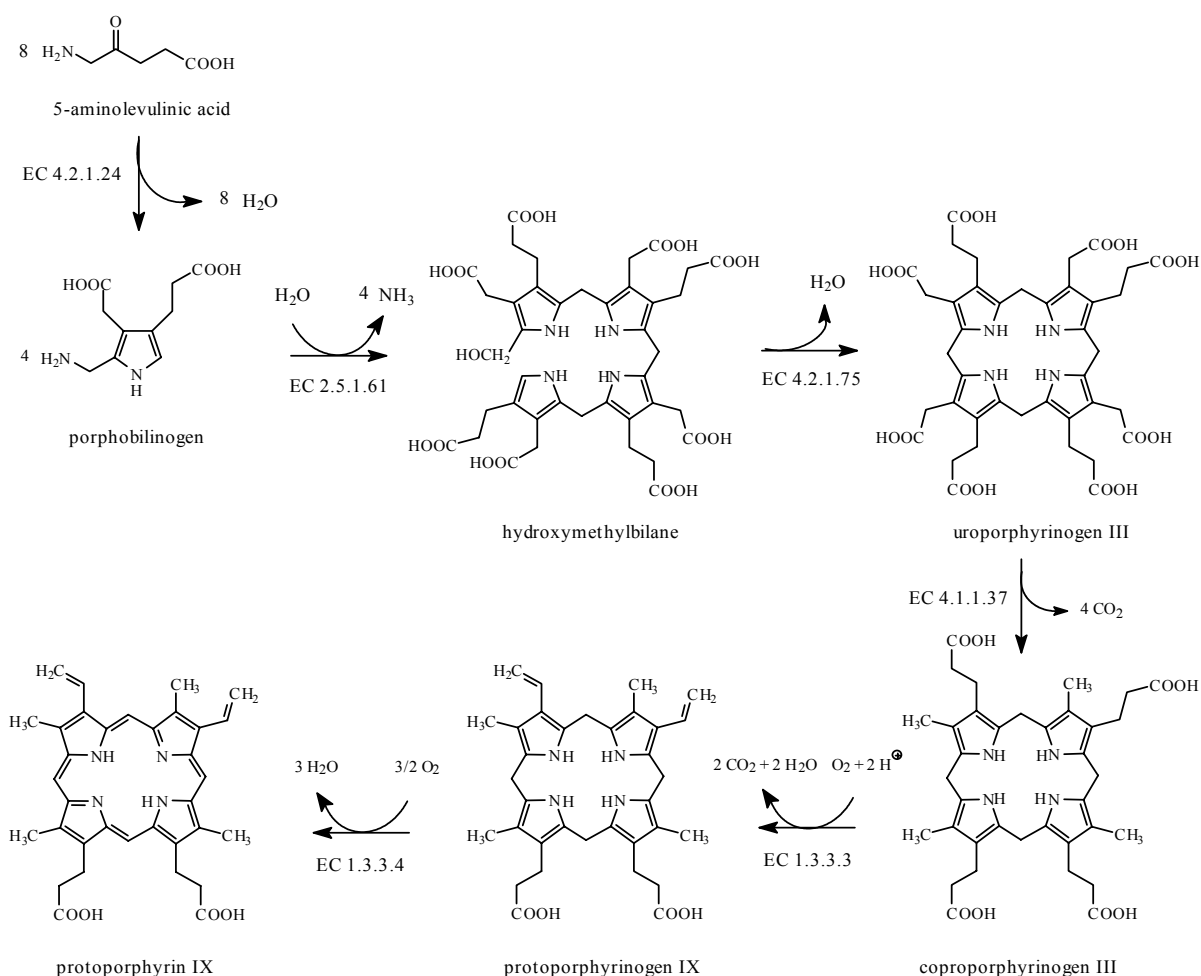

Figure 4

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[3]Coproporphyrinogen dehydrogenase (EC 1.3.99.22), occurring mainly in bacteria, differs from coproporphyrinogen oxidase (EC 1.3.3.3.) used by eukaryotes by using *S*-adenosyl-L-methionine (SAM, AdoMet) instead of oxygen as oxidant. The reaction starts by using an electron from the reduced form of the enzyme's [4Fe-4S] cluster to split SAM into methionine and the radical 5'-deoxyadenosin-5'-yl. This radical initiates an attack on the 2-carboxyethyl groups, leading to their conversion into vinyl groups. This conversion, $-CH-CH_2-COO^- = -CH=CH_2 + CO_2 + e^-$ replaces the electron initially used (KEGG 2007).

protoporphyrin IX. The oxidase reaction requires molecular oxygen and results in the loss of six hydrogen atoms, yielding a completely conjugated ring system, which is responsible for the characteristic colour of porphyrins (KEGG 2007). All the reaction steps up to this point are the same for the biosynthesis of both heme pigments and chlorophylls. There, however, the pathway branches and the fate of protoporphyrin IX depends on which metal is inserted into the centre of the molecule. If iron is inserted, the species ultimately becomes heme. If magnesium is inserted, protoporphyrin IX is converted into chlorophylls.

1.1.1 Hemes

In the pathway leading from protoporphyrin IX to the heme pigments, heme synthetase, also known as ferrochelatase (EC 4.99.1.1), incorporates Fe^{2+} ion into the macrocycle forming protoheme (heme) and the subsequent reaction of heme with proteins yields myoglobin, hemoglobin, and other heme-containing metalloproteins (cytochrome *a*, cytochrome *c*, catalase, peroxidase) (Figure 5). The heme biosynthetic pathway and its subcellular compartmentation are probably identical in all mammalian cells[4].
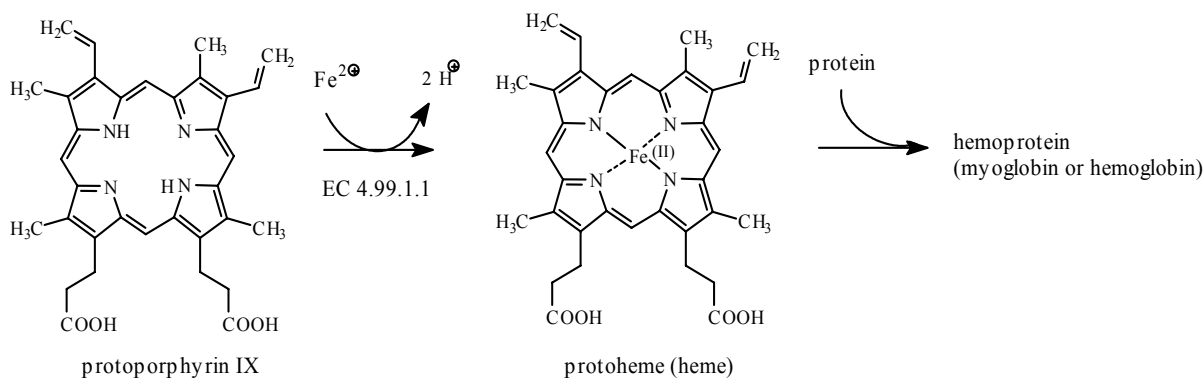
1.1.2 Chlorophylls

In the pathway leading from protoporphyrin IX to chlorophylls (Figure 6), the next step is the insertion of Mg^{2+} ions by protoporphyrin IX magnesium-chelatase (EC 6.6.1.1), which yields magnesium-protoporphyrin IX. The following step is the esterification of the propionic acid side chain carboxyl (attached at C-13 of the ring C of protoporphyrin IX) by *S*-adenosyl-L-methionine (SAM, AdoMet), which is catalysed by magnesium protoporphyrin IX methyltransferase (EC 2.1.1.11). The next phase of the chlorophyll biosynthetic pathway is the formation of the fifth ring (ring E) by cyclisation of the propionic acid side chain to form divinylprotochlorophyllide. The pathway, catalysed by magnesium-protoporphyrin IX monomethylester (oxidative) cyclase (EC 1.14.13.81), involves the oxidation of the propionic acid side chain at C-13' via 13'-hydroxy-magnesium-protoporphyrin IX 13-methyl ester and 13'-oxo-magnesium-protoporphyrin IX 13-methyl ester using NADPH and oxygen. The next step is the *trans*-reduction of the double bond in the ring D using three molecules of NADPH, which yields divinylchlorophyllide *a*. The product has the (7*S*,8*S*)-configuration. This process is driven by light in angiosperms and is carried out by an enzyme called protochlorophyl-

Figure 5

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[4]Although heme is synthesised in virtually all animal tissues, the principal sites of synthesis are erythroid cells that will give rise to erythrocytes (about 85%) and hepatocytes (accounting for nearly all the rest of heme synthesis) (The Medical Biochemistry Page 2006). The 5-aminolevulinic acid biosynthesis takes place in the mitochondria and the next steps up to the formation of coproporphyrinogen III proceed in the cytosol. The cytoplasmic coproporphyrinogen III is then transported to the mitochondria, in an ATP-dependent process. The final three steps of the biosynthetic pathway, including the formation of protoporphyrinogen IX, protoporphyrin IX, and protoheme, proceed in the mitochondria. In green plants, 5-aminolevulinic acid biosynthesis takes place in the stroma of the plastid and all subsequent reactions associated with the chlorophyll biosynthesis are loosely bound to the plastid membranes.
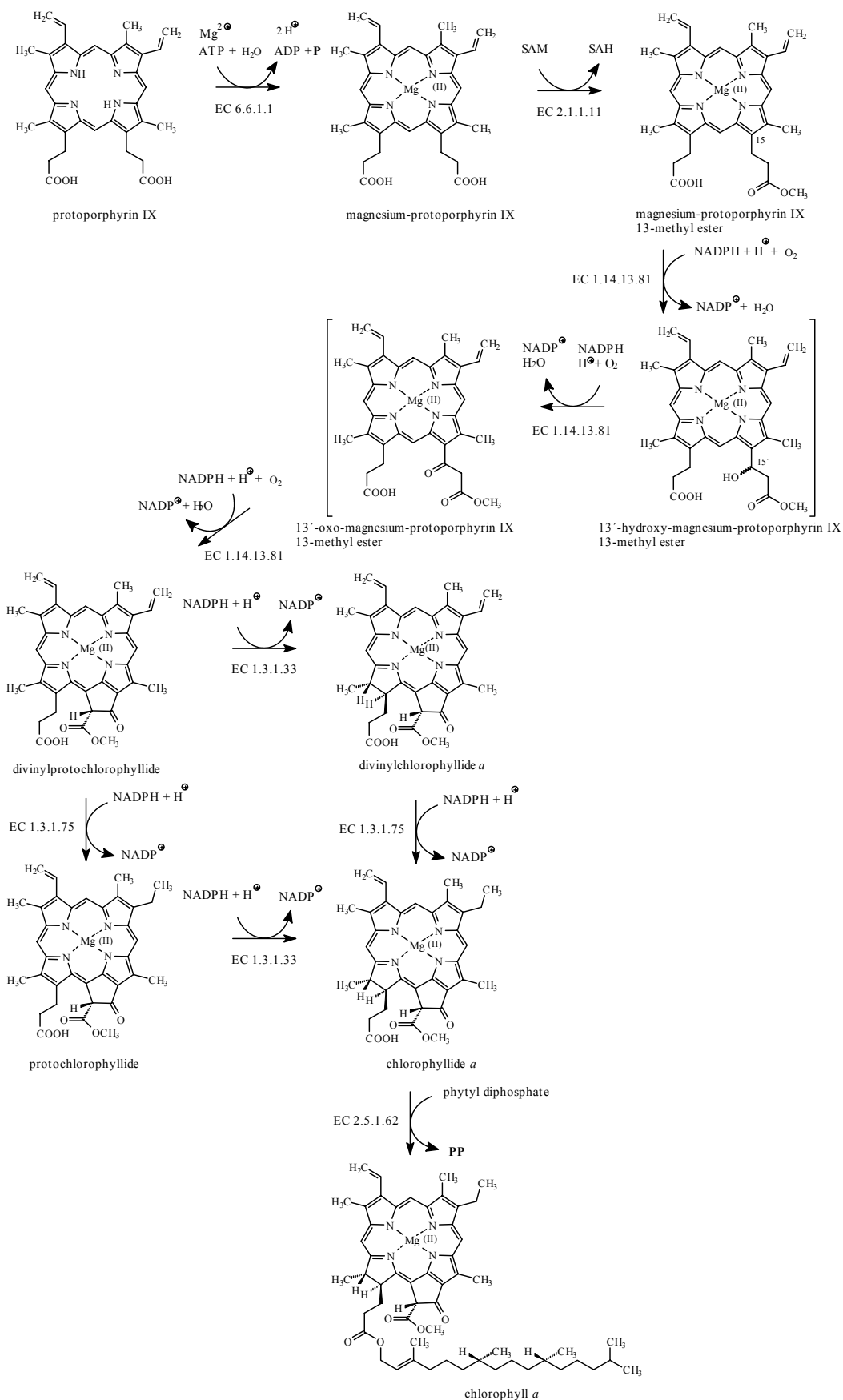
protoporphyrin IX

magnesium-protoporphyrin IX

magnesium-protoporphyrin IX
13-methyl ester

13′-oxo-magnesium-protoporphyrin IX
13-methyl ester

13′-hydroxy-magnesium-protoporphyrin IX
13-methyl ester

divinylprotochlorophyllide

divinylchlorophyllide *a*

protochlorophyllide

chlorophyllide *a*

chlorophyll *a*

Figure 6

lide reductase (EC 1.3.1.33)[5]. The vinyl group at C-8 of the ring B of divinylchlorophyllide *a* is then reduced, using NADPH, to the ethyl group by the action of divinylchlorophyllide *a* 8-vinyl-reductase (EC 1.3.1.75). This enzyme also reduces divinyl protochlorophyllide to protochlorophyllide in some species, providing an alternative pathway. Protochlorophyllide, analogously to divinylprotochlorophyllide, is then reduced to chlorophyllide *a* using protochlorophyllide reductase (EC 1.3.1.33). The final step in the chlorophyll biosynthetic pathway leading to chlorophyll *a* is the attachment of the phytyl residue from phytyl diphosphate, which is catalysed by an enzyme called chlorophyll synthase (EC 2.5.1.62).

Chlorophyllide *a* can be oxidised at the methyl group at C-7 of the ring B to 7-hydroxychlorophyllide *a* and further to chlorophyllide *b* with the formyl group instead of the methyl group. The successive reactions are catalysed by chlorophyllide *a* oxygenase (EC 1.13.12.14). A specific chlorophyll synthetase (EC 2.5.1.-) then catalyses the attachment of the phytyl residue in chlorophyllide *b* using phytyl diphosphate (Rudiger 2002; BioCyc Database Collection 2007).

2 MELANINS

Melanins are an important group of pigments widely distributed in all living organisms. Melanins are classified into three groups, the eumelanins (produced by polymerisation of a nitrogenous melanogen), the pheomelanins (obtained from polymerisation of a sulphurated melanogen), and the allomelanins (generated by polymerisation of polyphenols). Hybrids of the above mentioned first two groups can be easily formed as mixtures or copolymers. The formation of melanins (melanogenesis) is a rare radical known process occurring *in vivo* (Zecca *et al.* 2001; Halaban 2002; Langfelder *et al.* 2003; Slominski *et al.* 2005; Plonka & Grabacka 2006).

Black or brown eumelanins[6] and yellow or red pheomelanins are frequently prominent in skin, hair, feathers, and scales and occur also in membranous tissues in mammals, birds, reptiles, amphibians, fish, and other organisms. They occur in different states of oxidation and aggregation, accompanied sometimes by other pigments, which is responsible for innumerable shades of black, brown, yellow, and red colours frequently observed in animals. They also play an important background role in the display of iridescent (interference) colours frequently seen, for example, in the skin of butterflies, reptiles, and fish. Brown or black allomelanins are common in some fungi and in higher plants.

2.1 Eumelanins

The starting compound in the biosynthesis of eumelanins is L-tyrosine. It is hydroxylated either by (6*R*)-5,6,7,8-tetrahydro-L-biopterin (also known as L-*erythro*-5,6,7,8-tetrahydrobiopterin or BH_4)-dependent monooxygenase enzyme tyrosine hydroxy-

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[5]Anoxygenic photosynthetic bacteria carry out this reaction without light, using a completely different set of enzymes. Cyanobacteria, algae, lower plants, and gymnosperms contain both the light-dependent protochlorophyllide oxidoreductase pathway and the light-independent pathway. Seedlings of angiosperms grown in complete darkness lack chlorophyll, because the protochlorophyllide oxidoreductase enzyme requires light. These etiolated plants turn green very rapidly when exposed to light.

[6]The basic functions of melanins are still a matter of controversy and speculation. Melanogenesis probably evolves parallely in various groups of living organisms to provide protection from environmental stress conditions. Eumelanin, the prominent form of melanins in humans, has the important role of the skin protector because it acts as UV and visible light absorber. Furthermore, it takes part in reactions involving modulations of the redox states of pigment cells, in liver and spleen of lower vertebrates it has cytoprotective functions against reactive oxygen species and lipid hydroperoxides. Melanogenesis in pathogenic fungi and bacteria correlates with an increased virulence, melanins act as electron acceptors and in atmospheric nitrogen fixation of bacteria.

In human skin, eumelanins and pheomelanins are produced in the basal layer of the epidermis by neural crest derived specialised cells (melanocytes) where the melanin is located in specialised cytoplasmic vacuoles (melanosomes) often bound to proteins and metals. Some fungal melanin is found as a part of the cell wall, some melanin is found in association with the fibrillar matrix, which extends out from the cell wall of many fungi. Notably, the ink sack of the common cuttlefish (*Sepia officinalis*) contains an intensely black suspension of extracellular melanin granules that become the typical pigment of special sauces consumed in the Mediterranean region.

lase (EC 1.14.16.2) or by tyrosinase (EC 1.14.18.1) to 3,4-dihydroxy-l-phenylalanine (l-DOPA, l-dopa, levodopa) (IUBMB 2007; Velíšek *et al.* 2006). Dopa is further oxidised to dopaquinone (Figure 7) by tyrosinase (EC 1.14.18.1) and up to this point the biosynthetic pathways of eumelanins and pheomelanins are undistinguishable.

In the pathway leading to eumelanins, dopaquinone, in the absence of thiols, undergoes intramolecular cyclisation to form leucodopachrome, which is oxidised by the action of tyrosinase (EC 1.14.18.1) to dopachrome. The zink enzyme l-dopachrome isomerase (EC 5.3.3.12) transforms dopachrome to 5,6-dihydroxyindole-2-carboxylic acid; alternatively, dopachrome spontaneously decarboxylates

to 5,6-dihydroxyindole. These dihydroxyindoles are then oxidised to their respective quinone derivatives (melanogens) and undergo oxidative polymerisation to eumelanins. Similar pigments (called dopa-melanins) occur in some fungi.

2.2 Pheomelanins

In the presence of thiols such as cysteine (Cys) or glutathione (GSH) and under the catalysis by glutathione reductase (EC 1.8.1.7) and γ-glutamyl transpeptidase (EC 2.3.2.2) (Velíšek *et al.* 2006), dopachinone yields cysteinyldopa. Further oxidation leads to pheomelanin production via benzothiazine intermediates (Figure 7).
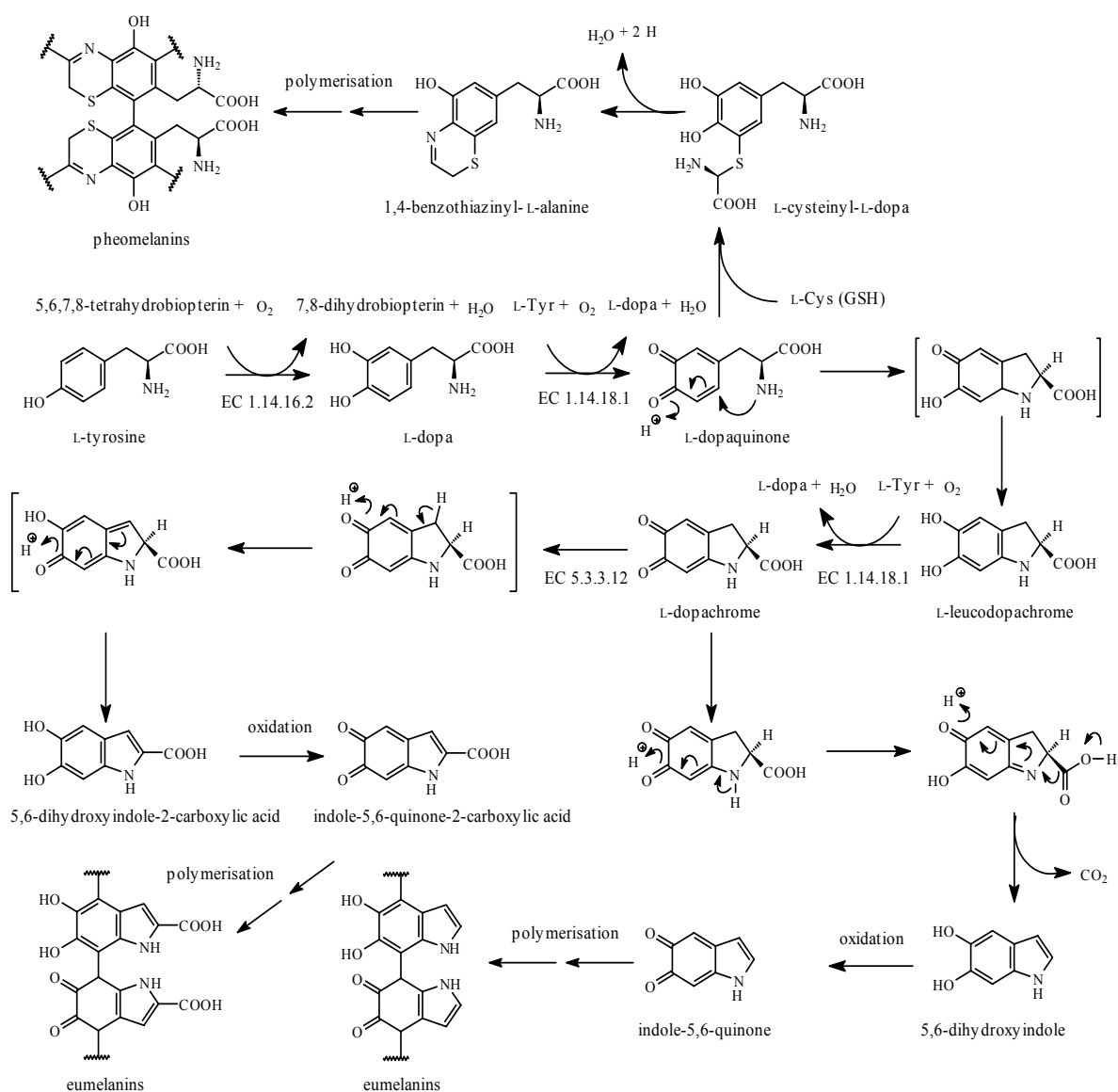
Figure 7

2.3 Allomelanins

Allomelanins are the most heterogenous group of melanins. They comprise nitrogen-free macromolecular polymers of simple phenols, usually dark brown to black pigments of fungi and higher plants. They are chemically unrelated to the true eumelanins and pheomelanins of animals (Hendry & Houghton 1992). The precise biosynthetic origin of most of the allomelanins[7] is unknown but they appear, structurally at least, to be related to polymers of simple phenols and their quinones.

The biosynthesis of the so-called dihydroxynaphthalene melanins (DHN-melanins) in microorganisms proceeds via the acetate-polymalonate (polyketide) pathway from malonyl-CoA, which acts as the starter and extender unit for the multienzyme protein complex polyketide synthase (see Chapter 2.3.1) (Figure 8). The condensation product, polyketoester (pentaketide), formed from one malonyl-CoA as the starter group and four malonyl-CoA extension units, undergoes intramolecular aldol condensation, dehydration favoured by the formation of conjugated system
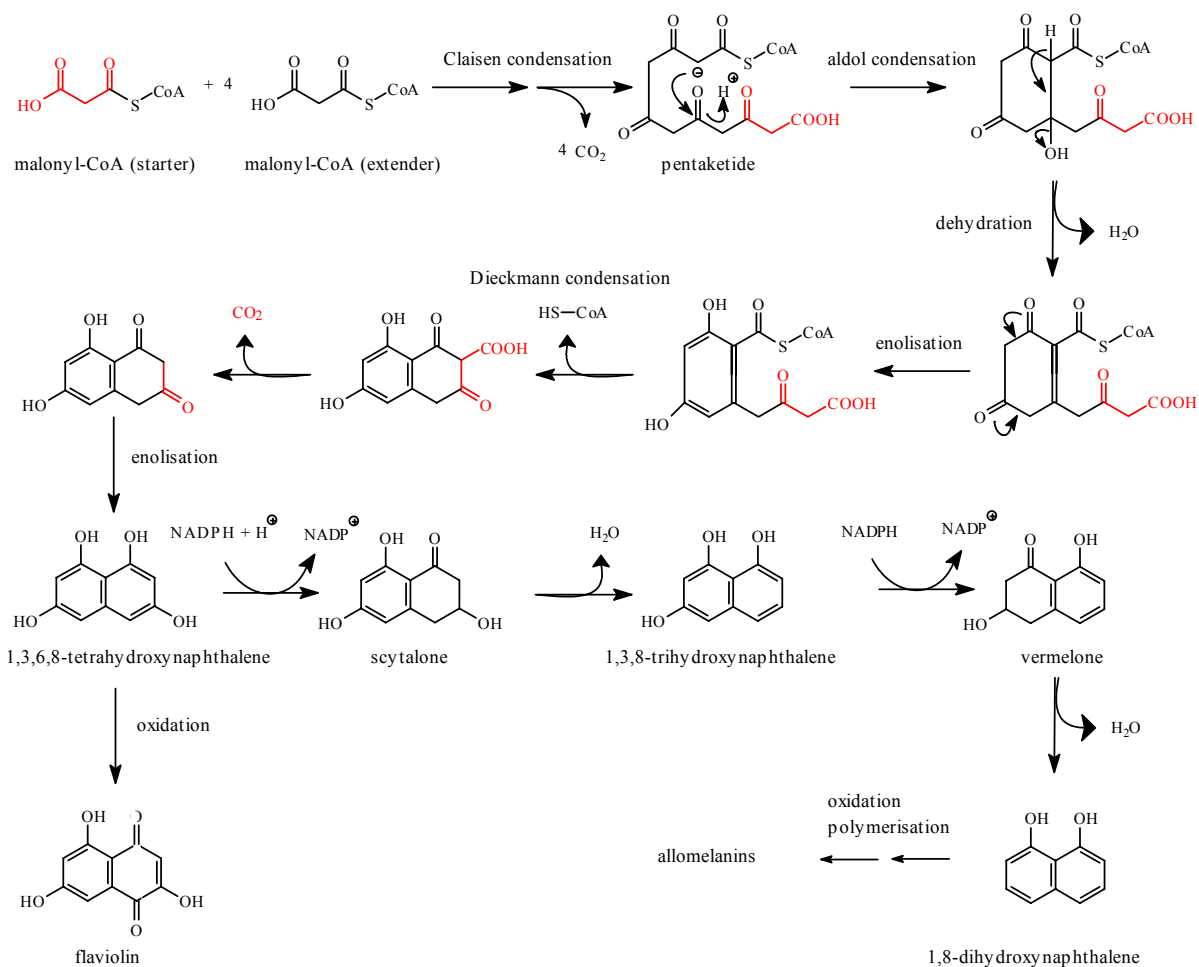

Figure 8

[7]Some species of bacteria produce and secrete pyomelanin, a product of homogentisic acid polymerisation. The main enzymes engaged in pyomelanin production are tyrosine transaminase (EC 2.6.1.5), which catalyses the formation of 4-hydroxyphenylpyruvic acid from tyrosine and 2-oxoglutaric acid, and 4-hydroxyphenylpyruvate decarboxylase (EC 1.13.11.27), which catalyses homogentisic acid formation from 4-hydroxyphenylpyruvic acid (Velíšek & Cejpek 2007a). Another melanogenic microbial enzyme, 4-hydroxyphenylacetic acid hydroxylase (EC 1.14.13.3), catalyses hydroxylation of 4-hydroxyphenylacetic acid and other aromatic compounds, which leads to the formation of *o*-benzoquinones and other *o*-quinone derivatives, which then polymerise spontaneously to allomelanin-like polymers (Plonka & Grabacka 2006).

and enolisation favoured by the formation of aromatic ring. The Dieckmann condensation of the product formed (analogously to the formation of 1,4-dihydroxy-2-naphthoic acid, the precursor of phylloquinone, Velíšek & Cejpek 2007a) is then followed by eliminations of HS-CoA and CO_2, and enolisation to yield 1,3,6,8-tetrahydroxynaphthalene. This compound is oxidised by the action of 1,3,6,8-tetrahydroxynaphthalene reductase (EC 1.1.1.152) to scytalone. Its dehydration (catalysed by scytalone dehydratase, EC 4.2.1.94) is favoured by the formation of a conjugated system yielding 1,3,8-trihydroxynaphthalene. Analogously, trihydroxynaphthalene is oxidised by the action of 1,3,8-trihydroxynaphthalene reductase (EC 1.1.1.152) to vermelone, which, after dehydration (scytalone dehydratase, EC 4.2.1.94) and enolisation forms the building stone of allomelanins, 1,8-dihydroxynaphthalene. Subsequent steps are thought to involve dimerisation of the dihydroxynaphthalene molecules, followed by polymerisation, possibly catalysed by laccase (EC 1.10.3.2). The pathway may vary in individual fungi (Langfelder *et al.* 2003). Low activity of 1,3,6,8-tetrahydroxynaphthalene reductase in some microorganisms (e.g. members of the genus *Streptomyces*) leads to the accumulation of the yellow pigment flaviolin (2,5,7-trihydroxynaphtho-1,4-quinone) formed by oxidation of 1,3,6,8-tetrahydroxynaphthalene. Analogously, 1,3,8-trihydroxynaphthalene yields on oxidation at C-4 yellow 2-hydroxyjuglone (Wheeler & Klich 1995; Langfelder *et al.* 2003).

Many other polyphenols can become the starting compound in the biosynthesis of allomelanins in microorganisms, e.g. homogentisic acid (pyomelanins), 4-hydroxyphenylacetic acid etc. (Velíšek & Cejpek 2007a). Quinones formed in large amounts by soil microorganisms and/or by oxidation of phenols readily react with phenols, amino compounds, etc. and by further oxidative process some brown, high molecular weight compounds (humic acids) are obtained.

In higher plants, similar reactions of phenolic substrates (such as tyrosine, dopa, phenolic acids, their depsides such as chlorogenic acid, catechins etc.) lead to the formation of numerous *o*-benzoquinones (1,2-benzoquinones) and *p*-benzoquinones (1,4-benzoquinones) (Figure 9). The enzymes involved comprise monophenol oxidases such as tyrosinases with cresolase activity (EC 1.14.18.1), *o*-diphenolases (EC 1.10.3.1) with catecholase
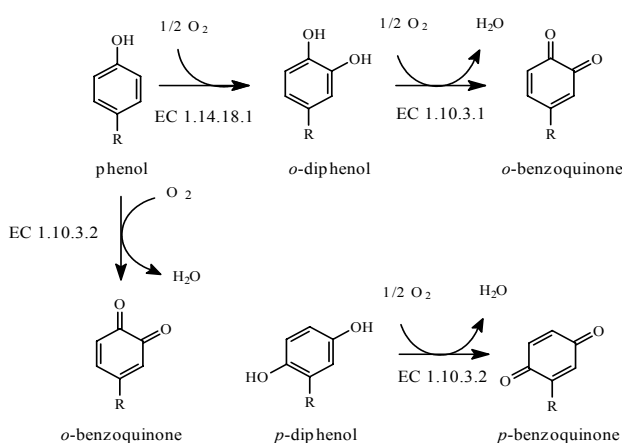

Figure 9

activity, and laccases (EC 1.10.3.2) oxidising both *o*-diphenols and *p*-diphenols. 1,2-Benzoquinones are often red, while 1,4-benzoquinones are mainly yellow compounds (e.g. dopaquinone is pink, the chlorogenic acid derived 1,2-benzoquinone is orange and the 1,2-benzoquinone derived from catechin is yellow).

Monophenols can be oxidised to the corresponding *o*-benzoquinones also non-enzymatically, e.g. by hydrogen peroxide, which forms as a product of enzymatic reactions (some oxidases, superoxiddismutase, EC 1.15.1.1), by ascorbic acid autoxidation catalysed by transient metals, and by autoxidation of cuprous ions in acid medium ($O_2 + 2\,Cu^+ + 2\,H^+ \rightarrow H_2O_2 + 2\,Cu^{2+}$). Diphenols are then readily oxidised to benzoquinones by hydrogen peroxide, oxygen (especially in neutral and alkaline solutions), cupric ions or hydroperoxyl radicals that form as the products of lipid autoxidation (Velíšek 2002).

Subsequent spontaneous condensation reactions of benzoquinones with the parent diphenols (leading to dimers) and other nucleophilic reagents (e.g. amino acids and proteins) lead to the formation of dark allomelanine-like brown to black nitrogen-free polymers and nitrogen- and sulphur-containing pigments. Some of these reactions are schematically depicted in Figure 10. The formation of these pigments during the normal development of plants is a well-known phenomenon commonly observed in senescent leaves, seedpods, dead cells of bark, seed coats, and pericarps. Furthermore, many plant tissues darken rapidly on storage (e.g. banana), injury (e.g. potatoes on peeling) and processing (e.g. fermentation of tea leaves) in the course of these so called enzymatic browning reactions (Velíšek 2002).
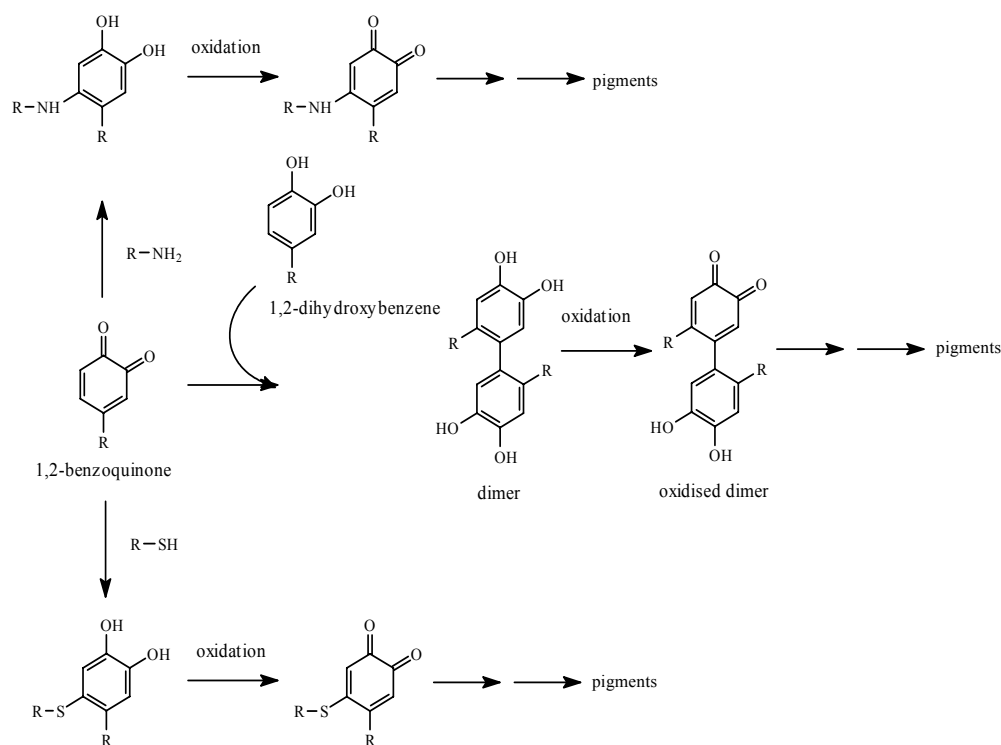
Figure 10

2.3.1 Biosynthesis of aromatic polyketides

The biosynthesis of aromatic polyketides, a class of secondary metabolites in all organisms, shares striking similarities with the fatty acid biosynthesis (Velíšek & Cejpek 2006a). Polyketides are synthesised by sequential reactions catalysed by a collection of enzyme activities called polyketide synthases (PKSs)[8], large multifunctional protein that contains a coordinated group of active sites. Component domains of PKSs consist of acyl transferases (for the loading of starter, extender and intermediate acyl units), acyl carrier domain (with an SH group), a serine-attached 4'-phosphopantetheine (ACP, which hold the growing macrolide as a thiol ester), 3-ketoacyl synthase (catalysing chain extension), 3-keto reductases (responsible for the first reduction), dehydratase (elimination of water to give an unsaturated thiolester), enoyl

reductase (the final reduction to full saturation), and finally a thiolesterase (product release and cyclisation) (World of Polyketides 2007).

The biosynthesis occurs in a stepwise manner from simple 2-, 3-, or 4-carbon building blocks acting as starters. The common starters are acetyl-CoA (*S*-acetyltransferase, EC 2.3.1.38) and its activated derivatives malonyl-CoA and methylmalonyl-CoA. The major extender units are acetyl-CoA, malonyl-CoA, methylmalonyl-CoA, propionyl-CoA, butyryl-CoA, isobutyryl-CoA, pentanoyl-CoA, hexanoyl-CoA, octanoyl-CoA, benzoyl-CoA, cinnamoyl-CoA, *p*-coumaroyl-CoA, caffeoyl-CoA, feruoyl-CoA etc.). *S*-malonyltransferase (EC 2.3.1.39) provides the malonyl groups for polyketide biosynthesis and the action of β-oxoacyl-ACP synthase (EC 2.3.1.41) yields the poly-β-keto chain, $-[CH_2-C(=O)]_n-$ (Dewick 2002; World of Polyketides 2007). The key chain-building

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[8]Numerous organisms employ PKSs to produce pigments, antibiotics, toxins, pheromones, and other products of secondary metabolism. At least 3 architecturally different types of PKSs have been discovered. The type I systems (PKS I) are typically involved e.g. in the biosynthesis of DHN-melanins, certain antibiotics (macrolides) and aflatoxins in microorganisms. The type II systems catalyse the formation of compounds that require aromatisation and cyclisation, but not extensive reduction or reduction/dehydration cycles. These PKSs are involved e.g. in the biosynthesis of bacterial aromatic natural products. The type III PKSs are responsible for the synthesis of polyhydroxyphenols in bacteria and chalcones and stilbenes in plants (World of Polyketides 2007).

step is an iterative decarboxylative condensation analogous to the chain elongation step of classical fatty acid biosynthesis but the pathways to fatty acids (Velíšek & Cejpek 2006a) and aromatic polyketides branch early. Unlike in fatty acid biosynthesis, however, in which each successive chain elongation step is followed by a fixed sequence of the oxo group reduction (3-oxoacyl-ACP reductase, EC 1.1.1.100), dehydration (3-hydroxyacyl-ACP dehydratase, EC 4.2.1.61), and enoyl reduction (enoyl-ACP reductase, EC 1.3.1.10) as schematically shown in Figure 11, the individual chain elongation intermediates of polyketide biosynthesis undergo all, some, or none of these functional group modifications, resulting in a striking level of chemical complexity in the products. Additional degrees of complexity arise from the use of different starter units and chain elongation units as well

as the generation of new stereoisomers (World of Polyketides 2007).

2.3.2 Dieckmann condensation

The Dieckmann condensation is the base-catalysed intramolecular condensation of diesters (intramolecular nucleophilic acyl substitution) to give cyclic β-keto esters (Figure 12). The intermolecular equivalent reaction is the Claisen condensation (Velíšek & Cejpek 2006a). This means the enolate component and the carbonyl component are parts of a single, larger molecule (enolates are good nucleophiles and the ester carbonyl carbon are electrophilic). The enzyme catalysis obviates the need for a base and esterification of the second carboxyl to achieve the formation of β-keto ester (IUPAC 2006).
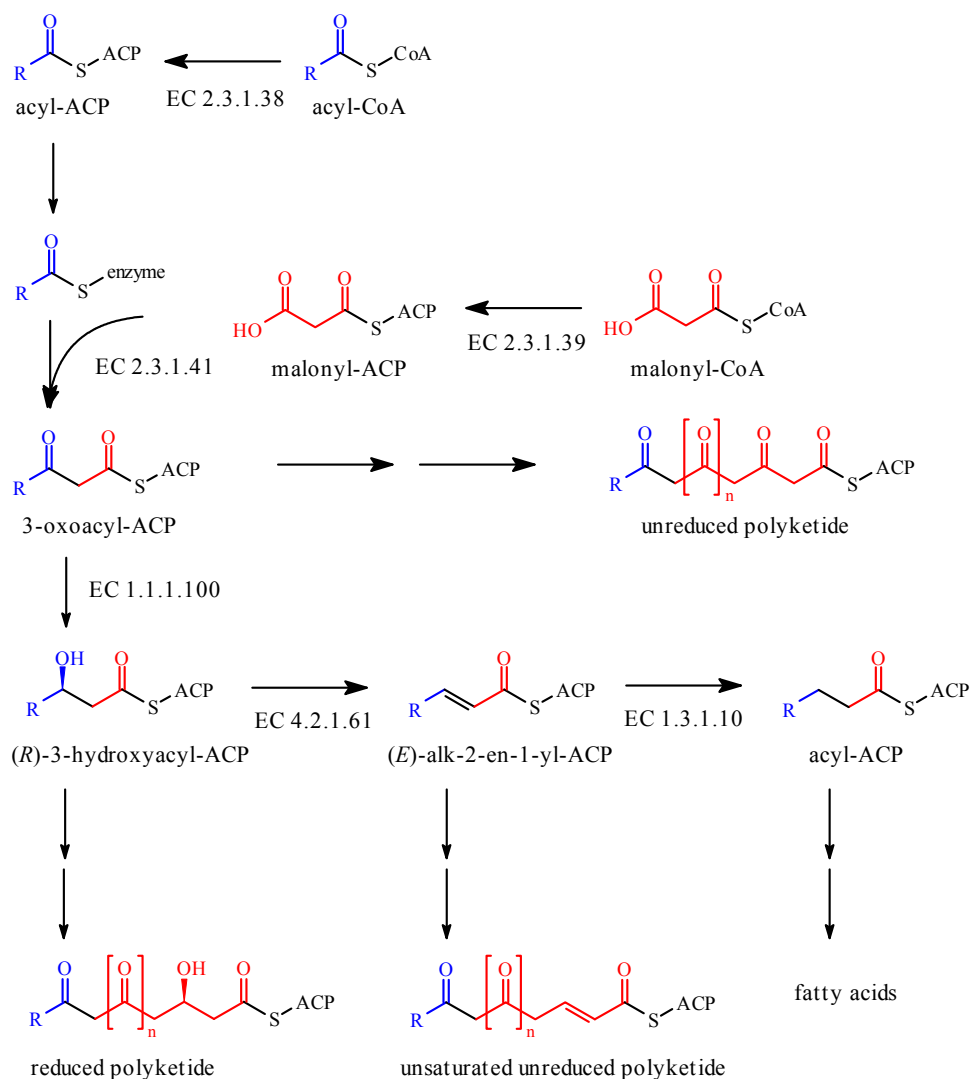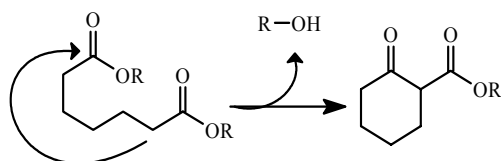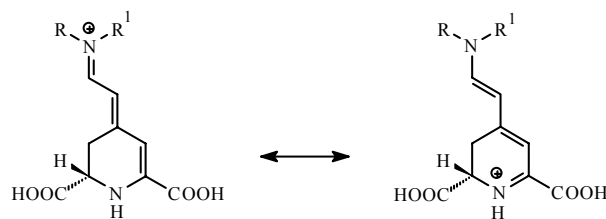

Figure 11

Figure 12

Figure 13

3   BETALAINS

The betalains are a class of more than 100 water soluble pigments comprising the violet betacyanins and the yellow to orange betaxanthins that occur in the form of various glycosides and acylglycosides. Betalains have a limited distribution within the plant kingdom, occurring only in those plant species confined to the order *Caryophyllales*, notably the red beet varieties (*Beta vulgaris* subsp. *vulgaris*, *Chenopodiaceae*) where they substitute for the more diverse family of phenylalanine-derived plant pigments anthocyanins (the flavonoids occurring in all other families of flowering plants). However, there are two exceptions, the *Caryophyllaceae* and *Molluginaceae* plants that accumulate anthocyanins instead of betalains. Betalains also occur in some higher fungi, belonging to the genus *Amanita* (*Basidiomycetes*) such as fly agaric (fly mushroom, *A. muscaria*). Betalains show antioxidant and radical scavenging activities (Hendry & Houghton 1992; Strack *et al.* 2003).

All betalains have the same basic structure (Figure 13). The chromophore with a system of conjugated double bonds is a substituted dihydropyridine. The individual pigments differ in the structure of R and R^1 substituents that are either aliphatic or become a part of *N*-heterocycles. Betalains arise as immonium conjugates of betalamic acid with cyclo-3-(3,4-dihydroxyphenyl)-L-alanine (cyclo-dopa), amino acids, or amines, respectively (Hendry & Houghton 1992; Strack *et al.* 2003).

The biosynthesis[9] of betalains (Strack *et al.* 2003) starts from L-tyrosine (Figure 14), which is hydroxylated to 3,4-dihydroxy-L-phenylalanine (L-DOPA, L-dopa, levodopa) by the action of tyrosinase (EC 1.14.18.1). Dopa is then oxidised by the same enzyme (EC 1.10.3.1)[10] to dopaquinone and dopa 4,5-dioxygenase (EC 1.13.11.-)[11] transforms dopa by extradiol 4,5-ring-cleavage to 4,5-seco-dopa. Dopaquinone spontaneously forms cyclo-dopa whereas spontaneous cyclisation of 4,5-seco-dopa yields betalamic acid, the central intermediate in the formation of all betalains and their yellow chromophore. Spontaneous condensation of betalamic acid with cyclo-dopa, involving the formation of an aldimine bond, yields betanidin,

[9]In plants, betalains are synthesised in the cytoplasm and then stocked in vacuoles. The synthesis is highly regulated and depends on the plant development and tissue. They mainly accumulate in flowers, fruits, and occassionaly in vegetative tissues. The average amount of betalains in the red beet is about 0.1% (fresh weight) and can reach even 0.2% in some varieties (Hendry & Houghton 1992).

[10]Fenoloxidases comprise 2 subgroups of enzymes associated with oxidation of phenols. Monophenol oxidases (EC 1.14.18.1), more commonly known as tyrosinases, are a group of copper proteins that catalyse hydroxylation of phenols in a two-step process. Phenols are first oxidised to 1,2-dihydroxybenzenes (*o*-diphenols) and then subsequent oxidation of *o*-diphenols yields *o*-benzoquinones (*o*-diphenol oxidase, EC 1.10.3.1). Tyrosinase from *Portulaca grandiflora* also catalyses the oxidation of cyclo-dopa to dopachrome, which can be reduced back to cyclo-dopa by ascorbic acid (Schliemann *et al.* 1998). Laccases (EC 1.10.3.2), a group of multi-copper proteins of low specificity, act on both *o*- and *p*-diphenols producing the corresponding quinones.

[11]Dioxygenases introduce both atoms from molecular O_2 into the substrate, and are frequently involved in the cleavage of bonds including aromatic rings. Cyclic peroxides (dioxethanes) are likely to be intermediates. Oxidative cleavage of aromatic rings typically employs catechol (1,2-dihydroxy) or quinol (1,4-dihydroxy) substrates. In the case of catechols, cleavage may be between or adjacent to the two hydroxyls, giving products containing aldehyde and/or carboxylic acid functionalities (Dewick 2002). The mushroom enzyme dopa dioxygenase has two sorts of dioxygenase activity as it produces both 4,5-seco-dopa and 2,3-seco-dopa (Strack *et al.* 2003).
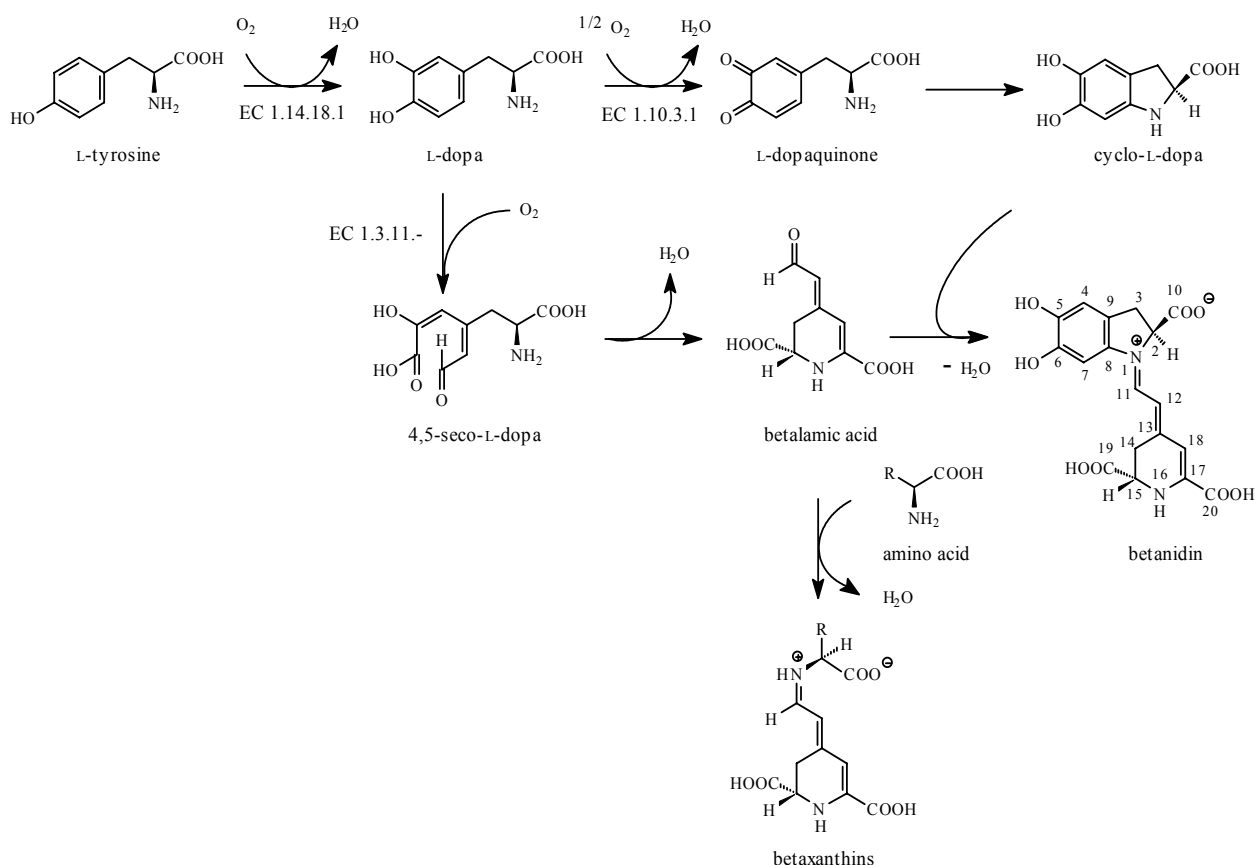
Figure 14

the aglycone of most betacyanins. The minor product of this condensation reaction is isobetanidin, differing only by the absolute configuration at the C-15 chiral centre.

The spontaneous condensation of betalamic acid with an amino acid or amine, again involving the formation of an aldimine bond, generates betaxanthins (Schliemann *et al.* 1999) (Figure 14). The dominating betaxanthin of the red beet is vulgaxanthin I, (*S*)-glutamine-betaxanthin (Figure 15), which represents about 95% of the red beet yellow pigments. It is accompanied by vulgaxanthin II (derived from L-glutamic acid) and betalamic acid (Hendry & Houghton 1992). The yellow beet varieties (*Beta vulgaris* subsp. *vulgaris*) contain a set of betaxanthins including indicaxanthin, i.e. (*S*)-proline-betaxanthin, and immonium conjugates of betalamic acid with L-aspartic acid, γ-aminobutyric acid, L-serine, L-valine, L-leucine, L-isoleucine, L-phenylalanine, L-dopa, and L-tryptophan. Similar set of betaxanthins

were found to occur in the yellow edible fruits of cactus pear (*Opuntia ficus-indica*, *Cactaceae*) (Strack *et al.* 2003).

Different betaxanthins, the muscaaurins I–VII, have been identified as the pigments responsible for the characteristic red-orange colour of the caps of several *Amanita* species. Muscaaurin I and II are derived from unusual nonprotein amino acids, ibotenic acid and stizolobic acid, respectively. The other muscaaurins are derivatives of common proteinogenic amino acids (Michelot & Melendez-Howell 2003).

Ibotenic acid (pantherin, agarin, α-toxin), i.e. 2-amino-3-hydroxy-5-isooxazoleacetic acid[12], and its decarboxylation product muscimol (pyroibotenic acid, β-toxin), i.e. 5-aminomethyl-3-hydroxyisoxazole, are the active principles responsible for the poisoning syndrome due to *A. muscaria* and *A. pantherina*. Ibotenic acid is biosynthesised from L-glutamic acid, probably via (2*S*,3*R*)-3-hydroxyglutamic acid (*threo*-3-hydroxy-

[12]In *A. muscaria*, the keto and enol tautomers of ibotenic acid occur at the ratio of 96:4 in favour of the enol form. On average, the ibotenic acid content is about 0.005% of the spores and 0.017% in fresh caps (Størmer *et al.* 2004).
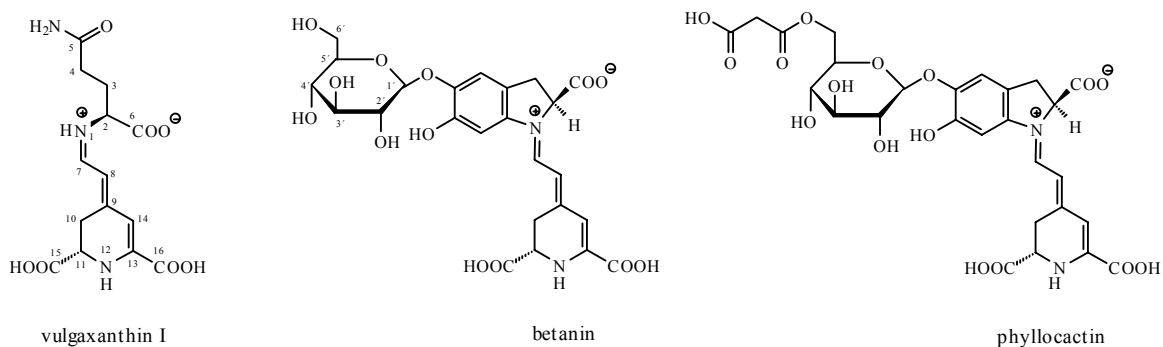
vulgaxanthin I   betanin   phyllocactin

Figure 15

glutamic acid) and its amide[13], respectively. The simplified reaction scheme of the biosynthesis of ibotenic acid and some of its derivatives is shown in Figure 16. Muscazone, i.e. 2-amino-2,3-dihydro-2-oxo-5-isooxazoleacetic acid, probably forms by photo-rearrangement of ibotenic acid. The chemical frame of (*R*)-4-hydroxypyrrolid-2-one (found as a constituent of *A. muscaria*) is common in some micromycetes; these metabolites exhibit a potent biological activity against bacteria and other fungi (Michelot & Melendez-Howell

2003). Tricholomic acid, a closely related dihydro derivative of ibotenic acid, has been identified in *Tricholoma muscarium*.

In fly agaric (*A. muscaria*), other amanitas, and some higher plants, the carbonyl group of 4,5-*se-co*-dopa can be oxidised to a carboxylic group by aldehyde dehydrogenase (EC 1.2.1.-) in the presence of NADP$^+$ or NAD$^+$, which is, under the spontaneous formation of a δ-lactone, transformed to stizolobic acid (l-2-amino-6-carboxy-2-oxo-2*H*-pyran-4-propionic acid) (Musso 1982; Saito
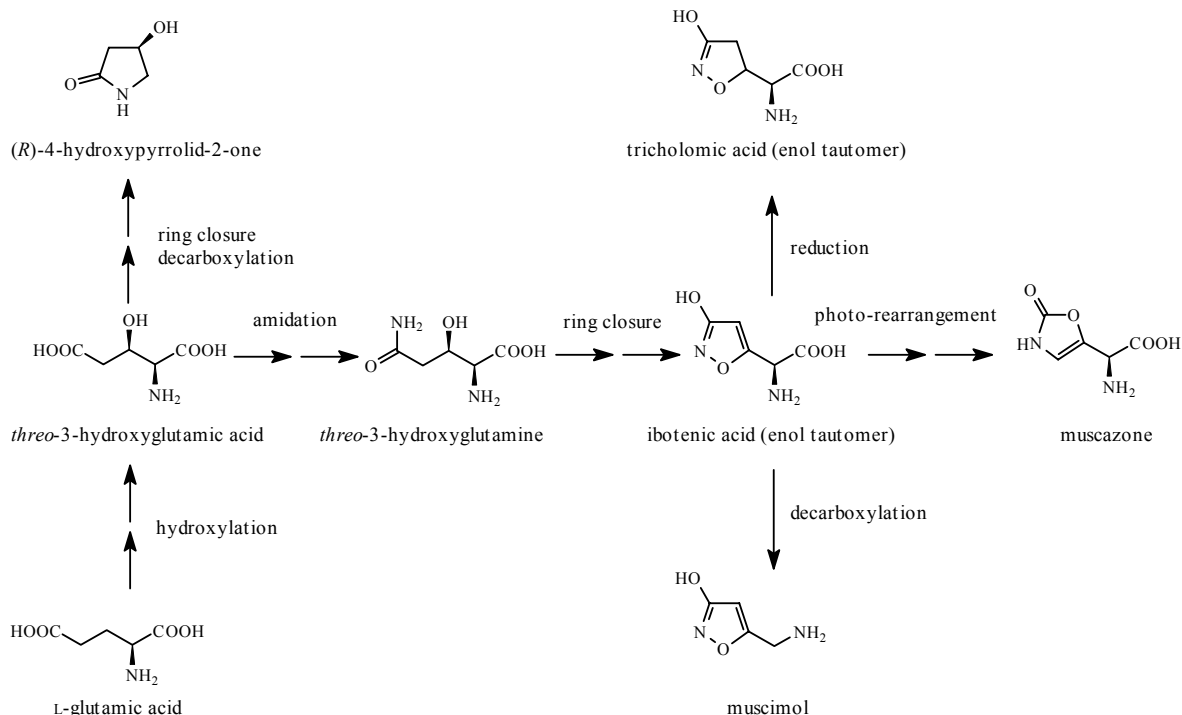

Figure 16

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[13]Analogously, bacterial peptidoglycan biosynthesis involves the incorporation of d-glutamic acid, which forms from l-glutamic acid (glutamate racemase, EC 5.1.1.3), but in some cases (in a few coryneform bacteria) it is hydroxylated to *threo*-3-hydroxyglutamate (Schleifer *et al.* 1967). In some organisms the 2-carboxyl group of d-glutamate is modified by amidation (Schleifer & Kandler 1967).
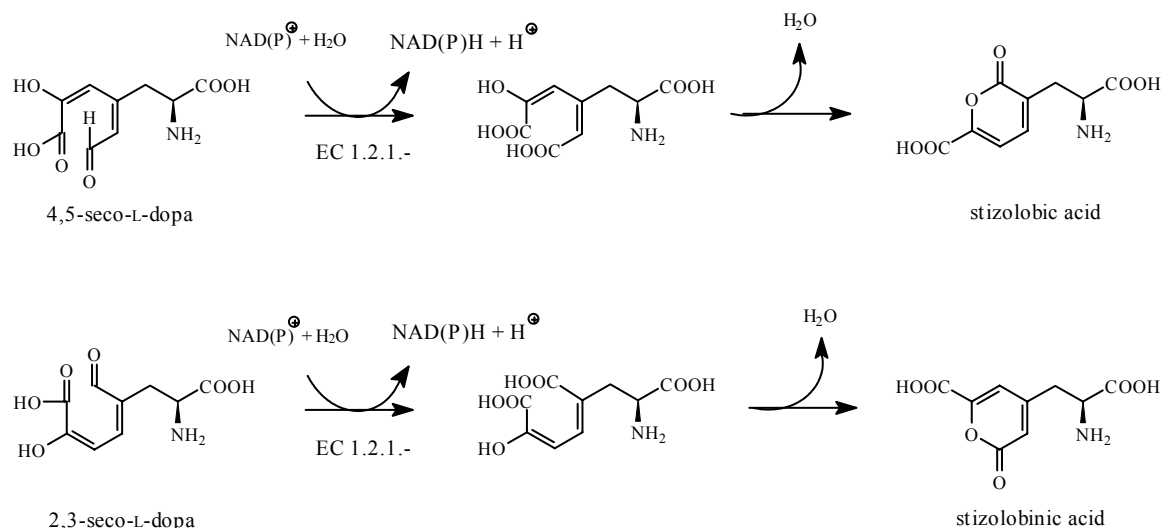
Figure 17

& Komamine 1976; Bugg & Winfield 1998). Stizolobinic acid (l-2-amino-6-carboxy-2-oxo-2*H*-pyran-3-propionic acid) analogously forms from 2,3-*seco*-dopa (Figure 17).

A. muscaria and mushrooms of the *Hygrocybe* family (e.g. *H. conica*) synthesise a second pigment besides betalain, muscaflavin (derived from dihydroazepine), and store it in the cuticle of the cup. Muscaflavin, the yellow pigment not found in betalain-containing plants, is synthesised from dopa which is first transformed to 2,3-*seco*-dopa by 2,3-extradiol cleavage catalysed by dopa 2,3-dioxygenase[13]. The spontaneous cyclisation of 2,3-*seco*-dopa generates muscaflavin. Analogously to betalamic acid, muscaflavin can spontaneously condense with an amino acid under the formation of an aldimine bond, yielding hygroaurins (Mueller *et al.* 1997; Strack *et al.* 2003) (Figure 18).

The basic betacyanin and betaxanthin structures can be modified in numerous ways, conjugation reactions like glycosylation or acylation are common. The majority of betacyanins are acylated under catalysis of a suitable transferase by ferulic acid or less frequently by cinnamic acid and malonic acid on their glycoside part via an ester linkage. For example, glucosylation of betanidin in position C-5 by betanidin 5-*O*-glucosyltransferase forms betanin, i.e. (15*S*)-betanidin-5-*O*-β-d-glucopyranoside, the major red beet pigment representing 75–95% of all red beet betalains (Figure 15). Betanin is accompanied by some minor red pigments such as isobetanin, (15*R*)-betanidin-5-*O*-β-d-glucopyranoside, prebetanin (betanin 6'-sulfate), and isoprebetanin (isobetanin 6'-sulfate) (Hendry & Houghton 1992). Betanin also occurs in the edible fruits of cactus pear (*Opuntia ficus-indica*, *Cactaceae*) as the major red pigment. Betanin and phyllocactin (6'-*O*-malonylbetain, Figure 15), which is formed from betanin by the action of malonyltransferase, are the major betacyanins from the edible fruit pulp of vine cactus (*Hylocereus polyrhizus*, *Cactaceae*) (Strack *et al.* 2003).
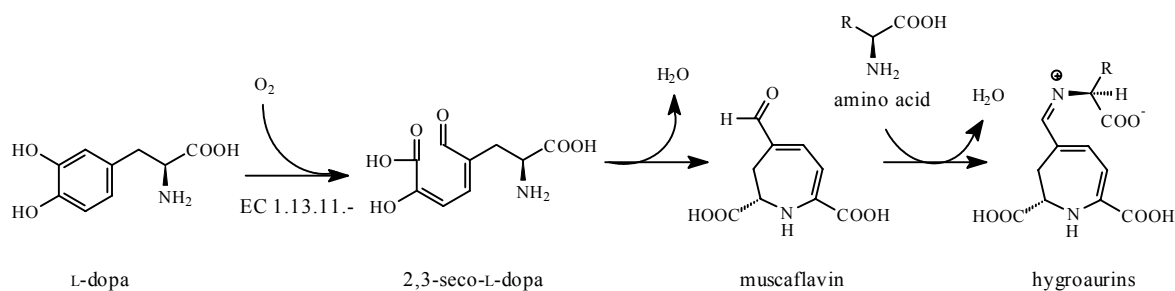

Figure 18

4 QUINONES

Quinones comprise a group of about 400 yellow, red, brown, and black pigments occurring in different parts of microorganisms, algae, lichens, higher fungi, higher plants, and even in some insects. The natural quinoid pigments occurring in foods are mostly derived from benzo-1,2-quinone (1,2-dioxobenzene), benzo-1,4-quinone (1,4-dioxobenzene), naphtho-1,4-quinone (1,4-dioxonaphthalene), and anthra-9,10-quinone (9,10-dioxoanthracene) (Figure 19) but they are, with some exceptions, only of little importance.

In many cases, quinones are found as the corresponding colourless reduced forms (hydroxy and dihydroxy derivatives) and their *O*- and *C*-glycosides that are formed by the action of various glycosyltransferases (EC 2.4.1.-). Quinones form from these glycosides under catalysis by various glycosidases (e.g. *β*-glucosidase, EC 3.2.1.21) and oxidases. Quinones can be synthesised from very different starting substances and via different biochemical pathways (the acetate pathway also called acetate-polymalonate pathway or polyketide pathway, the shikimate pathway[14], the mevalonic acid pathway, and the 1-deoxy-D-xylulose phosphate pathway). Most of the naturally occurring quinone pigments contain phenolic or methoxyl substituents. Many natural quinones occur as oligomers formed by coupling of two or more quinone molecules. The oligomers further differ in the points through which monomers are attached. There are many uncertainties about the exact biosynthetic pathways, especially in plants,

and the participating enzymes. Potential biosynthetic schemes proposed for some quinones are further illustrated.

4.1 Benzoquinones

1,4-Benzoquinone structures often appear in nature as the final products of oxidation of mono- and polycyclic aromatic nuclei. The carbon atoms of such rings can derive from acetic acid and malonic acid (e.g. in the polyketide quinones), from glucose (through shikimic acid) or from mevalonic acid (e.g. in the group of terpene quinones).

Important representatives of benzo-1,4-quinones are ubiquinones and plastoquinones that have been already reviewed (Velíšek & Cejpek 2007a). Numerous *o*-benzoquinones (1,2-benzoquinones) and *p*-benzoquinones (1,4-benzoquinones) form in food as a result of enzymatic browning reactions (Chapter 2.3). Other simple benzo-1,2-quinones and benzo-1,4-quinones are only of minor importance.

The red, violet, and brown terphenylquinones (benzo-1,4-quinones substituted at C-2 and C-5 with phenyl groups), their leucoforms (the corresponding dihydroxy derivatives), and alkylated derivatives are widespread in lichens (e.g. the family *Stictaceae*), lichenising and higher fungi and accompanied by their yellow and orange transformation products pulvinic acids, related butenolides, and other transformation products. The terphenylquinone polyporic acid, its homologues and derivatives are, for example, also produced by some higher fungi (*Macromycetes*, notably by
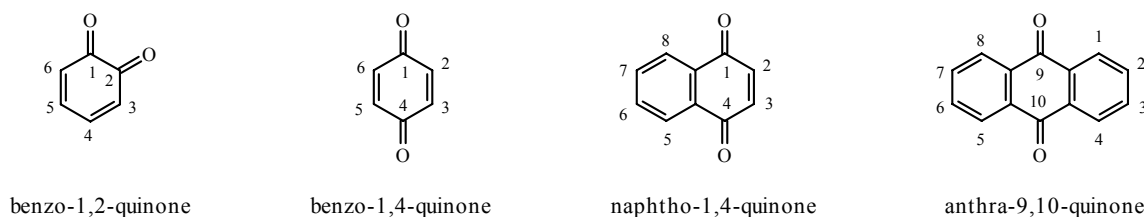
benzo-1,2-quinone   benzo-1,4-quinone   naphtho-1,4-quinone   anthra-9,10-quinone

Figure 19

---

[14]The shikimate pathway employed by microorganisms and plants, but not by animals, provides a route to the essential amino acids phenylalanine, tyrosine, and tryptophan via the central intermediates shikimic acid and chorismic acid (Velíšek & Cejpek 2006b). Shikimic acid is further employed for the biosynthesis (via 1,4-dihydroxy-2-naphthoic acid) of biologically active menaquinones produced by certain bacteria, phylloquinone distributed in higher plants, naphtho-1,4-quinone nucleus of the group of terpenoid quinones that cover plastoquinones, tocopherols, tocotrienols via homogentisic acid, and ubiquinones (coenzymes Q) via 4-hydroxybenzoic acid. The biosynthetic pathways leading to these biologically active compounds were already reviewed (Velíšek & Cejpek 2007a).
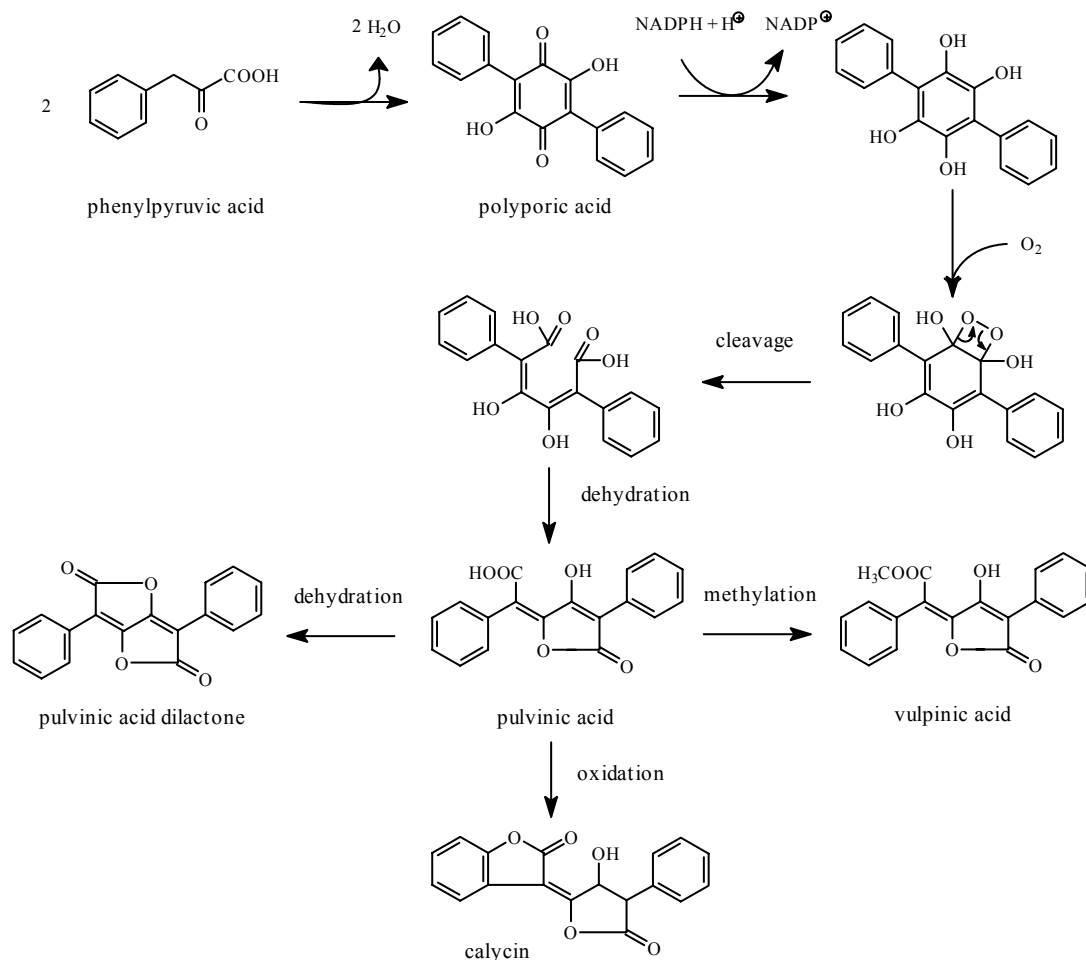
Figure 20

the genus *Amanita*, *Anthracophyllum*, *Boletopsis*, *Paxillus*, *Polyporus*, *Suillus* etc.) and found in their fruit bodies where they contribute to the cap colour (GILL 1996, 1998).

The dark violet polyporic acid is formed from the joining of two phenylpyruvic acids (the product of the shikimate pathway) (Figure 20). The benzoquinone/hydroquinone ring of polyporic acid then opens by oxidation (probably by the action of a dioxygenase-like enzyme, forming by isomerisation the intermediate 2,5-diphenyl-3-hydroxy-4-oxohex-2-enoic acid), which yields by

muscarufin

Figure 21

dehydration the lactone pulvinic acid. Further transformations lead to pulvinic acid dilactone, 3,6-diphenylfuro[3,2-*b*]furan-2,5-dione, which can be methylated (using SAM) to form vulpinic acid or oxidised to calycin (MOSBACH *et al.* 1974). Muscarufin (Figure 21), probably a putative derivative of terphenylquinone, is the principal coloured component of the *Amanita muscaria* caps (MICHELOT & MELENDEZ-HOWELL 2003).

4.2 Naphthoquinones

Shikimic acid is the precursor of the allelopathic substance, naphtho-1,4-quinone juglone, which is found naturally in the leaves, roots, and bark of plants in the *Juglandaceae* family, particularly the black walnut (*Juglans regia*), where it occurs in the glycosylated reduced form as 4-*β*-D-glucopyranoside of 1,4,5-trihydroxynaphthalene (hydrojuglone, 1,5-dihydroxy-4-naphthalenyl-*β*-D-glucopyranoside) (DUROUX *et al.* 1998). The juglone formation is the result of either the glucoside hydrolysis
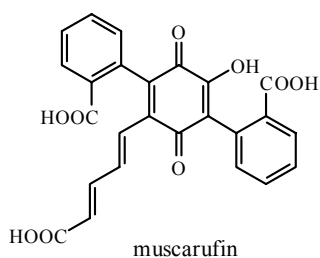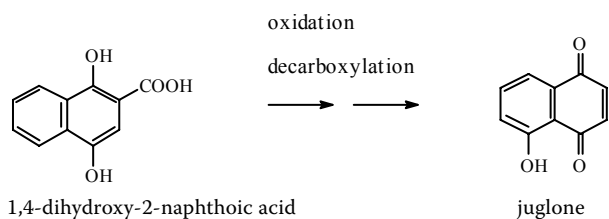
1,4-dihydroxy-2-naphthoic acid

juglone

Figure 22

and 1,4,5-trihydroxynaphthalene oxidation, or the oxidation of the glucoside followed by its hydrolysis. Because of its tendency to create dark orange-brown stains, juglone has also found some usage as a colouring agent for foods and cosmetics, such as hair dyes. Analogously to the biosynthesis of phylloquinone (VELÍŠEK & CEJPEK 2007a), the biosynthesis of juglone (5-hydroxynaphtho-1,4-quinone) (Figure 22) and its isomer lawsone (2-hydroxynaphtho-1,4-quinone) in *Lawsonia inermis*, syn. *L. alba*[15] and other plants proceeds via 1,4-dihydroxy-2-naphthoic acid, presumably by an oxidative sequence in which hydroxyl replaces the carboxyl.

Another shikimic acid metabolite, 4-hydroxybenzoic acid, is the precursor of alkannin, the colouring ingredient of the dye alkanet. Alkannin occurs in the outer surface of the roots of *Alkanna tinctoria* (*Boraginaceae*) and it is also found in many traditional medicinal plants of the *Boraginaceae* family (mainly in the genus *Anchusa, Echium, Lithospermum*). Nowadays, alkanet is mainly used for food colouring and cosmetics. In the pathway to alkannin, 4-hydroxybenzoic acid from the shikimic acid pathway condenses with geranyl diphosphate from the mevalonate and the 1-deoxy-D-xylulose phosphate pathways (BRIGHAM *et al.* 1999), which is followed by decarboxylation, oxidation and cyclisation reactions to yield alkannin (Figure 23).

The orange homologue of juglone is plumbagin (5-hydroxy-2-methylnaphtho-1,4-quinone), occurring e.g. in *Plumbago officinalis* (*Plumbaginaceae*), *Drosera rotundifolia* (*Droseraceae*), and many other plants of the *Plumbaginaceae, Droseraceae, Ebenaceae,* and *Euphorbiace* families in bound forms, which is an example of naphtho-1,4-quinones formed entirely via the acetate-polymalonate
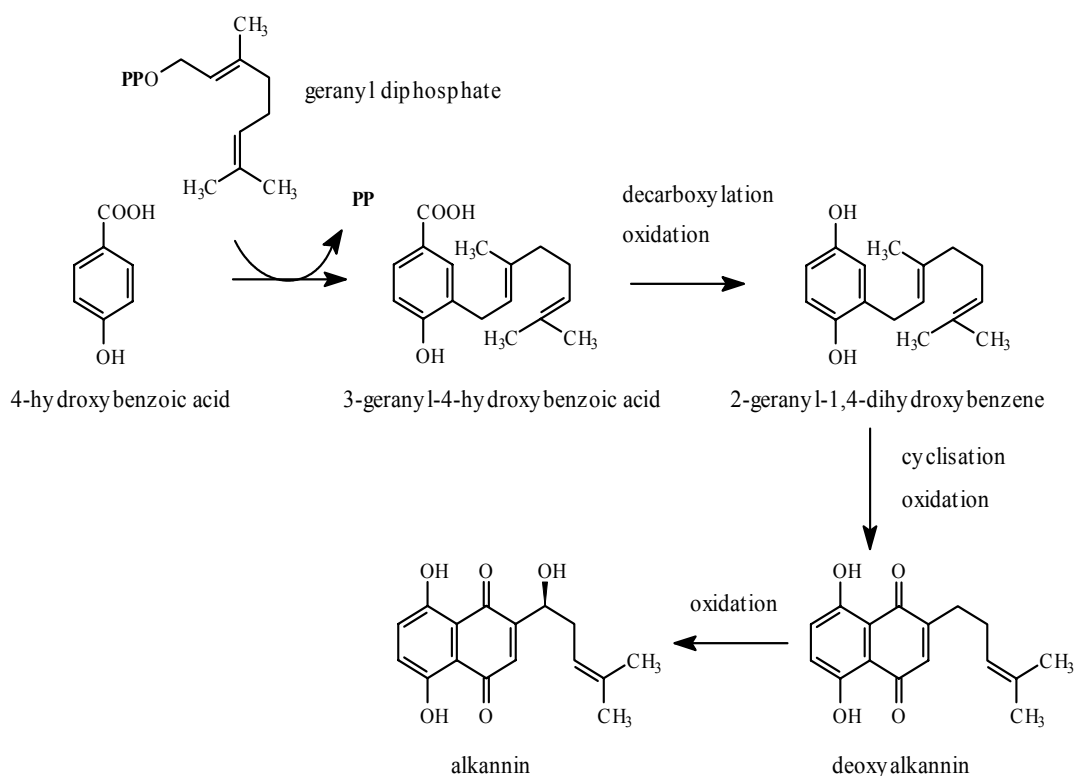

geranyl diphosphate

4-hydroxybenzoic acid   3-geranyl-4-hydroxybenzoic acid   2-geranyl-1,4-dihydroxybenzene

alkannin   deoxyalkannin

Figure 23

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[15]The plant produces a red-orange dye henna. The dye has an affinity to proteins and thus has been used to dye skin, hair, fingernails, leather, silk, and wool.
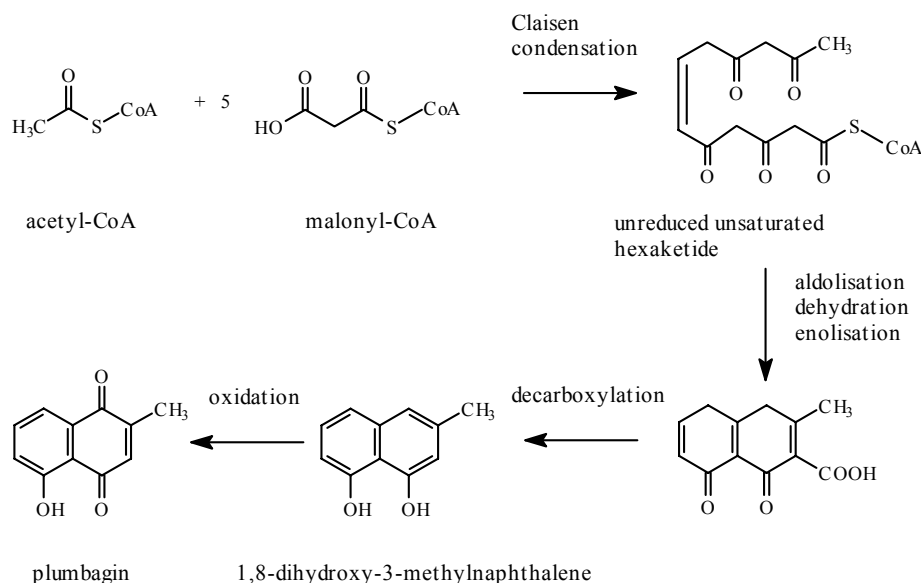
Figure 24

pathway (Mallavadhani *et al.* 1998) (Figure 24). Like juglone, it is physiologically active as it affects mucous membranes and stains the skin.

Naphthoquinones belonging to the vitamin K group (phylloquinone, menaquinones) have been already reviewed (Velíšek & Cejpek 2007a).

4.3 Anthraquinones

Anthraquinones represent the largest group of natural quinones. They occur in some plants (e.g. aloe, senna, rhubarb), fungi, lichens, and in insects and a wealth of structures are known with varying numbers of hydroxyl and other substituents. Several hundreds occur in flowering plants, where they are found especially in the families *Fabaceae* (*Cassia*), *Liliaceae* (*Aloe*), *Polygonacaeae* (*Rheum*), *Rhamnaceae*, *Rubiaceae* (*Rubia*), and *Sclophula-riaceae.* Anthraquinones are often accompanied by anthranol, anthrahydroquinone derivatives, their oxoforms, anthrone, and oxanthrone derivatives (Figure 25) that mainly occur in the form of various glycosides. Many natural anthraquinone derivatives tend to have laxative effects.

In plants, two main biosynthetic pathways leading to anthraquinones have been described, the shikimate (chorismate) pathway and the polyketide pathway (Harborne 1980; Manitto 1981; Dewick 2002). The shikimate pathway is used to produce anthraquinones with only one hydroxylated ring like 1,2-dihydroxylated anthraquinones (also called *Rubia* type anthraquinones, e.g. yellow orange alizarin and red purpurin). The plant *Rubia tinctorium* was used as a natural dye in the textile industry and also as a food colouring agent. The biosynthesis of alizarin (1,2-dihydroxyanthra-9,10-quinone) is analogous to the biosynthesis of the naphtho-1,4-quinone juglone as it proceeds via 1,4-dihydroxy-2-naphthoic acid. In the pathway to alizarin (Figure 26), the quinone formed from 1,4-dihydroxy-2-naphthoic acid condenses with dimethylallyl diphosphate (formed from acetyl-CoA) yielding naphtho-1,4-quinone, which is called catalponone. The cyclisation (the methyl from
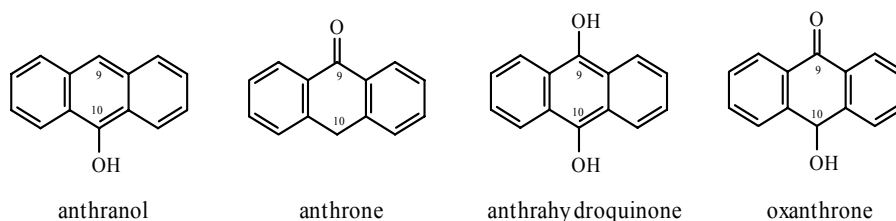

Figure 25

dimethylallyl diphosphate

1,4-dihydroxy-2-naphthoic acid   naphtho-1,4-quinone-2-carboxylic acid   catalponone

cyclisation
decarboxylation

oxidation
decarboxylation

alizarin   2-methylanthra-9,10-quinone
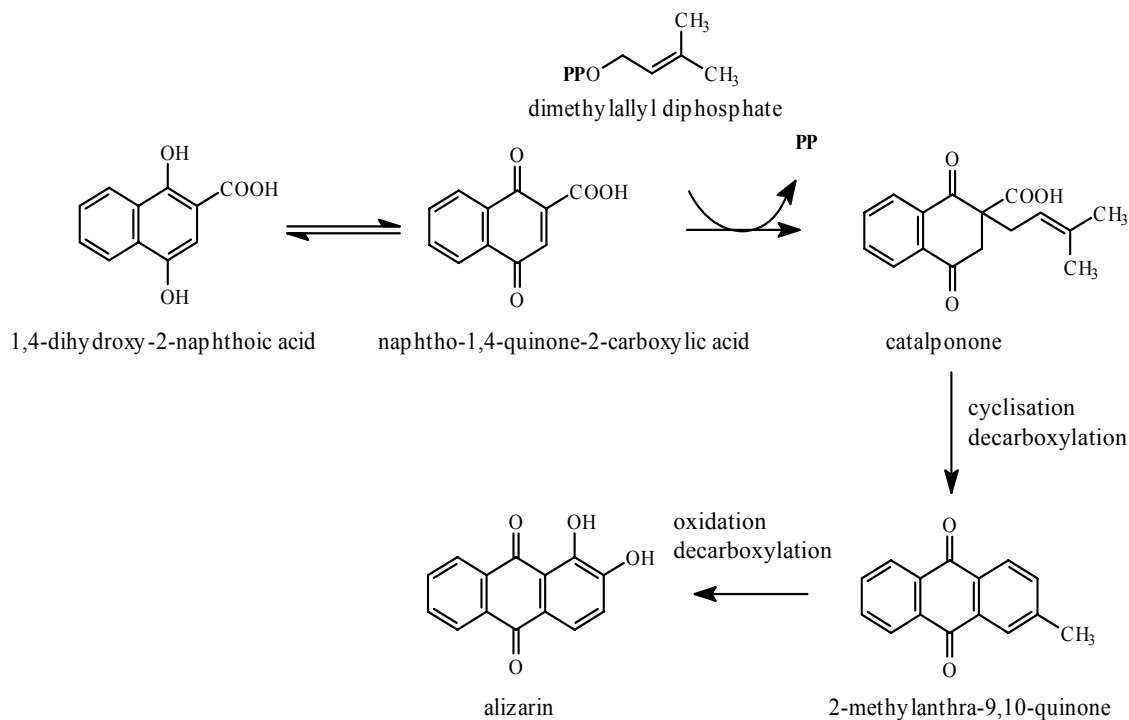
Figure 26

the isoprenyl substituent is removed presumably by the oxidation-decarboxylation sequence, i.e. via hydroxymethyl, formyl, and carboxyl groups) then yields 2-methylanthra-9,10-quinone, which is oxidised to alizarin. The aglycone alizarin is then bound to the *β*-anomer of primeverose (6-*O*-*β*-D-xylopyranosyl-D-glucopyranose) building up ruberythric acid (DERKSEN *et al*. 2003).

The polyketide pathway forms anthraquinones with both rings hydroxylated. Anthraquinones produced via the polyketide pathway are, for example, emodin, chrysophanol, aloe-emodin, and rhein that occur characteristically in several plants used medicinally as a source of purgative drugs, i.e. the petioles of rhubarb, rhizomes of various *Rheum* species, bark of buckthorn (*Rhamnus cathartica*), and dried bark of *R. purshiana* (cascara). They are present either as glycosides (e.g. emodin as its 8-*β*-D-glucopyranoside) or in the reduced form (as anthrone) or dimeric reduced form (as bianthrone).

The assembly of the anthraquinone skeleton is achieved in a step-wise sequence (Figure 27) (DEWICK 2002). After the polyketide is folded, the ring at the centre of the structure is formed first, followed in turn by the next two rings. Decarboxylation appears to take place before aromatisation of the last-formed ring and the tetrahydroanthracene intermediate such as atrochrysone is

involved. This dehydrates to emodin anthrone prior to introduction of the extra carbonyl oxygen as a last transformation is the production of anthraquinones.

Many of the naturally occurring glycosylated bianthrones, which are the active purgative agents, are mixed dimers derived from more than one parent quinone. For example, in rhubarb species several different bianthrones like emodin bianthrone, physcion bianthrone, sennoside, and many more were found, especially in roots (TEUSCHER & LINDEQUIST 1994). Among more highly condensed derivatives of emodin bianthrone is hypericin, a red photodynamic pigment (photosensitising agent) widely present in the flowers of *Hypericum* species (*Hypericaceae*). A one-electron oxidation of the emodin anthrone benzylic methylene group and/or the oxidation of its phenolic tautomer emodin anthranol allows oxidative coupling of the radicals thus formed to give emodin bianthrone (Figure 28). Further oxidative steps lead to dehydrodianthrone and then allow coupling of the aromatic rings through protohypericin to give a naphthodianthrone hypericin (DEWICK 2002).

The females of the cochineal insects belonging to the genus *Dactylopius* (e.g. *D. coccus*, syn. *Coccus cacti*) represent the insect-containing anthraquinones not sequestered from the food. A deep red crimson or carmine dye is extracted from
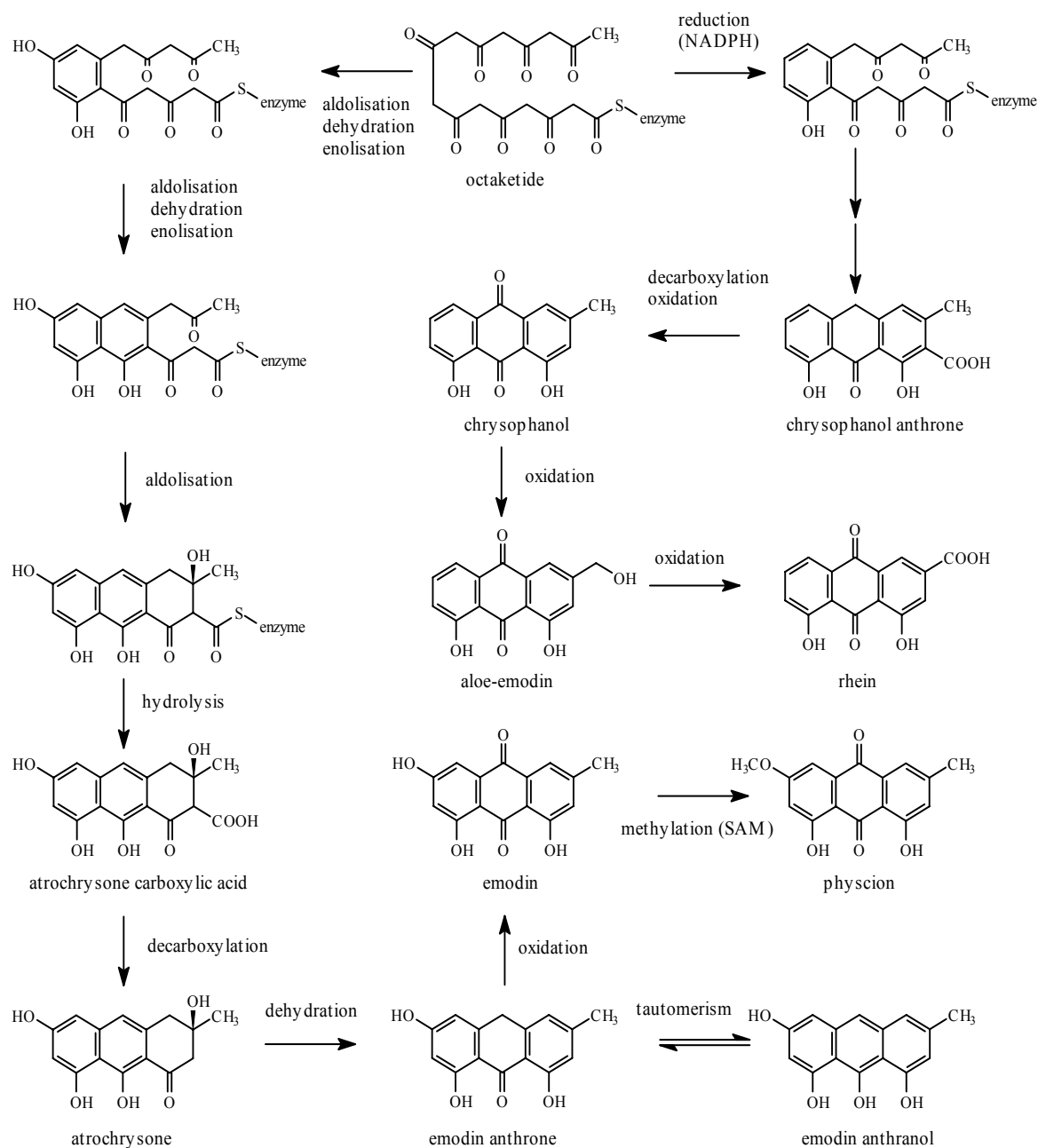
Figure 27

the female insects and used to produce scarlet, orange, and other red tints in some foodstuffs[16]. The main colouring principle of carmine dye is the anthraquinone *C*-glucoside carminic acid,

7-*α*-ᴅ-glucopyranosyl-9,10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxoanthracenecarboxylic acid, having kermesic acid as the aglycone (Figure 29).

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[16]There are two principal forms of cochineal dye: cochineal extract is a colouring made from the raw dried and pulverised bodies of insects and carmine is a more purified colouring made from the cochineal. Cochineal extract natural carminic-acid content is usually 19–22%. The water-soluble form is used in alcoholic drinks with calcium carmine; the insoluble form is used in a wider variety of products. Together with ammonium carmine they can be found in meat, sausages, processed poultry products (meat products cannot be coloured in the United States unless they are labeled as such), surimi, marinades, alcoholic drinks, bakery products, cookies, desserts, icings, pie fillings, jams, preserves, gelatin desserts, juice beverages, varieties of cheddar cheese and other dairy products and sauces.
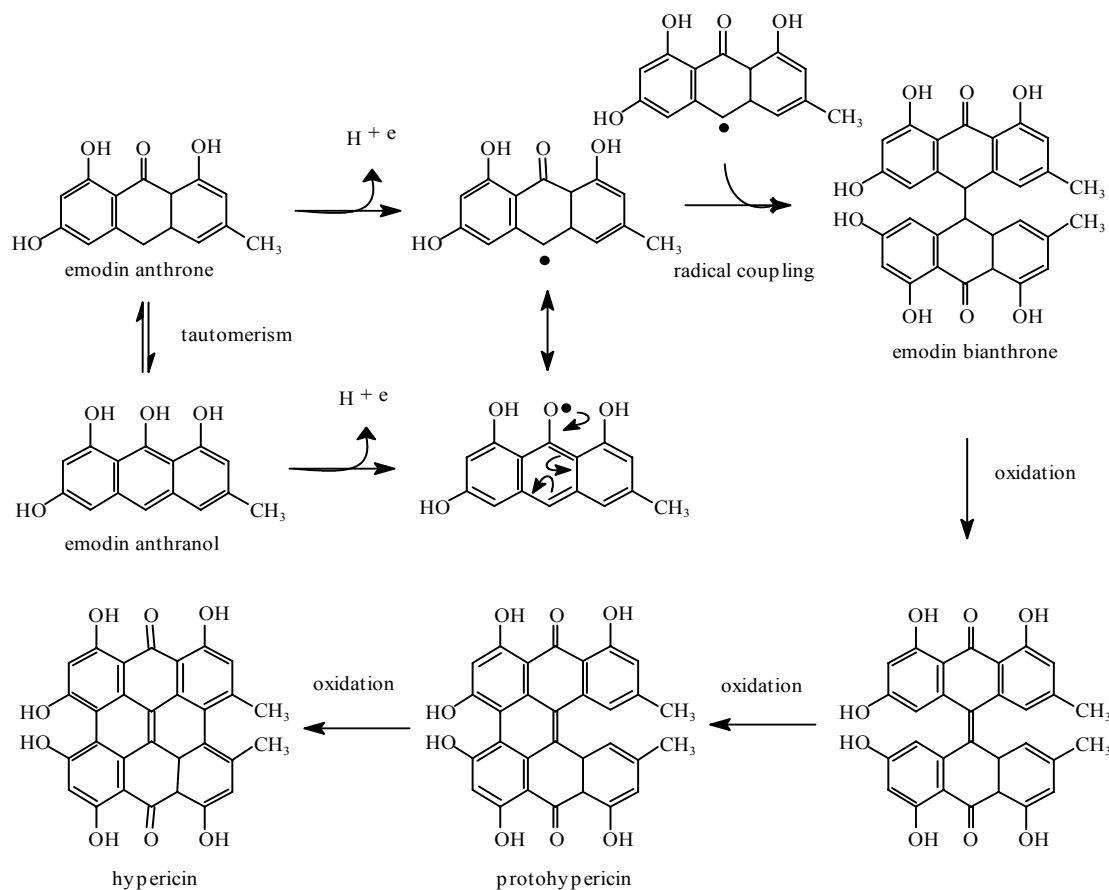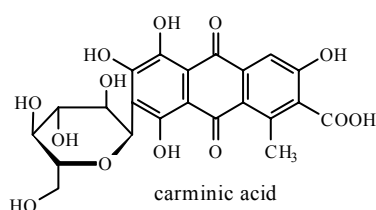
Figure 28

Figure 29

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
CoA	coenzyme A as a part of a thioester
Cys	L-cysteine

Dopa	L-dihydroxyphenylalanine
Glu	L-glutamic acid
GSH	reduced glutathione
Lys	L-lysine
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
P	phosphoric acid
PKS	polyketide synthase
PP	diphosphoric acid
SAH	*S*-adenosyl-L-homocysteine (AdoHcy)
SAM	*S*-adenosyl-L-methionine (AdoMet)
Tyr	L-tyrosine

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