

Biosynthesis of Food Constituents: Vitamins.

1. Fat-Soluble Vitamins – 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 fat-soluble vitamins (vitamin A, vitamin D, vitamin E, vitamin K, the corresponding provitamins, and the closely related ubiquinones and plastoquinones) in animals, plants, and microorganisms. Extensively used are reaction schemes, sequences, and mechanisms with the enzymes involved, with detailed explanations using chemical principles and mechanisms.

Keywords: biosynthesis; vitamin A; vitamin D; vitamin E; vitamin K; retinal; 3,4-didehydroretinol; retinoids; 7-dehydrocholesterol; cholecalciferol; ergosterol; ergocalciferol; tocopherols; tocotrienols; ubiquinones; plastoquinones; phyloquinone; menaquinones

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

amino acids and a number of phenylpropanoids, whereas their side-chains are formed by non-mevalonate pathways.

1 VITAMIN A

Carotenoid cleavage products known as apocarotenoids are widespread in living organisms as they exert key biological functions. In animals, apocarotenoids function as vitamins, visual pigments, and important regulatory signalling molecules in cell division, growth and differentiation of tissues as well as in controls of reproduction (FRIEDRICH 1988; BLOMHOFF *et al.* 1991). In plants, apocarotenoids play roles of hormones, pigments, flavours, and defence compounds (WINTERHALTER & ROUSEFF 2001; GIULIANO *et al.* 2003).

The A group of fat-soluble vitamins historically includes vitamin A₁ (all-*trans*-retinol also known as retinol) and vitamin A₂ (3,4-didehydro-retinol also known as dehydroretinol). Vitamin A₂ has about 40% of the activity of vitamin A₁. These vitamins and their biologically active metabolites are known as retinoids. Since mammals do not synthesise retinoids *de novo*, they depend exclusively upon the alimentary supply of retinol, its derivatives, and precursors (provitamins A). Retinoids are found only in animal products. Eggs, dairy products, animal livers and kidneys, and fish liver oils (e.g. cod liver oil and halibut liver oil used as dietary supplements) are particularly rich sources. Provitamins A are widely distributed in plants and microorganisms that are capable of carotenoid biosynthesis (DEWICK 2002). Retinoids are important metabolites of carotenoids that have at least one non-hydroxylated ring system of the β -type, e.g. carotenes (β -carotene, α -carotene, and γ -carotene) and xanthophylls (β -cryptoxanthin and echinenone). Green vegetables (e.g. spinach) and rich fruit sources (carrots, apricots, mangoes)

provide adequate levels of provitamins A that are synthesised within the plastids.

In mammals, provitamins A are transformed into vitamin A by the oxidative cleavage of the β -type carotenoids taken in the diet. The cleavage of β -carotene occurs in the mucosal cells of the intestine, and is catalysed by an oxygen-dependent dioxygenase, β -carotene 15,15'-dioxygenase (EC 1.14.99.36). This reaction proceeds in three stages, epoxidation of the C15,C15'-double bond, hydration of the double bond leading to ring opening, and the oxidative cleavage of the diol formed. This symmetric or centric cleavage can yield two molecules of the intermediate aldehyde, all-*trans*-retinal (Figure 1). Other provitamins A give one molecule of all-*trans*-retinal¹.

The retinal formed is further metabolised² forming reversibly retinol (retinol dehydrogenase, EC 1.1.1.105), and then its esters with long-chain fatty acids (phosphatidylcholine-retinol O-acyltransferase, EC 2.3.1.135), the storage forms of retinal³. Irreversible oxidation of the retinal carbonyl group by the metalloflavoprotein (FAD)

¹Of the carotenoids, β -carotene is the most potent retinol precursor, yet is six fold less effective on a weight basis than retinol, resulting from incomplete absorption and conversion (BLOMHOFF *et al.* 1991). One retinol equivalent (RE) is equal to 1 μ g of retinol, 6 μ g of β -carotene, or 12 μ g of mixed carotenes.

²Retinal binds a major intestinal protein, cellular retinol-binding protein, which protects it from oxidation into retinoic acid, but allows it to be reduced into retinol by the microsomal retinol dehydrogenase (NAPOLI 1996, 1999). Retinol formed by hydrolysis of retinyl esters in the intestinal lumen during uptake also complexes with retinol-binding protein. The protein-retinol complex then serves as a substrate for the conversion of retinol into retinyl esters. The retinyl esters (predominantly but not exclusively retinyl palmitate) are incorporated into chylomicrons, along with triacylglycerols, cholesteryl esters, carotenoids, and other fat-soluble vitamins, secreted into lymph, and transported to the livers for storage. Once in the hepatocytes (retinyl esters are also stored in the lungs and bone marrow), retinyl esters undergo hydrolysis to release free retinol, which then binds with retinol-binding protein. This complex is mostly transferred to the hepatic stellate cells and retinol is reesterified. Mobilisation of retinol from these cells involves ester hydrolysis and complexation of free retinol with retinol-binding protein before excretion into plasma. Inside the target cells, retinol (having no direct known biological activity) is converted into hormonally active products, e.g. retinal and retinoic acid.

The functional retinoids fall into two categories (NAPOLI 1996). The first category comprises the cofactor (having its role in vision), 11-*cis*-retinal, covalently bound to the protein opsin to form rhodopsin. The second category includes the humoral agents that regulate gene expression. This category includes the retinoids derived from all-*trans*-retinal, i.e. all-*trans*-retinoic acid, 9-*cis*-retinoic acid, and 3,4-didehydroretinoic acid, which is derived from all-*trans*-3,4-didehydroretinol, and 14-hydroxy-*retro*-retinol.

³To date, two retinol-esterifying enzymes have been described (FORTUNA *et al.* 2001). The first enzyme, phosphatidylcholine-retinol O-acyltransferase (EC 2.3.1.135, requires a fatty acyl group from the *sn*-1 position of phosphatidylcholine as acyl donor), esterifies free or bound (cellular-retinol-binding-protein) retinol in the majority of tissues. The second enzyme, acyl-CoA:retinol O-acyltransferase (EC 2.3.1.76, acts on palmitoyl-CoA and other long-chain acyl derivatives of HS-CoA), catalyses esterification of free retinol in the mammary gland.

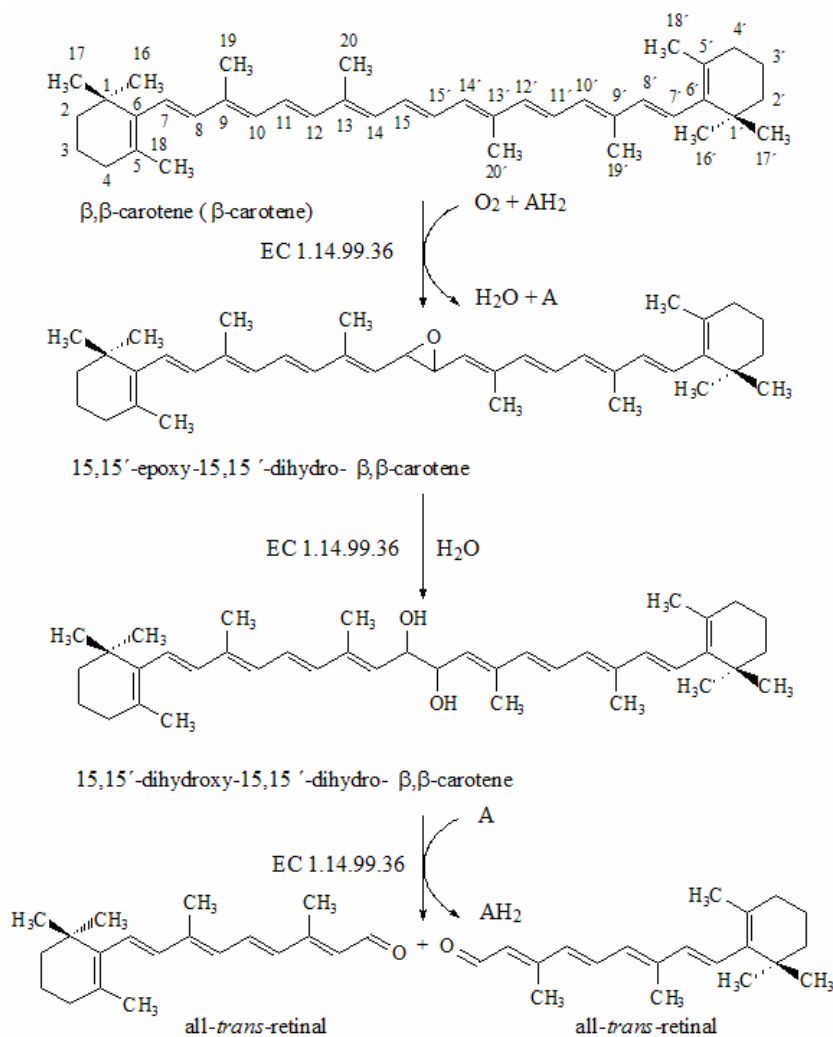


Figure 1

(retinal dehydrogenase, EC 1.2.1.36) gives all-*trans*-retinoic acid, the oxidation of the β -ring yields 3,4-didehydroretinol, which contains cyclohexadiene ring system (Figure 2). A survey of the most common names of the major retinoids and their precursors is given in Table 1 (IUPAC). Structures of several other important retinoids are given in Figure 3.

Although β -carotene cleaved at the central double bond is capable of giving rise to two molecules of retinol, there is evidence that cleavage can also occur at other double bonds⁴, so-called asymmetric or excentric cleavage. This asymmetric

cleavage leads to the formation of two molecules of β -apo-carotenals with different chain lengths. The asymmetric cleavage of β -carotene at the C11', C12' double bond results in the formation of β -apo-12'-carotenal and β -ionilideneacetaldehyde (11-apo- β -caroten-11-al), the C9', C10' double bond cleavage yields β -apo-10'-carotenal and β -ionone (9-apo- β -caroten-9-one), and the C7', C8' double bond cleavage gives β -apo-8'-carotenal and β -cyclocitral (7-apo- β -caroten-7-al) (Figure 4). Further chain shortening then produces retinal, but only one molecule is produced per molecule of β -carotene. Some of the β -apo-carotenals (e.g. β -ionone and

⁴ The properties of this enzyme (requires bile salts and Fe^{2+}) have been a subject of controversy because formerly it was considered to be a β -carotene 15,15'-monooxygenase (EC 1.13.11.21). Furthermore, both symmetric and asymmetric cleavage of β -carotene was reported. Recently, a second type of β -carotene dioxygenase has been described, which catalyses exclusively the asymmetric oxidative cleavage at the 9',10' double bond of β -carotene. Besides β -carotene, also lycopene can be oxidatively cleaved by this enzyme (KIEFER *et al.* 2001).

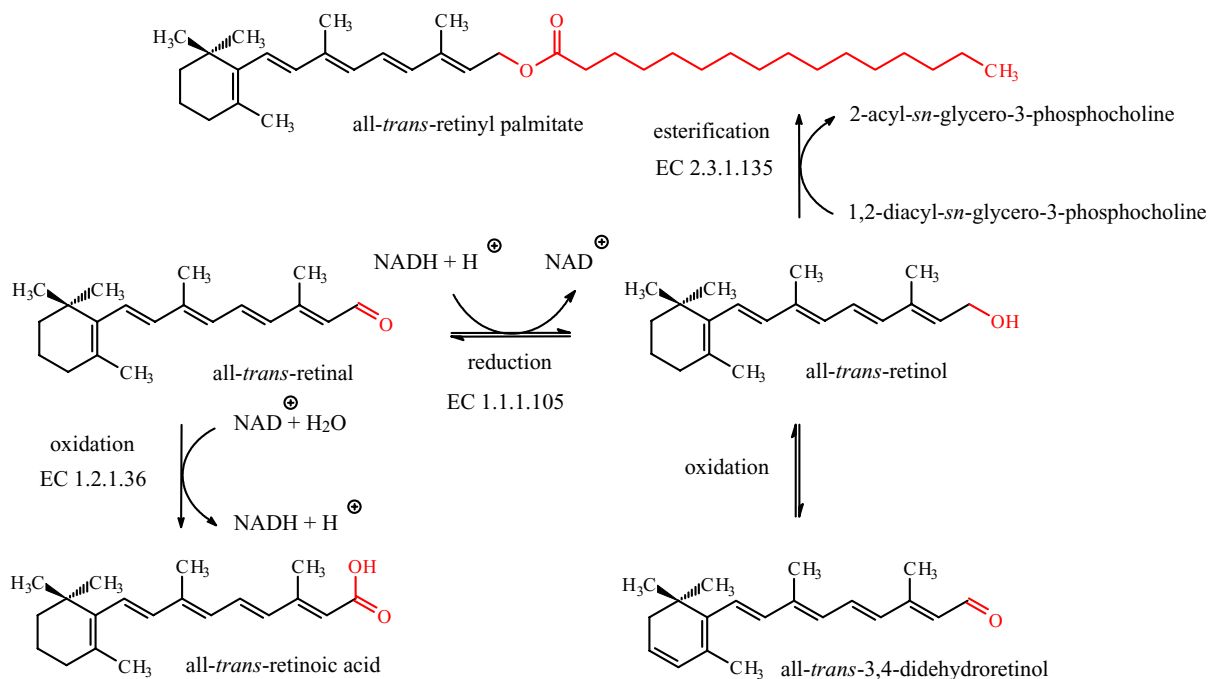


Figure 2

β -cyclocitral) are known as flavour-active components of many fruits and vegetables (WINTERHALTER & ROUSEFF 2001).

β -Apo-carotenals (i.e. β -apo-8'-carotenal, β -apo-10'-carotenal, and β -apo-12'-carotenal) can be oxidised to the corresponding β -apo-carotenoic acids or split at the C15, C15' double bond to all-*trans*-retinal. The β -apo-carotenoic acids, in turn, can be degraded either by a mechanism similar

to β -oxidation of fatty acids β -oxidation (VELÍŠEK & ČEJPEK 2006b) or by splitting at the C15, C15' position, which produces all-*trans*-retinoic acid (LIU *et al.* 1997; KIEFER *et al.* 2001).

Fresh-water fishes contain considerable amounts of vitamin A₂ in addition to vitamin A₁. In this respect they differ from marine fishes and from birds and mammals, which appear to have only vitamin A₁. It was found that, except the above pathway, fresh-

Table 1. Trivial, specific, semi systematic, or systematic names of the major carotenoids and retinoids

Name
α -carotene, β,ϵ -carotene
β -carotene, β,β -carotene
γ -carotene, β,ψ -carotene
β -cryptoxanthin, 3-hydroxy- β,β -carotene
all- <i>trans</i> -3,4-didehydroretinol, 3,4-didehydroretinol, vitamin A ₂ , (2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)nona-2,4,6,8-tetraen-1-ol
echinenone, β,β -caroten-4-one
all- <i>trans</i> -retinal, retinal, retinene, vitamin A ₁ aldehyde, 15-apo-caroten-15-al, (2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenal
all- <i>trans</i> -retinoic acid, retinoic acid, tretinoin, vitamin A ₁ acid, (2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-carboxylic acid
all- <i>trans</i> -retinol, retinol, vitamin A ₁ , (2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-ol

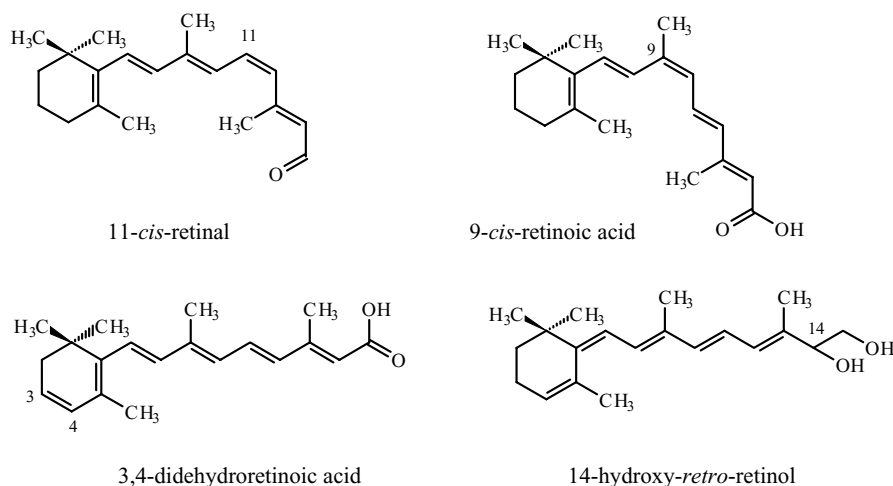


Figure 3

water fishes are able to convert lutein, also known as 3,3'-dihydroxy- α -carotene, i.e. (3*R*,3'*S*,6'*R*)- β , ϵ -caroten-3,3'-diol, to anhydrolutein in the intestine. Anhydrolutein is then split to all-*trans*-3,4-dehydroretinol and all-*trans*-3-hydroxyretinol, after which the latter can be converted to all-*trans*-3,4-dehydroretinol (Figure 5). β -Carotene is converted to all-*trans*-retinol as in birds and mammals (FRIEDRICH 1988).

2 VITAMIN D

The biological functions of vitamin D, achieved largely through a steroid hormone-like mecha-

nisms, are usually divided into classical roles which include the regulation of blood calcium and phosphate concentrations by actions in the intestine, bone, and kidney, and nonclassical roles which include cell differentiation and antiproliferative actions on various cell lines, especially bone marrow, skin, and intestine (JONES & MAKIN 2000).

Vitamin D₃ (cholecalciferol) is obtained in the diet from liver and dairy products such as butter, cream, and milk, while large amounts can be found in fish liver oils. Further requirements in animals are produced photochemically from the immediate precursor of cholesterol, 7-dehydrocholesterol, by the sun irradiation of the skin (FRIEDRICH 1988)

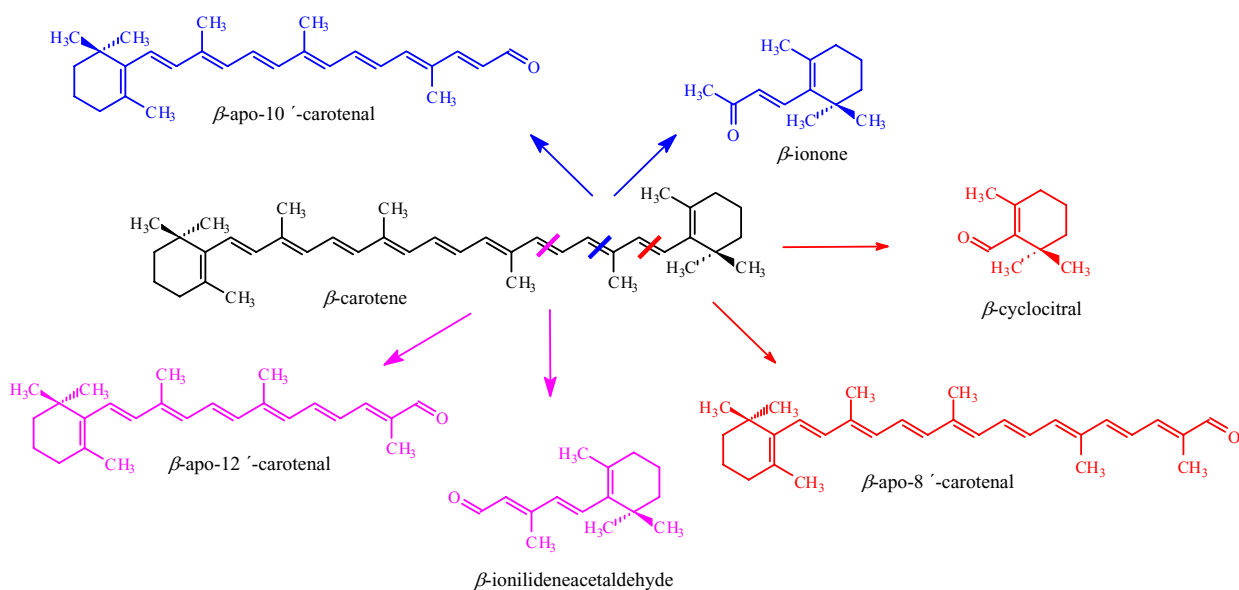


Figure 4

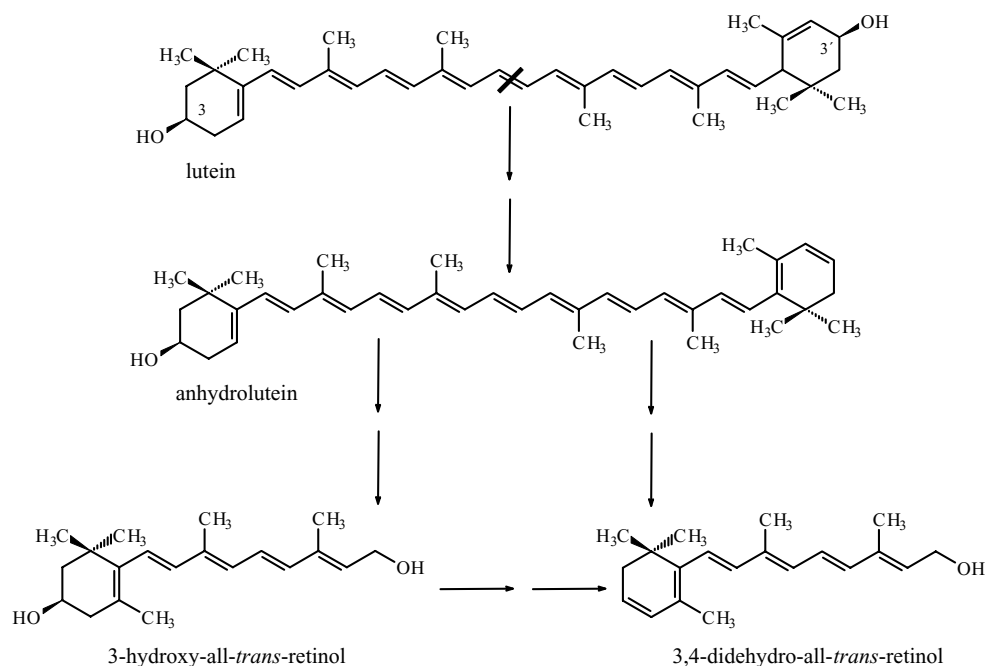


Figure 5

(Figure 6). A photochemical reaction (absorption of light energy by π -electron system) allows electrocyclic opening of 7-dehydrocholesterol ring to yield precholecalciferol (previtamin D_3). Once it is formed, it is slowly transformed by a thermal 1,7-hydrogen shift to cholecalciferol. The 1,7-hydrogen

shift should be considered as an extended version of an allylic isomerisation (Figure 7), however, there is intramolecular transfer of the proton rather than employing an external source (DEWICK 2002).

Cholecalciferol is not itself the active form of the vitamin D. A specific globulin known as vita-

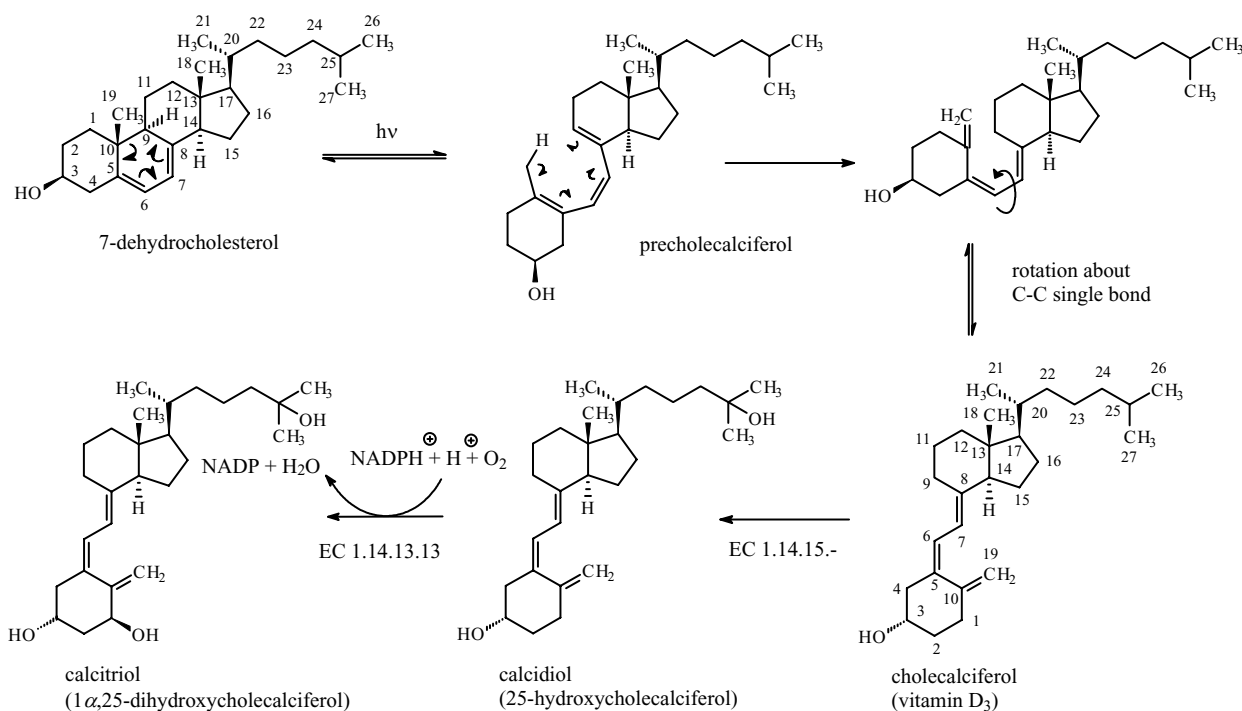


Figure 6

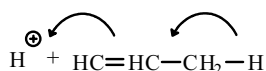


Figure 7

min D binding protein transports cholecalciferol from the skin to the liver for storage or the first step of activation, i.e. hydroxylation to calcidiol (25-hydroxyvitamin D₃) by the enzyme cholecalciferol 25-hydroxylase (from the sub-sub class of oxidoreductases, EC 1.14.15.-) (Figure 6). Calcidiol is subsequently transported to kidneys for the second step of activation, hydroxylation to calcitriol (1 α ,25-dihydroxyvitamin D₃), which is catalysed by the 25-hydroxycholecalciferol 1 α -hydroxylase enzyme (calcidiol monooxygenase, which is an active part of cytochrome P₄₅₀, EC 1.14.13.13).

Many other vitamin D₃ metabolites have been reported over the years (JONES & MAKIN 2000). In the kidneys, calcidiol can be transformed into 24(R),25-dihydroxyvitamin D₃, and in the kidneys and other target tissues (skin, bone, intestine, and parathyroid gland) into 1 α ,24(R),25-dihydroxy-

vitamin D₃ and this compound to calcitroic acid (Figure 8).

7-Dehydrocholesterol, cholecalciferol, calcidiol, and calcitriol have been found in several members of the *Solanaceae* family (e.g. *Solanum glaucophyllum*, syn. *S. malacoxylon*, a plant native to South America, which causes pathological calcinosis in grazing animals) as well as species belonging to other families. The metabolic pathways leading to the individual compounds are largely unknown (CURINO *et al.* 2001).

Vitamin D₂ (ergocalciferol) may be obtained from ergosterol (Figure 8) in exactly the same way in which 7-dehydrocholesterol yields cholecalciferol. It was found in plants and fungi, including *Saccharomyces cerevisiae* yeasts and higher fungi (*Basidiomycetes*)⁵ (SENATORE 1992; MATILLA *et al.* 1994).

Current trivial names, recommended trivial names, and systematic steroid names (IUPAC) of the major vitamin D compounds are summarised in Table 2.

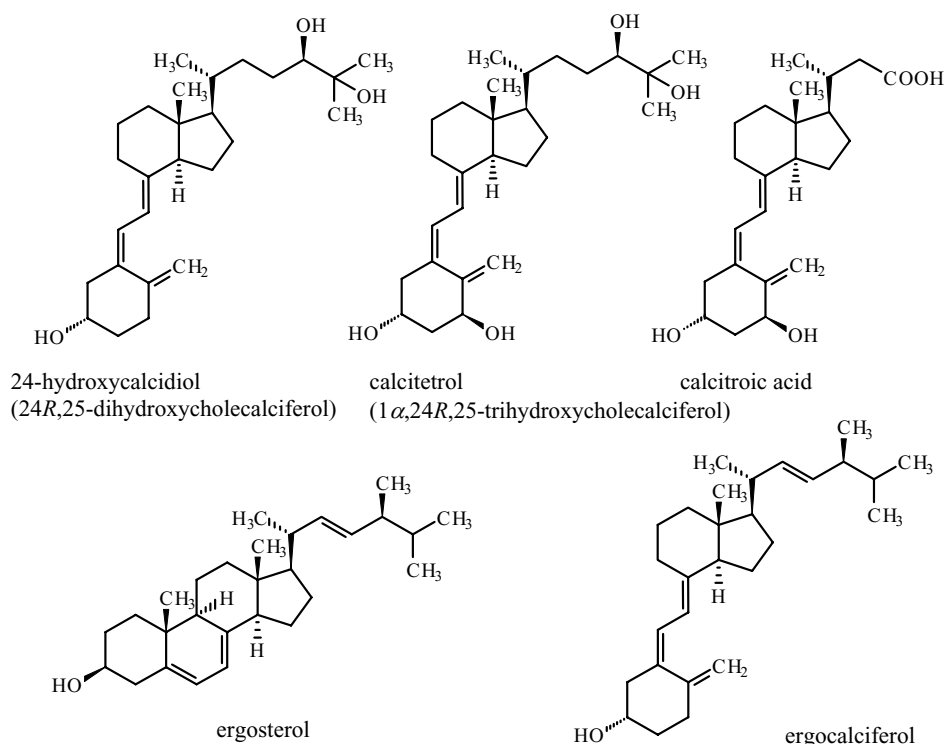


Figure 8

⁵Other compounds with vitamin D activity have also been synthesised, such as vitamin D₄ from 22,23-dihydroergosterol, vitamin D₅ from 7-dehydrositosterol, vitamin D₆ from 7-dehydrostigmasterol, and vitamin D₇ from 7-dehydrocampesterol. Vitamin D₁ was an early preparation, later shown to be a mixture of vitamin D₂ and a photochemical by-product lumisterol (9 β ,10 α -ergosterol).

Table 2. Nomenclature for the major vitamin D compounds and their precursors⁶

Trivial name	Systematic steroid name
7-dehydrocholesterol	7-dehydrocholest-5-en-3 β -ol
(6Z)-tacalcinol, precholecalciferol, previtamin D ₃	(6Z)-9,10-secocholesta-5(10),6,8-trien-3 β -ol
cholecalciferol, calcinol, vitamin D ₃	(5Z,7E)-9,10-secocholesta-5,7,10(19)-trien-3 β -ol
25-hydroxycholecalciferol, calcidiol	(5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-3 β ,25-diol
1 α ,25-dihydroxycholecalciferol, calcitriol	(5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-1,3 β ,25-triol
1,24R-dihydroxycholecalciferol, 24R-hydroxycalcidiol	(5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-1,3 β ,24-triol
1,24R,25-trihydroxycholecalciferol, calcitriol	(5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-1,3 β ,24,25-tetrol
lumisterol	(22E)-9 β ,10 α -ergosta-5,7,22-trien-3 β -ol
ergosterol	(22E)-ergosta-5,7,22-trien-3 β -ol
ergocalciferol, ercalcinol, vitamin D ₂	(5Z,7E,22E)-9,10-secoergosta-5,7,10(19),22-tetraen-3 β -ol

3 TERPENOID QUINONES

The biologically active *p*-quinones with terpenoid side-chains are very closely related substances. They are potentially derivable by the oxidation of suitable phenolic compounds, quinols (hydroquinones, 1,4-dihydroxybenzenes). Accordingly, *p*-quinones can be formed from phenolic systems generated by either the acetate or shikimate pathways. There exist three types of closely related *p*-quinone nuclei, i.e. methyl-substituted benzo-1,4-quinone, methyl- and methoxy-substituted benzo-1,4-quinone, and (methyl-substituted) naphtho-1,4-quinone, and two types of side-chains (phytyl or derived phytyl, and multiprenyl). The most important methyl-substituted benzo-1,4-quinones are known as plastoquinones, methyl- and methoxy-substituted benzo-1,4-quinones as ubiquinones, and (methyl-substituted) naphtho-1,4-quinones as vitamin K. On reduction, the *p*-quinones yield the corresponding quinols, and

each of these has an isomer formed by the ring closure; these are known as chromenols and chromanols, respectively. The most important methyl-substituted chromanols are known as vitamin E (IUPAC). The interrelationships are shown in Figure 9 (*R* = terpenoid side-chain). Ubiquinones and plastoquinones show different biological activities; nevertheless, they are not counted among fat-soluble vitamins.

3.1 Vitamin E

Vitamin E is thought to be involved in many essential processes in animals and plants. The best-characterised function of vitamin E in mammals is to act as a lipophilic free radical scavenger, thereby effectively inhibiting lipid oxidation. The function of vitamin E in plants is far from being clear. Similarly as in animal cells, vitamin E acts as an antioxidant, thus it protects the plant from oxygen toxicity⁷. Recently, other possible functions, like a

⁶ Systematic nomenclature of vitamin D derivatives utilises the obvious relationship to steroids, and the term *seco* (ring opened) is incorporated into the root name. The numbering system for steroids is also retained, thus 7-dehydrocholesterol becomes a derivative of cholestane, namely 7-dehydrocholest-5-en-3 β -ol or (3S)-7-dehydrocholest-5-en-3-ol, and vitamin D₃ becomes a derivative of 9,10-secocholestane, namely (5Z,7E)-9,10-secocholesta-5,7,10(19)-trien-3 β -ol or (3S,5Z,7E)-9,10-seco-cholesta-5,7,10(19)-trien-3-ol.

⁷ Tocopherols and tocotrienols are amphiphatic molecules: the hydrophobic prenyl chain associates with membrane lipids and the polar chromanol ring is exposed to the membrane surface. They scavenge lipid peroxy radicals, thereby preventing the propagation of lipid peroxidation in membranes, and the ensuing products tocopheroxyl and tocotrienoxyl radicals, respectively, are recycled back to tocopherols and tocotrienols by the concerted action of other antioxidants. Furthermore, tocopherols and tocotrienols protect lipids and other membrane components by both physical quenching and chemical reaction with singlet oxygen. The scavenging of singlet oxygen by α -tocopherol in chloroplasts results in the formation of, among other products, α -tocopherol quinone, a known contributor to cyclic electron transport in thylakoid membranes, therefore providing photoprotection for chloroplasts.

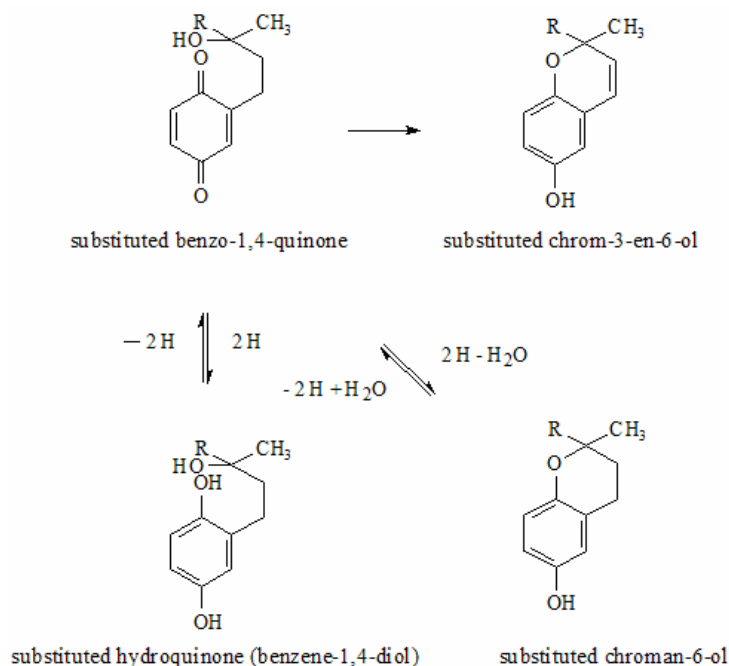


Figure 9

Table 3. Nomenclature of tocopherols and tocotrienols

Trivial name	Semi systematic name	Abbreviation
α -tocopherol	5,7,8-trimethyltolcol	α -T
β -tocopherol	5,8-dimethyltolcol	β -T
γ -tocopherol	7,8-dimethyltolcol	γ -T
δ -tocopherol	8-methyltolcol	δ -T
α -tocotrienol	5,7,8-trimethyltocotrienol	α -T-3
β -tocotrienol	5,8-dimethyltocotrienol	β -T-3
γ -tocotrienol	7,8-dimethyltocotrienol	γ -T-3
δ -tocotrienol	8-methyltocotrienol	δ -T-3

role in thylakoid membrane (rigidity and fluidity regulation), and intracellular signalling have been suggested (MUNNÉ-BOSCH & ALEGRE 2002).

The name vitamin E comprises the biologically active lipid-soluble chroman-6-ols (benzopyran-6-ols) collectively called tocochromanols. Among the tocochromanols are four tocopherols, which possess a saturated phytyl side chain bound to a chromanol ring, and four tocotrienols, which have

an unsaturated geranylgeranyl side chain⁸. Based on the number and positions of methyl groups at the chromanol ring, four forms of tocopherols and tocotrienols, respectively, can be distinguished (Table 3).

Only plants and some cyanobacteria are able to synthesise vitamin E while most prokaryotes and yeasts form very little or none vitamin. α -Tocopherol is the predominant form of vitamin E in

⁸Tocopherols are derived from tocol, (2*R*,4'*R*,8'*R*)-3,4-dihydro-2-methyl-2-(4',8',12'-trimethyltridecyl)-2*H*-1-benzopyran-6-ol. The systematic name of tocotrienol is (2*R*,3'*E*,7'*E*,4'*R*,8'*R*,11'*E*)-3,4-dihydro-2-methyl-2-(4',8',12'-trimethyltrideca-3',7',11'-trienyl)-2*H*-1-benzopyran-6-ol.

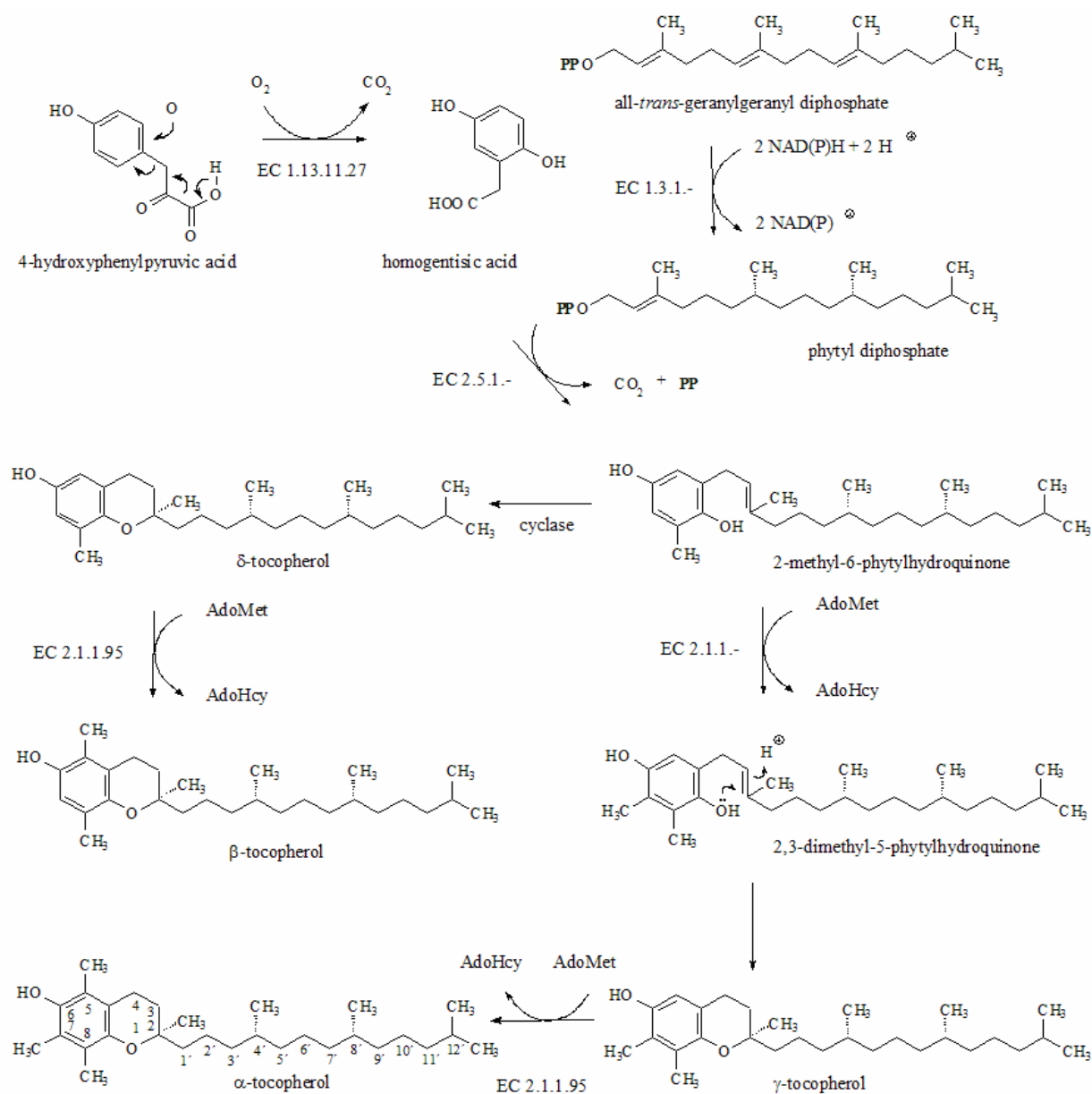


Figure 10

green parts of higher plants, and is synthesised and localised mainly in plastids, whereas generally in non-photosynthetic tissues (e.g. seeds), γ -tocopherol is the major form (MUNNÉ-BOSCH & ALEGRE 2002).

Tocopherols in plants are biosynthesised from the precursors of two pathways: the non-mevalonate isoprenoid pathway and the shikimate pathway (HIRSCHBERG 1999; DEWICK 2002; HOFIUS &

SONNEWALD 2003). The non-mevalonate pathway provides a hydrophobic side-chain, phytyl diphosphate⁹, produced from all-*trans*-geranylgeranyl diphosphate by a step-wise reduction catalysed by geranylgeranyl reductase (EC 1.3.1.-). The shikimate pathway provides the chromanol ring via 4-hydroxyphenylpyruvic acid¹⁰ and homogentisic acid (Figure 10). The latter reaction, catalysed by 4-hydroxyphenylpyruvate decarboxylase

⁹Phytyl is (2*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-enyl.

¹⁰4-Hydroxyphenylpyruvic acid is formed either irreversibly from prephenic acid or reversibly from L-tyrosine (VELÍŠEK & CEJPEK 2006a).

(EC 1.13.11.27), is a complex sequence involving hydroxylation, the migration of the side-chain, and decarboxylation. Homogentisate phytyltransferase (EC 2.5.1.-) then catalyses the condensation of homogentisic acid with phytyl diphosphate to form the first intermediate, 2-methyl-6-phytylhydroquinone (2-methyl-6-phytylplastoquinol or 6-phytyltoluquinol), the common precursor of all tocopherols. The next step is *C*-methylation (*ortho* to OH group) by 2-methyl-6-phytylhydroquinone methyltransferase (EC 1.1.1.-) of 2-methyl-6-phytylhydroquinone at *C*-3 to yield 2,3-dimethyl-5-phytylhydroquinone

(2,3-dimethyl-5-phytylplastoquinol), which is followed by cyclisation (catalysed by phytylquinol cyclase) to 6-membered ring via protonation of the double bond to yield γ -tocopherol. Finally, a second methylation by γ -tocopherol methyltransferase (tocopherol *O*-methyltransferase, EC 2.1.1.95) at *C*-5 yields α -tocopherol. It is still unclear how δ -tocopherol and β -tocopherol are produced, but their synthesis proceeds from 2-methyl-6-phytylhydroquinone and is probably analogous to the synthesis of γ -tocopherol and α -tocopherol, respectively.

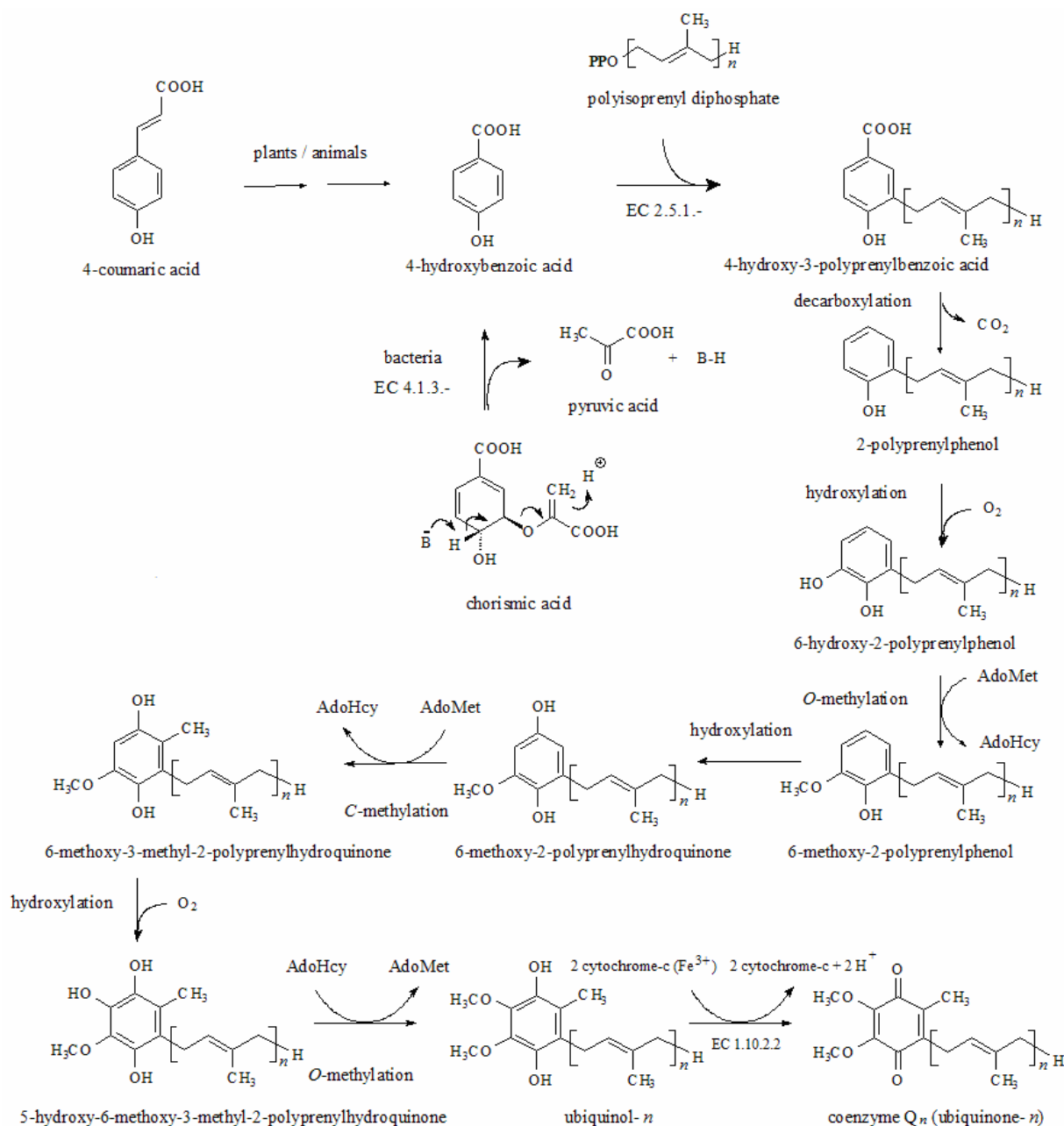


Figure 11

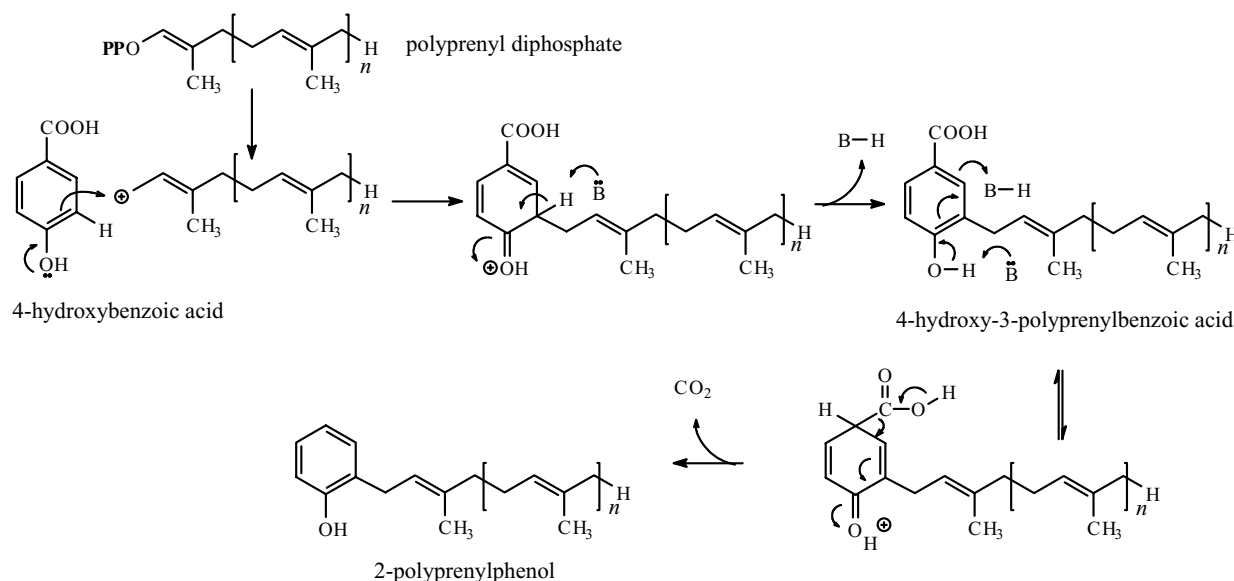


Figure 12

In the tocotrienol biosynthesis, homogentisic acid condenses with geranylgeranyl diphosphate to yield 2-methyl-6-geranylgeranylhydroquinone (6-geranylgeranyltoluquinol) which is then transformed to γ -tocotrienol. Methylation leads (primarily) to α -tocotrienol and (partially) to δ -tocotrienol and then to β -tocotrienol.

3.2 Ubiquinones

Ubiquinones (coenzyme Q) are found in almost all organisms and function as essential components of the respiratory chain. Coenzyme Q is the only low molecular-weight electron carrier in the mitochondrial electron transport system, which is not permanently attached to a protein. It is a benzo-1,4-quinone derivative with a long side-chain composed of a varying number of isoprene units ($n = 1-12$) in the dependence on species. Most organisms synthesise a range of compounds, of which those where $n = 7-10$ usually predominate. In mammals, the most common form contains ten isoprene units (coenzyme Q₁₀, ubi-quinone-10). Apart from this vital function, coenzyme Q₁₀ has antioxidative properties.

Coenzyme Q₁₀ is derived from 4-hydroxybenzoic acid (*p*-hydroxybenzoic acid), the origin of this compound, however, varies according to organisms (DEWICK 2002). Thus, bacteria are known to transform chorismic acid by enzymatic elimination of pyruvic acid (chorismate lyase, EC 4.1.3.-), whereas plants and animals utilise a route from *L*-phenylalanine or *L*-tyrosine (VELÍŠEK & CEJPEK 2006a) via 4-hydroxy-

cinnamic acid (4- or *p*-coumaric acid) (Figure 11). 4-Hydroxybenzoic acid is the substrate for C-alkylation *ortho* to the OH group with a polyisoprenyl diphosphate of appropriate chain length. The product then undergoes further transformation, the exact sequence of modifications, i.e. decarboxylation, hydroxylation (O₂), and O-methylation by *S*-adenosyl-*L*-methionine, varying in organisms and yielding 6-methoxy-2-polyprenylphenol. 6-Methoxy-2-polyprenylphenol is then hydroxylated to yield 6-methoxy-2-polyprenylhydroquinone, and coenzyme Q production then involves further C-methylation, hydroxylation, and O-methylation. The conversion of ubiquinol to quinone is thought to be non-enzymatic (MEGANATHAN 2001). When incorporated in the mitochondrial membrane, it is oxidised by ubiquinol-cytochrome-*c* reductase (complex III, EC 1.10.2.2) in the respiratory chain. Ubiquinol can also form ubiquinone in an NADH-dependent reduction reaction catalysed by ubiquinone reductase (EC 1.6.5.3).

The proposed mechanism of 4-hydroxybenzoic acid prenylation and 4-hydroxy-3-polyprenylbenzoic acid decarboxylation reaction is outlined in Figure 12 (MEGANATHAN 2001).

3.3 Plastoquinones

Plastoquinones are involved in the photosynthetic electron transport chain in plants. Similarly to tocopherols and tocotrienols, plastoquinones are produced from homogentisic acid by C-alkylation *ortho* to the OH group using polyisoprenyl diphosphate with $n = 3-10$, but most commonly

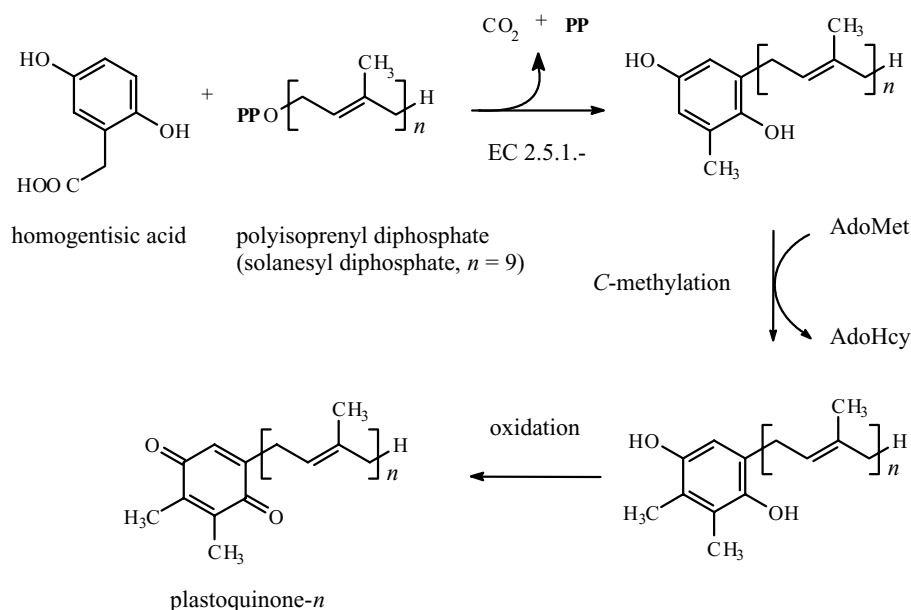


Figure 13

with $n = 9$, i.e. solanesyl diphosphate (Figure 13). Homogentisate phytyltransferase (EC 2.5.1.-) catalyses the prenylation reaction that adds solanesyl diphosphate to homogentisic acid in the plastoquinone-9 biosynthesis pathway. During the alkylation reaction, the CH_2COOH side-chain of homogentisic acid suffers decarboxylation, and the product is thus an alkyl methyl *p*-quinol

derivative. Further aromatic methylation (using AdoMet) and oxidation of the quinol to a quinone follow to yield the plastoquinone.

3.4 Vitamin K

Vitamin K comprises a number of fat-soluble naphtho-1,4-quinone derivatives, with vita-

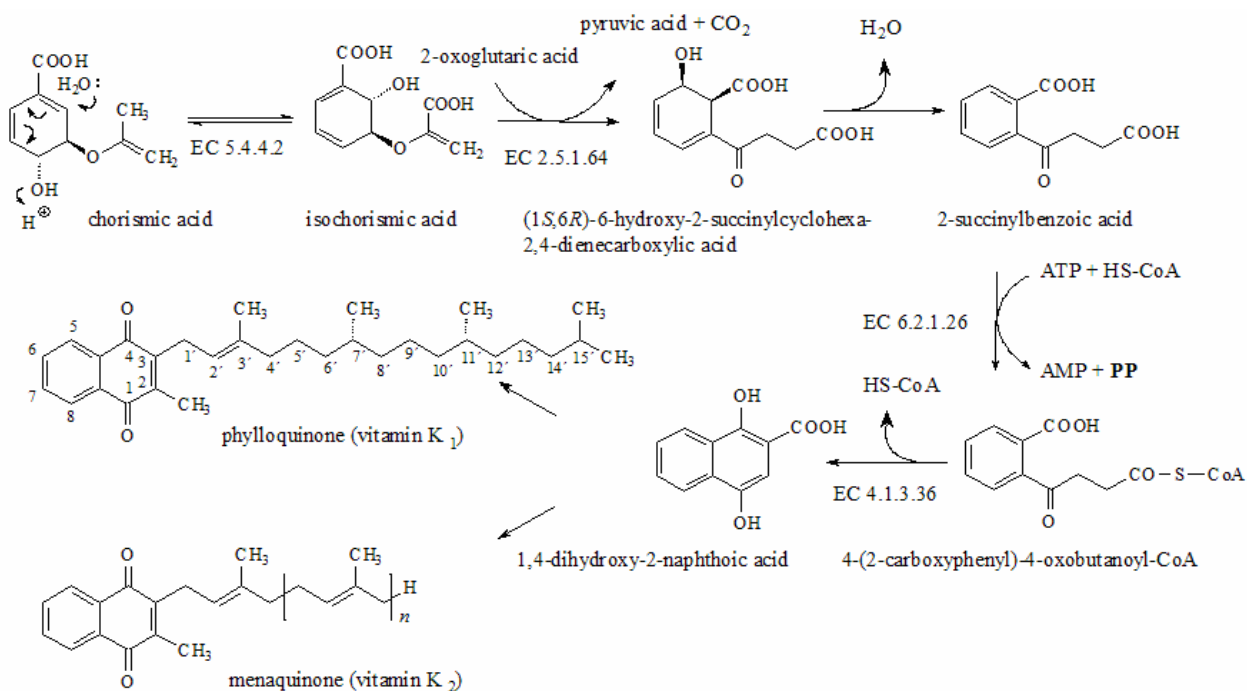


Figure 14

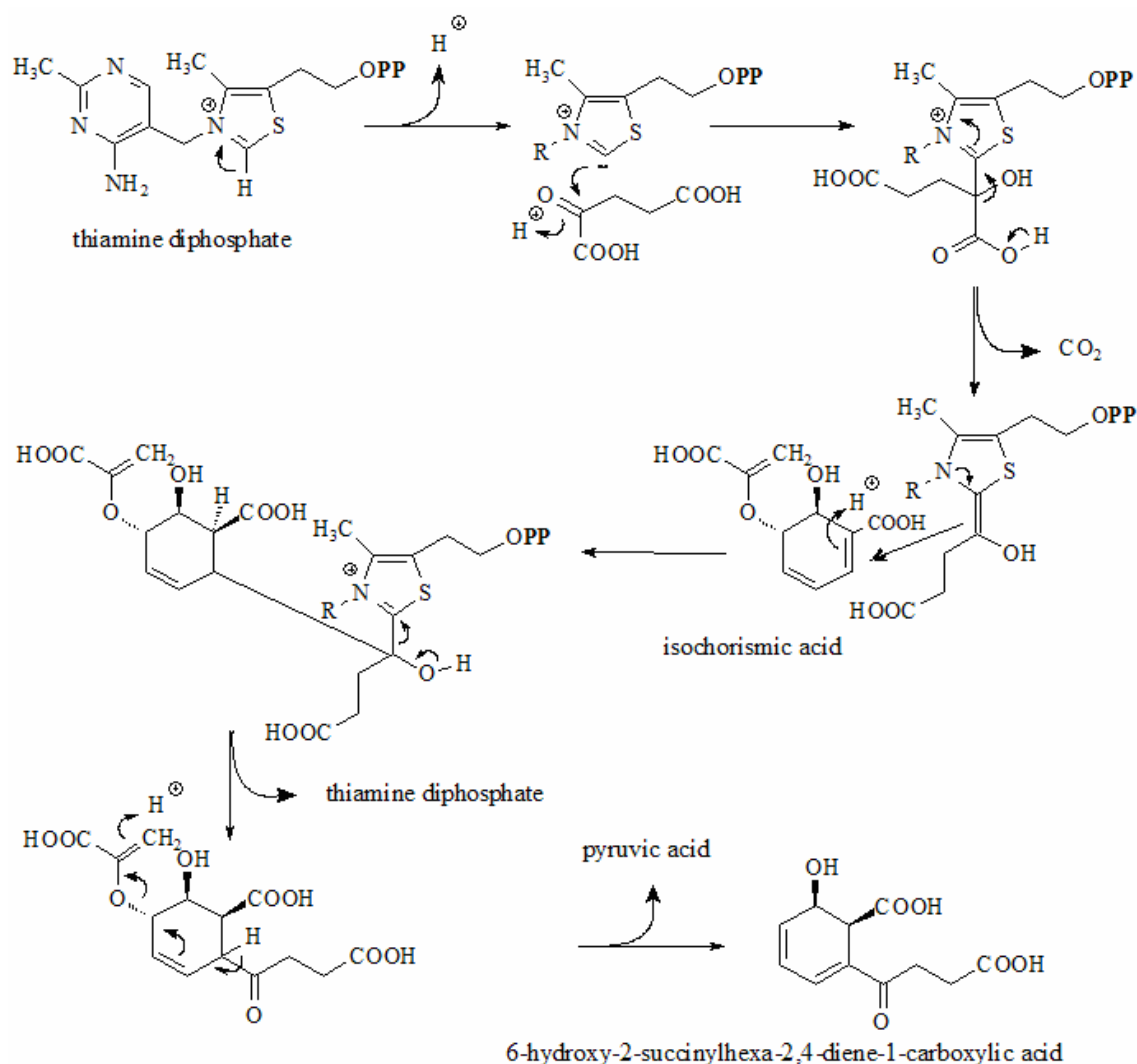


Figure 15

min K₁ (phyloquinone or phytylmenaquinone or 2-methyl-3-phytylnaphtho-1,4-quinone¹¹ being of plant origin, while vitamin K₂ (menaquinones or 2-methyl-3-multiprenylnaphtho-1,4-quinones) is produced by microorganisms. Vitamin K (coagulation vitamin) is involved in normal blood clotting processes in mammals, and deficiency would lead to haemorrhage¹². In green plants, phyloquinone is a normal component of the photosynthetic apparatus. It is also found in green and brown al-

gae. Phyloquinone has a diterpenoid side-chain, whereas the range of menaquinone structures tends to be rather wider with 1–13 isoprene units. Dietary vitamin K₁ is obtained from almost any green vegetable, while a significant amount of vitamin K₂ is produced by the intestinal microflora.

Phyloquinone and menaquinones are derived from chorismic acid via isochorismic acid (Figure 14). The isomerisation of chorismic acid to isochorismic acid proceeds via S_N2' reaction and

¹¹The systematic name of phyloquinone is (2'E,7'R,11'R)-2-methyl-3-(3',7',11',15'-tetramethylhexadecyl)naphtho-1,4-quinone.

¹²Blood clotting requires the posttranslational modification (carboxylation) of glutamic acid residues in the N-terminal portion of the protein prothrombin to γ-carboxyglutamates, generating bidentate ligands that allow the protein to bind other factors. This carboxylation requires carbon dioxide, molecular oxygen, and the reduced quinol form of vitamin K. During the carboxylation, the reduced vitamin K is oxidised to vitamin K 2,3-epoxide, and vitamin K is subsequently regenerated by reduction (phyloquinone reductase or menaquinone reductase, EC 1.6.99.2).

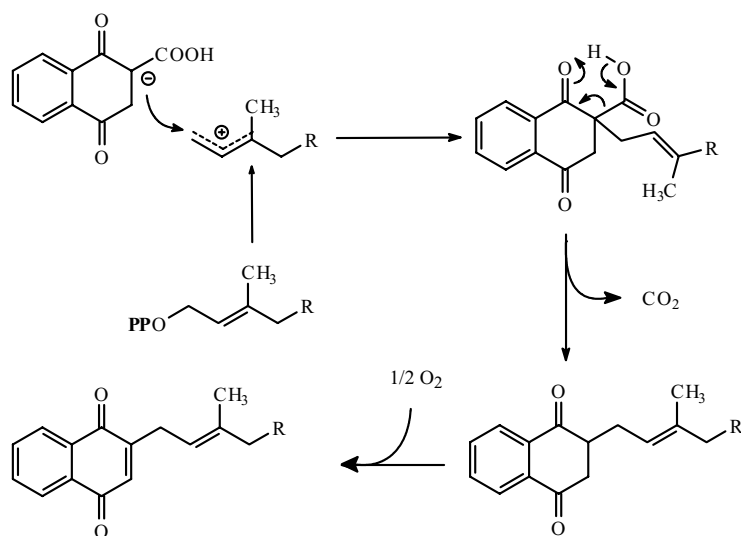


Figure 16

is catalysed by isochorismate synthase (EC 5.4.4.2) (MEGANATHAN 2001; DEWICK 2002; KEGG). Additional carbons for the naphthoquinone skeleton are provided by 2-oxoglutaric acid which is incorporated by a mechanism involving the cofactor thiamine diphosphate (Figure 15). The reaction is catalysed by 2-succinyl-6-hydroxycyclohexa-2,4-diene-1-carboxylate synthase (EC 2.5.1.64). 2-Oxoglutaric acid is decarboxylated in the presence of thiamine diphosphate to give the thiamine diphosphate anion of succinic semialdehyde, which attacks isochorismic acid in a Michael-type reaction. The loss of thiamine cofactor and the elimination of pyruvic acid followed by dehydration yield the intermediate *o*-succinylbenzoic acid. This is activated by the formation of a coenzyme A ester under catalysis by *o*-succinylbenzoate-CoA ligase (EC 6.2.1.26), and a Dieckmann-like condensation catalysed by naphthoate synthase (EC 4.1.3.36) allows the ring formation. The 1,4-dihydroxy-2-naphthoic acid is the more favoured tautomer from the hydrolysis of the coenzyme A ester. This compound is now the substrate for alkylation and methylation as seen with ubiquinones and plastoquinones. However, the terpenoid fragment is found to replace the carboxyl group, and the decarboxylated analogue is not involved. The transformation of 1,4-dihydroxy-2-naphthoic acid to the alkylated naphthoquinone appears to be catalysed by a single enzyme (not yet characterised, EC 2.5.1.-) (shown in Figure 16 using the diketo tautomer). This involves alkylation with phytyl diphosphate or polyprenyl diphosphate (formed

from geranylgeranyl diphosphate) (EC 2.1.1.-), decarboxylation of the resultant 2-oxoacid, oxidation to *p*-quinone, and finally methylation at C-2 using AdoMet. Menaquinones lacking the methyl group also occur and are described as demethylmenaquinones.

EC (Enzyme Commission) numbers and some common abbreviations

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

AH ₂	– hydrogen donor
AMP	– adenosine 5'-monophosphate
ATP	– adenosine 5'-triphosphate
CoA	– coenzyme A as a part of a thioester
FAD	– flavine adenine dinucleotide
PP	– diphosphoric acid
RE	– retinol equivalent
NADH	– nicotinamide adenine dinucleotide
NADPH	– nicotinamide adenine dinucleotide phosphate
SAH	– S-adenosyl-L-homocysteine (AdoHcy)
SAM	– S-adenosyl-L-methionine (AdoMet)

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