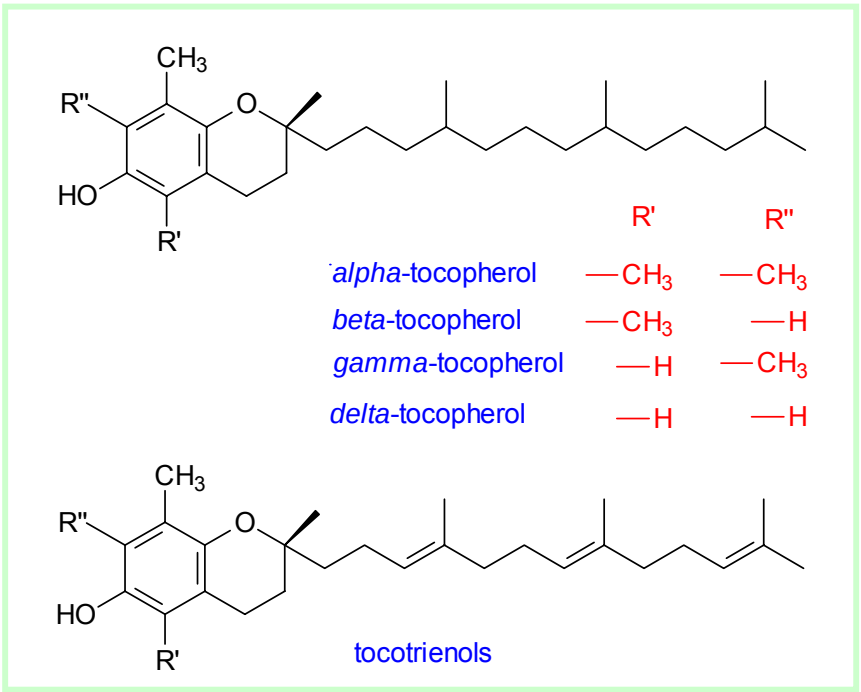
TOCOPHEROLS AND TOCOTRIENOLS

STRUCTURE, COMPOSITION, BIOLOGY AND ANALYSIS

1. Structure and Biosynthesis

Tocopherols constitute a series of related benzopyranols (or methyl tocols) that occur in plant tissues and vegetable oils and are powerful lipid-soluble antioxidants. In the tocopherols, the C_{16} side chain is saturated, and in the **tocotrienols** it contains three *trans* double bonds. Together, these two groups are termed the **tocochromanols**. In essence, the tocopherols have a 20-carbon phytyl tail (including the pyranol ring), and the tocotrienols a 20-carbon geranylgeranyl tail with double bonds at the 3', 7' and 11' positions, attached to the benzene ring. The side-chain methyl groups have *R,R,R* stereochemistry. The four main constituents of the two classes are termed - *alpha* (5,7,8-trimethyl), *beta* (5,8-dimethyl), *gamma* (7,8-dimethyl) and *delta* (8-methyl).



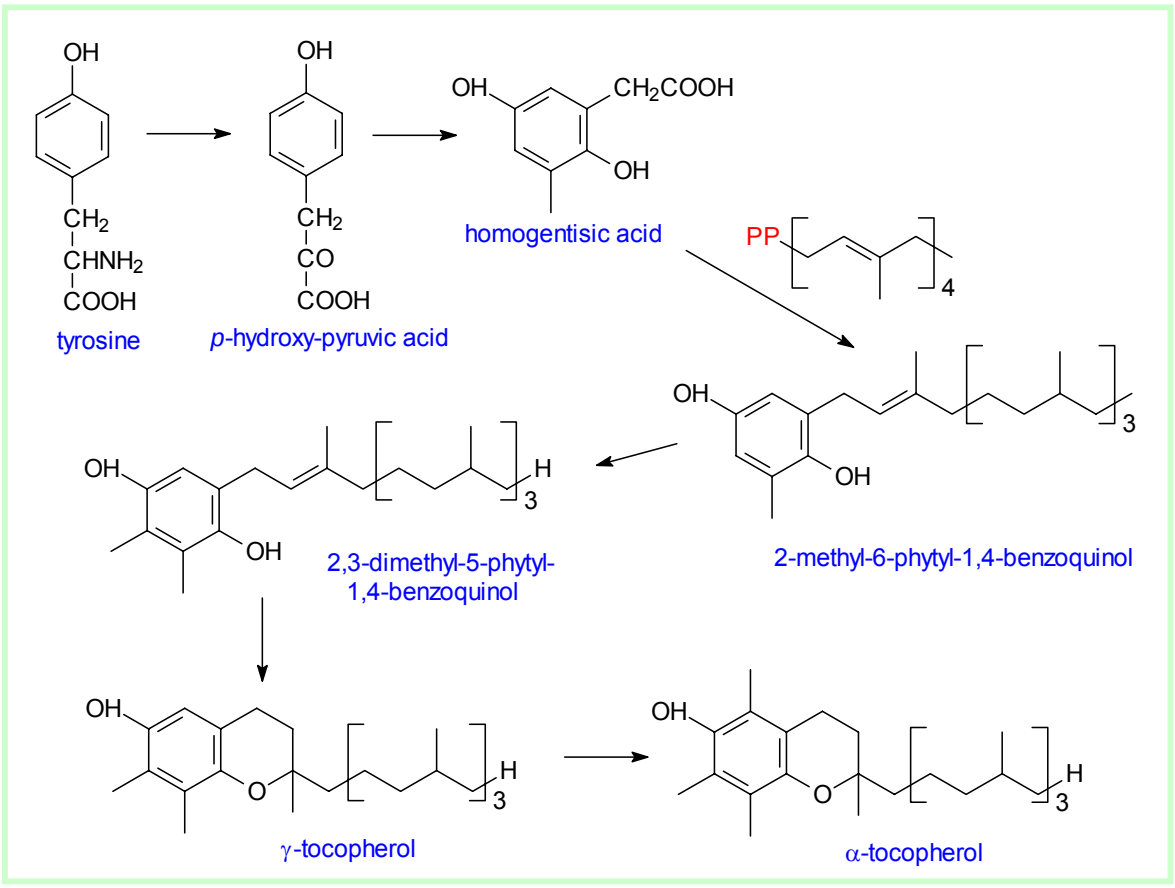
These compounds are only synthesised by plants and other oxygenic, photosynthetic organisms, but they are essential components of the diet of animals, and collectively they are termed 'vitamin E' (the individual tocopherols are 'vitamers'). In plants, there is a great range of tocochromanol contents and compositions, and photosynthetic plant tissues contain from 10 to 50 µg tocochromanols per g fresh weight. α-Tocopherol is often the main tocochromanol in leaves. Seed oils are a major source for the human diet and the compositions of tocopherols in some unrefined oils are listed in **Table 1**. Sunflower and olive oils are good sources of α-tocopherol and palm oil of the tocotrienols. In general, tocotrienols tend to be more abundant in seeds of monocots, such as wheat, rice and barley.

Table 1. Tocopherol and tocotrienol contents (mg/Kg) in some seed oils

	α-T*	β-T	γ-T	δ -T	α -TT*	β -TT	γ -TT	δ-TT
palm	89	-	18	-	128	-	323	72
soybean	100	8	1021	421	-	-	-	-
maize	282	54	1034	54	49	8	161	6
sunflower	670	27	11	1	-	-	-	-
rapeseed	202	65	490	9	-	-	-	-

* Abbreviations: T, tocopherol; TT, tocotrienol
Data from: Gunstone, F.D., Harwood, J.L. and Padley, F.B. *The Lipid Handbook (Second Edition)* (Chapman & Hall, London) (1994).

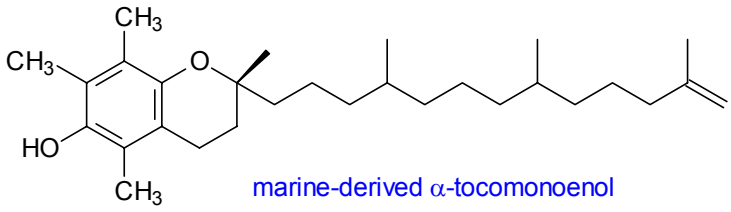
The mechanism of biosynthesis of tocopherols is well understood, and involves coupling of phytyl diphosphate with homogentisic acid (2,5-dihydroxyphenylacetic acid), followed by cyclization and methylation reactions.



The plant chloroplast is the site of biosynthesis, and the aromatic amino acid tyrosine can be considered the basic precursor. This is oxidized to *p*-hydroxypyruvic acid, which in the first committed step is converted to homogentisic acid by the enzyme *p*-hydroxyphenylpyruvate dioxygenase. Homogentisic acid is condensed with phytyl diphosphate in a reaction catalysed by a prenyl transferase to yield 2-methyl-6-phytyl-plastoquinol, which is first methylated to form 2,3-dimethyl-5-phytyl-1,4-benzoquinol and then converted by the enzyme tocopherol cyclase to γ-

tocopherol. A further methylation reaction produces α-tocopherol, while modifications to the pathway produce β- and δ-tocopherols and plastoquinones. Tocotrienols result from a similar series of reactions but with geranylgeranyl diphosphate as substrate in the condensation step.

Fish contain an unusual tocopherol that has been termed marine-derived α-tocomonoenol (a related isomer has been found in palm oil). It is found together with α-tocopherol in a wide range of marine fish species and appears to be a more efficient scavenger of free radicals at low temperatures.



marine-derived α-tocomonoenol

α-Tocopheryl phosphate has recently been detected at low levels in liver and adipose tissue, and it is possible that it may be a ubiquitous constituent of animal and plant tissue.

α-Tocopherol is a minor but ubiquitous component of the lipid constituents of animal cell membranes with estimates ranging from one molecule of tocopherol to from 100 to 1000 molecules of phospholipid, depending on the membrane. The hydrophobic tail lies within the membrane, as might be expected, and the polar head group is orientated towards the surface but probably below the level of the phosphate moieties of the phospholipids. There may be some limited hydrogen bonding between the hydroxyl groups and phosphate depending on the degree of hydration of the membrane. On the other hand, there is a strong affinity of α-tocopherol for polyunsaturated fatty acids, where the chromanol unit may interact with the double bonds, suggesting that tocopherol is located deep within the membrane.

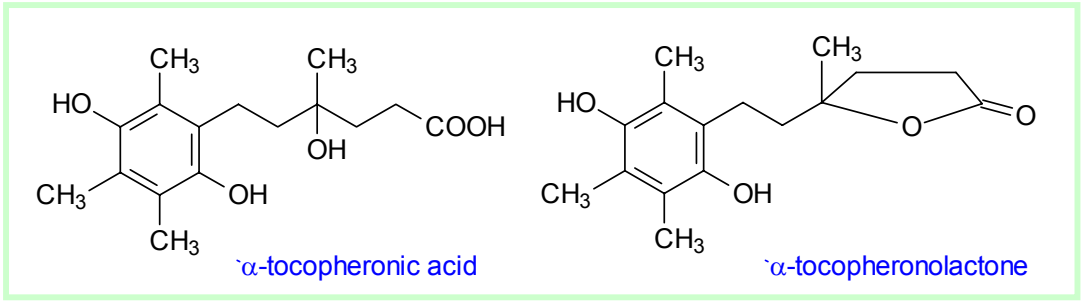
During the refining of vegetable oils, much of the natural tocopherols are lost or destroyed. Most commercial vitamin E is therefore prepared by chemical synthesis with trimethylhydroquinone and phytyl bromide as the precursors. The resulting product is a mixture of eight stereoisomers (from *R,R,R* to *S,S,S* methyl groups) of α-tocopherol, but it still has appreciable vitamin E activity. It is usually administered as the acetate derivative. Tocopherols are not usually regarded as effective antioxidants in the polyunsaturated seed oils of commerce, and at higher concentrations can even act as pro-oxidants, although the reasons for this are not understood. They are required in the developing seed (see below).

2. Tocopherols Metabolism in Animals

In animals, all tocopherols are absorbed to a similar extent in the intestines and are transported to the liver in chylomicrons mainly, but α-tocopherol is preferentially utilized and re-exported. This process is mediated by a specific tocopherol-binding protein in the liver that has a marked affinity for α-tocopherol, transferring it to the plasma **lipoproteins** (mainly the LDL and HDL in humans) for transport to other tissues (together with lesser amounts of γ-tocopherol). The "α-tocopherol salvage pathway" results in a 20- to 30-fold enrichment of α-tocopherol in plasma (average concentration 22-28 μM) relative to the other tocopherols. Transfer of tocopherols from the lipoproteins to peripheral tissues is promoted by the enzyme lipoprotein lipase. Concentrations of tocopherols can vary appreciably amongst tissues, with most in adipose tissue and adrenals, less in kidney, heart and liver, and least in the erythrocytes. Dietary tocotrienols are also absorbed in the intestines but less efficiently.

The process of conservation of one specific tocopherol appears to determine the relative vitamin E activities of the tocopherols and tocotrienols *in vivo*, rather than their individual potencies as antioxidants as measured in model systems *in vitro*. Only α-tocopherol (including synthetic material) or natural mixtures containing this can be sold under the label 'Vitamin E'. However, the tocotrienols are more potent antioxidants, *in vitro* at least, while γ-tocopherol (which is relatively abundant in skin) has some specific biological properties that are distinct from those of α-tocopherol.

Most of the tocochromanols other than α-tocopherol, together with any excess of the latter, are metabolized. The unwanted surplus may be excreted in the urine in the form of the so-called 'Simon metabolites', α-tocopheronic acid and α-tocopheronolactone, after oxidative cleavage of much of the phytyl tail. However, these are normally in the form of conjugates as sulfate or glucuronidate esters.



The first step in catabolism is ω-hydroxylation by cytochrome P450 (CYP4F2) at the 13' carbon to form a 13'-hydroxychromanol, followed by stepwise β-oxidation to cut off two or three carbon moieties from the phytyl chain in each cycle. Various carboxychromanol intermediates have been identified for all of the tocopherols together with sulfated forms of these in human cell cultures *in vitro*.

3. Tocopherols as Antioxidants

Although the syndrome associated with a lack of vitamin E in the diet of animals has been well known for decades, the mode of action and specific location of tocopherols in cell membranes are not clearly understood. Several theories have been proposed to explain their functions. Many argue that their primary task is to act as antioxidants to prevent free radical damage to unsaturated lipids or other membrane constituents and thence to tissues, while others now suggest that this may be secondary to more important biological functions (see below). That said there is no doubt that tocopherols are powerful antioxidants *in vitro* and surely have some such function *in vivo*. They are certainly extremely useful as antioxidants in non-biological systems, including foods, cosmetics, pharmaceutical preparations and so forth.

Because of their lipophilic character, tocopherols are located in the membranes or with storage lipids where that are immediately available to interact with lipid hydroperoxides. They react rapidly in a non-enzymic manner unlike many other cellular antioxidants, which are dependent on enzymes, to scavenging lipid peroxyl radicals, i.e. the chain-carrying species that propagate lipid peroxidation. In model systems *in vitro*, all the tocopherols (α > γ > β > δ) and tocotrienols are good antioxidants, with the tocotrienols being the most potent.

In general, the oxidation of lipids is known to proceed by a chain process mediated by a free radical, in which the lipid peroxyl radical serves as a chain carrier. In the initial step of chain propagation, a hydrogen atom is abstracted from the target lipid by the peroxyl radical as shown -

$$LOO^{\cdot} + LH \longrightarrow LOOH + L^{\cdot} \quad (1)$$

$$L^{\cdot} + O_2 \longrightarrow LOO^{\cdot} \quad (2)$$

- where LH is a lipid, $LOO^{\cdot}$ is the lipid peroxyl radical and LOOH is the lipid hydroperoxide

The main function of α-tocopherol is to scavenge the lipid peroxyl radical before it is able to react with the lipid substrate as –

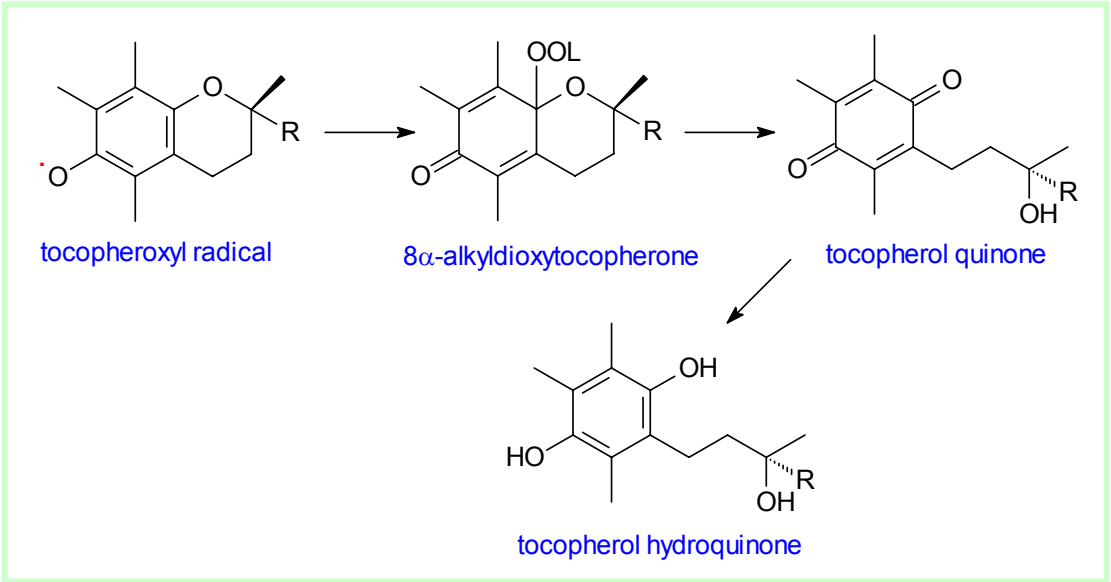
$$LOO^{\cdot} + TOH \longrightarrow LOOH + TOO^{\cdot} \quad (3)$$

- where TOH is tocopherol and $TOO^{\cdot}$ is the tocopheroxyl radical

It thus prevents propagation of the chain reaction. The potency of an antioxidant is determined by the relative rates of reactions (1) and (2). Studies of the relative rates of chain propagation to chain inhibition by α-tocopherol in model systems have demonstrated that α-tocopherol is able to scavenge peroxyl radicals much more rapidly than the peroxyl radical can react with a lipid substrate.

In biological systems, oxidant radicals can spring from a number of sources, including singlet oxygen, alkoxyl radicals, superoxide, peroxynitrite, nitrogen dioxide and ozone. α-Tocopherol is most efficient at providing protection against peroxyl radicals in a membrane environment.

When a tocopheroxyl radical is formed, it is stabilized by delocalisation of the unpaired electron about the fully substituted chromanol ring system rendering it relatively unreactive. This also explains the high first order rate constant for hydrogen transfer from α-tocopherol to peroxyl radicals. Reaction of the tocopheroxyl radical with a lipid peroxyl radical, as illustrated, yields 8α-substituted tocopherones, which are readily hydrolysed to 8α-hydroxy tocopherones that rearrange spontaneously to form α-tocopherol quinones. In an alternative pathway, the tocopheroxyl radical reacts with the lipid peroxyl radical to form epoxy-8α-hydroperoxytocopherones, which hydrolyse and rearrange to epoxyquinones. Tocopherol dimers and trimers may also be formed as minor products.



In plant and animal tissues, tocopherols can be regenerated from the tocopheroxyl radicals in a redox cycle mediated by a number of endogenous antioxidants, including vitamins A and C and coenzyme Q, and this must greatly extend their biological potency. Vitamin C (ascorbate) may be especially important in aqueous systems, although it may also act at the surface of membranes.

Suggestions that dietary supplements of vitamin E may reduce the rate of oxidation of lipids in low-density lipoproteins and thence the incidence or severity of atherosclerosis now appear to be unfounded, although benefits in some conditions have been claimed. Indeed, there are suggestions that excessive vitamin E supplementation may even be harmful. A recent study has suggested that relatively high doses of natural α-tocopherol over a long period are required to demonstrate a significant reduction in the levels of F_2 isoprostanes in the urine, which are considered to be the most reliable marker for oxidative stress *in vivo*. This subject is highly contentious and I prefer to leave it to the clinical experts.

In plants, tocopherols are most abundant in the membranes of the chloroplasts, where they were long believed to be the most important antioxidants, limiting the damage from photosynthesis-derived reactive oxygen species during conditions of oxidative stress, including high-intensity light stress. However, recent studies seem to suggest that they are just one of a number of different components that are involved in photo-protection. Certainly, any tocochromanol peroxy radicals formed must be converted back to the original compounds by the concerted action of other plant antioxidants. On the other hand, there is no doubt that tocopherols are essential for the control of non-enzymatic lipid peroxidation during seed dormancy and germination of seedlings. In their absence, elevated levels of malondialdehyde and phytoprostanes are formed, and there can be inappropriate activation of plant defense responses.

4. Other Biological Functions of Tocopherols

With the discovery that the antioxidant effects of various tocopherols and tocotrienols have little relation to their vitamin E activities has come the realization that they have many other functions in tissues, most of which are specific to α-tocopherol. Indeed, it has even been suggested that these are so important that tocopherol may be protected from functioning as an antioxidant in tissues *in vivo* through a network of cellular antioxidant defences. Only when other antioxidants are exhausted are the tocopherols utilized. However, there is no experimental proof of this hypothesis, although it is certainly true that most other vitamins are essential cofactors for specific enzymes or transcription factors.

α-Tocopherol is believed to be a gene regulator, causing up-regulation of mRNA or protein synthesis that could be the result of effects on gene transcription, mRNA stability, protein translation, protein stability and post-translational events. β-Tocopherol has no such properties. Effects have also been observed on genes connected with tocopherol catabolism, lipid uptake, collagen synthesis, cellular adhesion, inflammation and cell signalling. Vitamin E modulates the activity of several enzymes involved in signal transduction, perhaps through influencing protein-membrane interactions. For example, α-tocopherol inhibits the enzyme protein kinase C, which in turn reduces the release of reactive oxygen species in various ways and has effects on gene expression. It may also have secondary roles in stabilizing the structure of membranes, in regulating haem biosynthesis, in modulating the immune response, and as a participant in electron transport chains. Some non-antioxidant effects of γ-tocopherol in tissues in relation to reactive nitrogen oxide species have been observed, but the specificity of these is not yet certain.

Tocotrienols have been shown to have neuroprotective effects, to inhibit cholesterol synthesis and to reduce the growth of breast cancer cells *in vitro*. These properties are largely distinct from those of the tocopherols.

The biological function of α-tocopheryl phosphate is not known, but it has been suggested that it may be a storage or transport form or it could be involved in cellular signalling. Synthetic phosphate derivatives of γ-tocopherol and α -tocopheryl succinate are known to have potent anti-cancer properties.

In plants, there is evidence that tocopherols also play a part in intracellular signalling in that they regulate the amounts of jasmonic acid (see our web page on plant lipoxins) in leaves and so influence plant development and stress responses. Thus, by controlling the degree of lipid peroxidation in chloroplasts, they limit the accumulation of lipid hydroperoxides required for synthesis of jasmonic acid, which in turn regulates the expression of genes that affect a number of stress conditions. In addition, tocopherols are required for the development of the cell walls in phloem transfer cells under cold conditions.

5. Analysis

Tocopherols can be analysed by gas chromatography, both with flame-ionization and mass spectrometric detection, but the methods that are usually recommended involve high-performance liquid chromatography with fluorescence detection. Related methods are used for ubiquinones and the isoprenoid alcohols.

Recommended Reading

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