Food Sci. Technol. Int. Tokyo, **3** (4), 301-309, 1997

Review

Vitamin E: Mechanism of Its Antioxidant Activity

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Received August 8, 1997; Accepted August 26, 1997

The antioxidant activity of vitamin E (α-tocopherol) during the peroxidation of unsaturated lipids has been reviewed based on its reaction products. Free-radical scavenging reactions of α-tocopherol take place *via* the α-tocopheroxyl radical as an intermediate. If a suitable free radical is present, a non-radical product can be formed from the coupling of the free radical with the α-tocopheroxyl radical. The reaction products of α-tocopherol with lipid-peroxyl radicals are 8a-(lipid-dioxy)-α-tocopherones which are hydrolyzed to α-tocopherylquinone. If the supply of oxygen is insufficient, α-tocopherol can trap the carbon-centered radicals of lipids to form 6-*O*-(lipid-alkyl)-α-tocopherols. On the other hand, the dimer and trimer of α-tocopherol is formed by the bimolecular self-reaction of the α-tocopheroxyl radical in a reaction mixture containing a large amount of α-tocopherol. The other product-forming pathway yields isomeric epoxy-α-tocopherylquinones and their precursors, epoxyhydroperoxy-α-tocopherones, but the mechanism of this pathway remains unknown. The reaction products of other vitamin E compounds (γ- and δ-tocopherols) during lipid peroxidation are almost the same as those formed from the α-tocopherol. The tocopheroxyl radicals of γ- and δ-tocopherols prefer to react with each other to form dimeric products that are still effective as antioxidants.

Keywords: vitamin E, tocopherol, antioxidant, lipid peroxidation, autoxidation

Lipid peroxidation is a degradative, free radical mediated-process responsible for the development of objectionable odors and flavors in oils, fats, and foods (Frankel, 1980, 1991). Moreover, oxidation of polyunsaturated fatty acids of the biomembranes causes functional abnormalities and pathological changes (O'Brien, 1987). Vitamin E compounds (tocopherols and tocotrienols) are well recognized for their effective inhibition of lipid peroxidation in foods and living cells (Burton & Traber, 1990). Vitamin E is synthesized only by plants: therefore it is a very important dietary nutrient for humans and animals (Fryer, 1992).

Tocopherol isomers are chain-breaking antioxidants. The antioxidative activity of the tocopherols is related to scavenging the free radicals of unsaturated lipids (Burton & Ingold, 1981, 1986; Niki *et al.*, 1984; van Acker *et al.*, 1993; Kamal-Eldin & Appelqvist, 1996). α-Tocopherol, the most biologically active and abundant form of vitamin E *in vivo*, efficiently transfers a hydrogen atom to a lipid free radical, such as peroxyl, alkoxyl, and carbon-centered radicals, giving the corresponding non-radical product of the lipid and an α-tocopheroxyl radical. The α-tocopheroxyl radicals, once formed, react with a second free radical or each other to form a non-radical product. Each molecule of α-tocopherol consumes thus two lipid free radicals and terminates the free-radical chain reaction. To elucidate the free-radical scavenging mechanism of tocopherols, the reaction products of tocopherols with lipid free radicals have been investigated (Winterle *et al.*, 1984; Ham & Liebler, 1995, 1997; Liebler *et al.*, 1990, 1991; Liebler & Burr, 1992; Matsuo *et al.*, 1989; Yamauchi *et al.*, 1989, 1990a, 1990b, 1990c, 1993, 1994, 1995a).

This review describes the mechanisms of inhibition of lipid peroxidation by tocopherols, especially the most biologically active α-tocopherol, based on their reaction products.

α-Tocopherol as the Free Radical Scavenger

Lipid peroxidation (or autoxidation) is a chain reaction that proceeds in three stages: initiation, propagation, and termination (Frankel, 1980; Porter, 1986). In the initiation step, a carbon-centered lipid radical (an alkyl radical) is produced by the abstraction from a polyunsaturated fatty acid moiety. The initiation reaction can be catalyzed by heat, light, and transition metals (Chan, 1987). In the propagation step, the alkyl radical reacts with molecular oxygen at a very high rate, giving a peroxyl radical. The peroxyl radical, a chain-carrying radical, is able to attack another polyunsaturated lipid molecule. Although the initial peroxyl radical is converted to a hydroperoxide, this process produces a new alkyl radical, which is rapidly converted into another peroxyl radical. The chain reaction does not stop until the chain-carrying peroxyl radical meets and combines with another radical to form inactive products (termination step).

Figure 1 shows the reaction scheme of α-tocopherol during the autoxidation of unsaturated lipids. α-Tocopherol (**1**) is a chain-breaking antioxidant to inhibit the propagation step (Burton & Ingold, 1986). α-Tocopherol donates its phenolic hydrogen atom to a peroxyl radical and converts it to a hydroperoxide. The tocopheroxyl radical (**2**) that is formed is sufficiently stable to be unable to continue the chain and, instead, is removed from the cycle by reaction with another peroxyl radical to form an inactive, non-radical product (Erben-Russ *et al.*, 1987). Because each tocopherol molecule

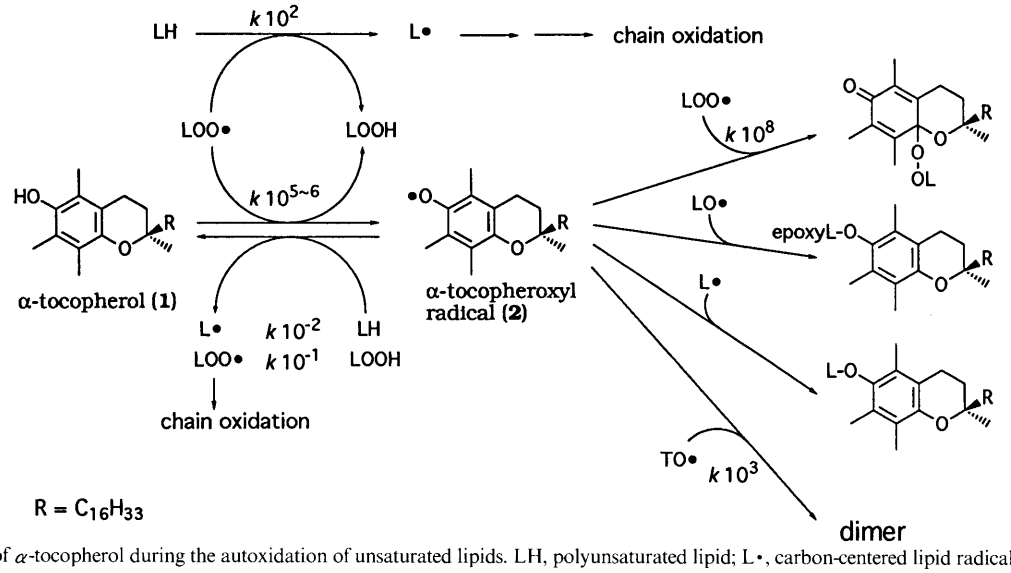

Fig. 1. Reaction of α-tocopherol during the autoxidation of unsaturated lipids. LH, polyunsaturated lipid; L•, carbon-centered lipid radical; LO•, lipid-alkoxyl radical; LOOH, lipid hydroperoxide; LOO• lipid-peroxyl radical; TO•, α-tocopheroxyl radical; k, rate constant in $M^{-1} s^{-1}$.

can trap two peroxyl radicals, the stoichiometric factor (n) for the four tocopherol isomers is theoretically considered to be equal to 2. The rate at which tocopherol isomers react with peroxyl radicals is a direct measure of their antioxidant efficiency (Burton & Ingold, 1981; Niki *et al.*, 1984; Mukai *et al.*, 1986). It has been determined that α-tocopherol is the most efficient chain-breaking antioxidant among the four isomers. α-Tocopherol also can react with alkoxyl radicals (Gardner *et al.*, 1972; Kaneko & Matsuo, 1985) or undergo self-coupling to form dimers and trimers (Csallany *et al.*, 1970; Yamauchi *et al.*, 1988). When oxygen is present in trace amounts, α-tocopherol can react directly with alkyl radicals (Evans *et al.*, 1992; Yamauchi *et al.*, 1993). α-Tocopherol at high concentrations induces the formation of lipid hydroperoxides (Peers *et al.*, 1981; Koskas *et al.*, 1984; Terao & Matsushita, 1986). The pro-oxidant effect of α-tocopherol was related to the reaction of α-tocopheroxyl radicals with lipids (Terao & Matsushita, 1986; Bowry & Stocker, 1993; Mukai *et al.*, 1993a, 1993b) The reaction products of α-tocopherol with lipid free radicals are described in the following sections.

Reaction Products of α-Tocopherol during the Autoxidation of Methyl Linoleate

α-Tocopherol inhibits the autoxidation of methyl linoleate in the bulk phase. α-Tocopherol at high concentrations, on the other hand, acts as a pro-oxidant during the autoxidation (Peers *et al.*, 1981; Terao & Matsushita, 1986). Figure 2 shows the possible pathways for the reactions of α-tocopherol with lipid free radicals. It has been reported that the products of α-tocopherol (**1**) formed under mild conditions of autoxidation are α-tocopherylquinone (**4**), α-tocopherol spirodiene dimer (**6**), and α-tocopherol trimer (**7**) (Csallany *et al.*, 1970; Yamauchi *et al.*, 1988). However, the other reaction products might also be formed during the autoxidation of unsaturated lipids.

Methyl linoleate containing 0.1 or 0.5 mol% α-tocopherol was autoxidized at 37°C or 60°C under air (Yamauchi *et al.*, 1995b). In this condition, the generated methyl linoleate-peroxyl radicals can be trapped by α-tocopherol. If the oxygen pressure in the reaction system is lowered, the produced carbon-centered radicals of methyl linoleate may also react with α-tocopherol. The reaction was run on two sample sizes, 0.5 g and 3 g, in a glass vial; the 0.5-g sample represents air-sufficient conditions and the 3-g sample air-insufficient conditions, respectively (Yamauchi *et al.*, 1993). When methyl linoleate containing 0.1 mol% α-tocopherol was autoxidized at 37°C, α-tocopherol could suppress the formation of methyl linoleate hydroperoxides (MeLOOH). The reaction products of α-tocopherol were a mixture of methyl 9-(8a-dioxy-α-tocopherone)-10(*E*),12(*Z*)-octadecadienoates and methyl 13-(8a-dioxy-α-tocopherone)-9(*Z*), 11(*E*)-octadecadienoates (**3**) and 4a,5-epoxy-8a-hydroperoxy-α-tocopherones (**5**). Thus, the reaction of peroxyl radicals with a low concentration of α-tocopherol under air-sufficient conditions gives the stable products (**3**) and terminates the autoxidation. Methyl linoleate containing 0.5 mol% α-tocopherol showed a linear accumulation of MeLOOH with depletion of the α-tocopherol. Therefore, α-tocopherol at a high concentration acts as a pro-oxidant (Cillard *et al.*, 1980; Terao & Matsushita, 1986). The main products were **3**, **5**, α-tocopherol dimer (**6**) and α-tocopherol trimer (**7**) in the 0.5-g sample. The formation of compounds **6** and **7** indicates that a bimolecular self-reaction of the α-tocopheroxyl radical occurs at high concentrations of α-tocopherol. In addition to these products, a mixture of methyl 9-(α-tocopheroxy)-10(*E*), 12(*Z*)-octadecadienoates and methyl 13-(α-tocopheroxy)-9(*Z*),11(*E*)-octadecadienoates (**8**) were formed in the 3-g sample. This indicates that the reaction between the carbon-centered radicals of methyl linoleate and α-tocopheroxyl radical occurs under air-insufficient conditions (Yamauchi *et al.*, 1993). The other product-forming pathway yields compound **5**, but the formation mechanism of this compound is unclear (Liebler *et al.*, 1990; Liebler & Burr, 1995).

The autoxidation at 60°C proceeded rapidly, and only trace amounts of 8a-(lipid-dioxy)-α-tocopherones (**3**) were de-

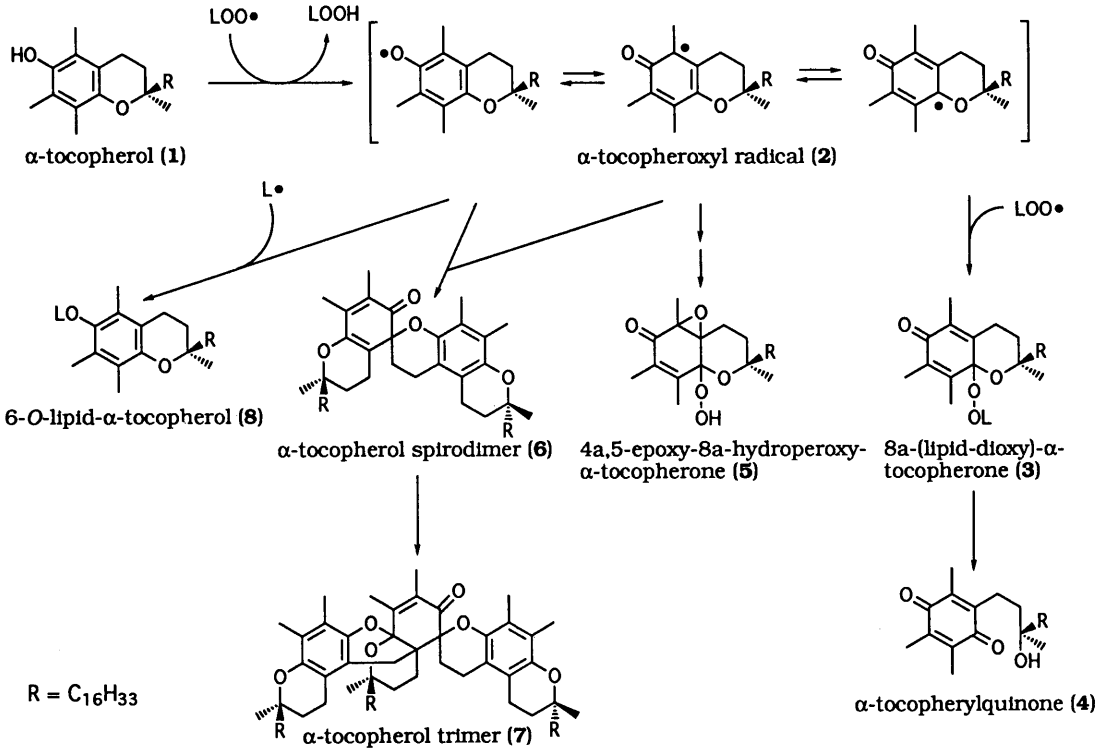

Fig. 2. Reaction products of α-tocopherol during the autoxidation of unsaturated lipids. L•, carbon-centered lipid radical; LOOH, lipid hydroperoxide; LOO•, lipid-peroxyl radical.

tected in the reaction mixture. The 6-*O*-alkyl-α-tocopherols (**8**) were the major products at 60°C under air-insufficient conditions, and the *E,E*-isomers were produced in addition to the *E,Z*-isomers (Yamauchi *et al.*, 1995b). This suggests that the carbon-centered radicals trapped by α-tocopheroxyl radicals at high temperature are produced by β-scission of methyl linoleate-peroxyl radicals (Chan *et al.*, 1978; Porter *et al.*, 1981).

Reaction of α-Tocopherol with Methyl Linoleate Hydroperoxides

Transition metal ions catalyze homolysis of lipid hydroperoxides that are cleaved to peroxyl radicals by metal ions in the oxidized state, such as Fe(III), whereas reduced metal ions, such as Fe(II), lead to alkoxyl radicals (Halliwell & Gutteridge, 1984; Sevanian & Hochstein, 1985). The free radicals produced in these processes are believed to stimulate the chain reaction of lipid peroxidation by abstracting further hydrogen atoms. If α-tocopherol is present, α-tocopherol can trap these radicals and can terminate lipid peroxidation (Gardner *et al.*, 1972; Kaneko & Matsuo, 1985; Yamauchi *et al.*, 1995a).

α-Tocopherol was reacted with methyl 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoate (13-MeLOOH) in the presence of an iron-chelate, Fe(III)-acetylacetonate, at 37°C in benzene (Yamauchi *et al.*, 1995a). The main products of α-tocopherol under air were methyl 13(*S*)-(8a-dioxy-α-tocopherone)-9(*Z*),11(*E*)-octadecadienoate (**3**), 4a,5-epoxy-8a-hydroperoxy-α-tocopherone (**5**), α-tocopherol dimer (**6**), and a mixture of methyl 9-(8a-dioxy-α-tocopherone)-12(*S*),13(*S*)-epoxy-10(*E*)-octadecenoate and methyl 11-(8a-dioxy-α-tocopherone)-12(*S*),13(*S*)-epoxy-9(*Z*)-octadecenoate (**10**). On the other hand, the reaction products under nitrogen atmosphere were a mixture of methyl 9- and 13-(α-tocopheroxy)-octadecadienoates (**8**) and a mixture of methyl 9-(α-tocopheroxy)-12(*S*),13(*S*)-epoxy-10(*E*)-octadecenoate and methyl 11-(α-tocopheroxy)-12(*S*),13(*S*)-epoxy-9(*E*)-octadecenoate (**9**), in addition to the 8a-(lipid-dioxy)-α-tocopherones (**3**).

Figure 3 shows the possible mechanisms of Fe(III)-catalyzed reaction of α-tocopherol with 13-MeLOOH. The first reaction is iron-dependent free radical formation by 13-MeLOOH being oxidized to a peroxyl radical or reduced to an alkoxyl radical (Halliwell & Gutteridge, 1984; Sevanian & Hochstein, 1985). It is also likely that the Fe(III) reacts very readily with α-tocopherol to give Fe(II) and an α-tocopheroxyl radical (Gardner *et al.*, 1972). This assertion is supported by the observation of significant amounts of α-tocopherol dimer (**6**), consistent with the presence of a high concentration of α-tocopheroxyl radical (Burton *et al.*, 1985). Furthermore, α-tocopherol can trap the peroxyl radical, giving a hydroperoxide and an α-tocopheroxyl radical. The alkoxyl radical produced by the Fe(II)-catalyzed decomposition of 13-MeLOOH then adds to the adjacent double bond to form the 12,13-epoxycarbon-centered radical (Dix & Marnett, 1981; Gardner, 1989; Wilcox & Marnett, 1993). In the presence of oxygen, the epoxycarbon-centered radical reacts with molecular oxygen to form peroxyl radicals and then reacts with the 8a-carbon radical of α-tocopherol (T•) to form 8a-(epoxylipid-dioxy)-α-tocopherones (**10**). The 13-peroxyl radical from 13-MeLOOH also reacts with T• to form

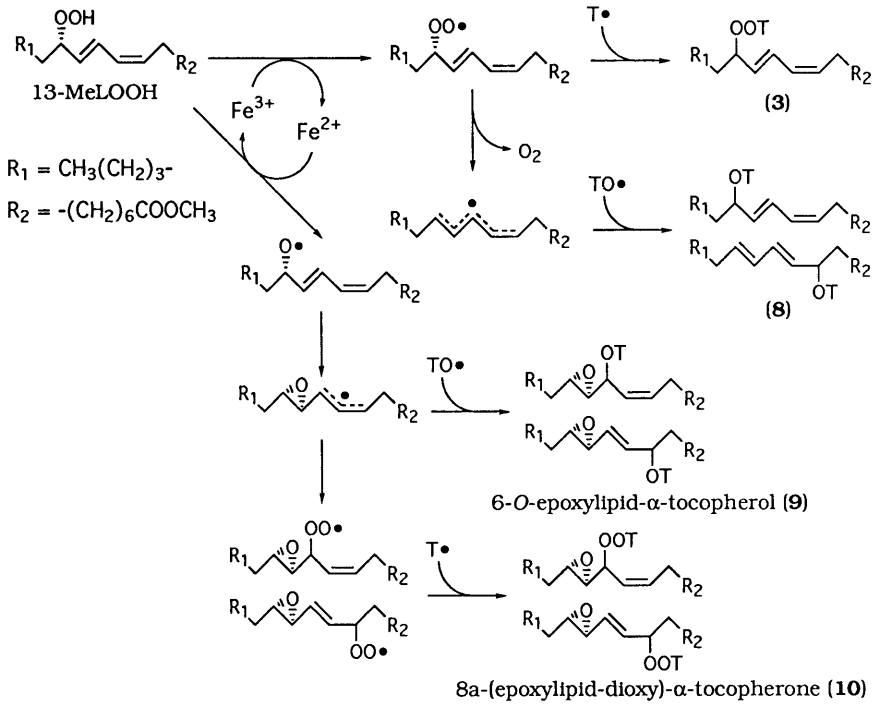

Fig. 3. Iron-catalyzed decomposition of methyl 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoate and the reaction with α-tocopherol. 13-MeLOOH, 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoate; TO•, the phenoxyl radical of α-tocopherol; T•, the 8a-carbon radical of α-tocopherol.

3. All the epoxycarbon-centered radicals produced are expected to react very rapidly with oxygen. When the oxygen pressure is lowered, the epoxycarbon-centered radical can directly react with the phenoxyl radical of α-tocopherol (TO•) and form 6-*O*-(epoxylipid)-α-tocopherones (**9**). In addition, radical elimination of the peroxyl radical may result in the formation of a carbon-centered radical and oxygen (Porter & Wujek, 1984), which then react with TO• to form **8** (Yamauchi *et al.*, 1995a). The peroxyl radicals from lipid hydroperoxide prefer to react with the 8a-carbon radical of α-tocopherol and the carbon-centered radicals react with the phenoxyl radical of α-tocopherol.

Lipid hydroperoxides can be dissociated into free radicals by heat during food processing (Gardner, 1987). α-Tocopherol can suppress further reactions of lipid hydroperoxides by donating a hydrogen atom to free radicals. It has been reported that α-tocopherol can suppress the thermal decomposition of methyl linoleate hydroperoxides and inhibit the formation of volatile and non-volatile decomposition products (Frankel & Gardner, 1989; Hopia *et al.*, 1996). The reaction products of α-tocopherol during the thermal decomposition of 13-MeLOOH were almost the same as those of α-tocopherol during the iron-catalyzed reactions between α-tocopherol and MeLOOH reported by Gardner *et al.* (1972), Kaneko and Matsuo (1985), and Yamauchi *et al.* (1995a). The main products were 6-*O*-epoxyalkyl-α-tocopherols (**5**) and 6-*O*-alkyl-α-tocopherols (**6**), and small amounts of dimer (**7**) and trimer (**8**) of α-tocopherol were also detected (Yamauchi *et al.*, unpublished data). Besides these products, methyl 13-hydroxy-9(*Z*),11(*E*)-octadecadienoate (**a**), methyl 13-oxo-9(*Z*),11(*E*)-octadecadienoate (**b**), and epoxy dimers of methyl linoleate with an ether bond (**c**) were produced as the reaction products of 13-MeLOOH (Hopia *et al.*, 1996; Yamauchi *et al.*, unpublished data).

The thermal decomposition of 13-MeLOOH and the reaction with α-tocopherol in bulk phase are illustrated in Fig. 4. The first reaction would be the formation of alkoxyl and peroxyl radicals of MeLOOH (Hamberg, 1975; Chan, 1987; Gardner, 1987). The alkoxyl radicals generally are known for their ability to abstract hydrogens. α-Tocopherol in the reaction mixture trapped the alkoxyl radical by hydrogen-atom transfer, giving an α-tocopheroxyl radical and hydroxy methyl linoleate (**a**). Because of the presence of unsaturation in the hydroperoxides, other reactions are evidently more competitive than hydrogen abstraction (Gardner, 1987). Alkoxyl radicals of methyl linoleate tend to rearrange into epoxyallylic radicals, even in the presence of compounds with a readily abstractable hydrogen, like α-tocopherol (Gardner *et al.*, 1972; Kaneko & Matsuo, 1985; Yamauchi *et al.*, 1995a). Without oxygen, the resulting epoxyallylic radical is susceptible to a variety of radical combination reactions; the epoxyallylic radicals react with α-tocopheroxyl radical to form 6-*O*-epoxyalkyl-α-tocopherols (**9**). If the production of the tocopheroxyl radical is insufficient, the epoxyallylic radicals could react with the alkoxyl radical of 13-MeLOOH to form dimeric products; epoxy dimers of methyl linoleate with an ether bond (**c**) appeared in the reaction mixture with low concentrations of α-tocopherol instead of **9**. The results indicate that alkoxyl radical rearrangement to epoxide is an important pathway in the homolysis of 13-MeLOOH by heat. The conversion of lipid hydroperoxides to the corresponding lipid ketones usually accounts for a relatively large portion of end products and they can be derived from hydroperoxides *via* alkoxyl

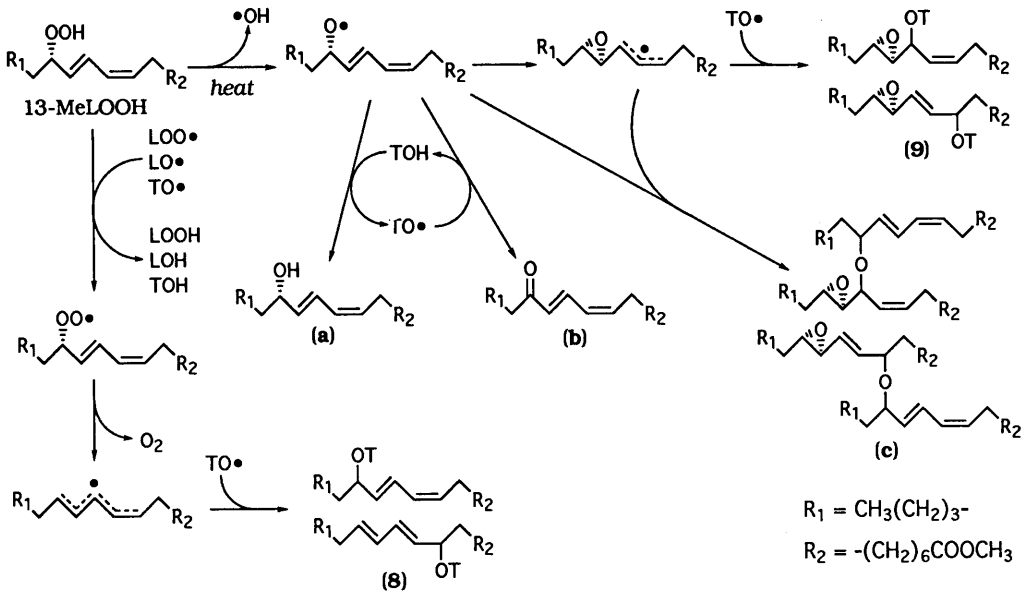

Fig. 4. Thermal decomposition of methyl 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoate and the reaction with α-tocopherol. 13-MeLOOH, 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoate; LOH, hydroxy lipid; LO•, lipid-alkoxyl radical; LOOH, lipid hydroperoxide; LOO•, lipid-peroxyl radical; TOH, α-tocopherol; TO•, the phenoxyl radical of α-tocopherol.

radicals (Gardner *et al.*, 1974; Hamberg, 1975). From 13-MeLOOH, the corresponding oxodiene, methyl 13-oxo-9,11-octadecadienoate (**b**), was obtained, and high concentrations of α-tocopherol accelerated the formations of **a** and **b**. Therefore, the catalytic reactions of α-tocopherol with an alkoxyl radical might produce hydroxy and ketodiene compounds (Hopia *et al.*, 1996).

The formation of a peroxyl radical is the other important pathway of the thermal decomposition of lipid hydroperoxides. Oxidation by higher oxidation states of transition metal ions is a pathway to form peroxyl radicals (Halliwell & Gutteridge, 1984). In the absence of a metal catalyst, the most likely process is that peroxyl radicals might be produced from hydroperoxides by hydrogen abstraction; the free radicals, such as peroxyl, alkoxyl, and α-tocopheroxyl radicals, could abstract the hydrogen atom from hydroperoxides (Gardner, 1987). The peroxyl radicals thus produced are unstable at high temperature, and radical elimination of the peroxyl radical results in the release of O_2 as a leaving group to form a carbon-centered radical (Porter & Wujek, 1984). The formation of the carbon-centered radical is substantiated by the finding that the isomerization of MeLOOH is accompanied by the exchange of the oxygen atoms of the hydroperoxy group with atmospheric oxygen (Chan *et al.*, 1978). The alkyl radical produced then reacts with an α-tocopheroxyl radical to form 6-*O*-alkyl-α-tocopherols (**8**) (Yamauchi *et al.*, 1995b). These results suggest that the elimination reaction of peroxyl radicals might occur during the thermal decomposition of lipid hydroperoxides.

Reaction of α-Tocopherol with Phospholipid-Peroxyl Radicals in Liposomes and *in vivo* Systems

Phospholipids are important structural components in biological membranes, and they are targets of lipid peroxidation. Phospholipid liposomal systems have been employed to model the peroxyl-radical trapping reactions of α-tocopherol in biological membranes (Liebler *et al.*, 1991; Liebler & Burr, 1992; Ham & Liebler, 1995; Yamauchi *et al.*, 1994, 1996). The effectiveness of α-tocopherol depends not only on its ability to trap peroxyl radicals but also on the ability of the resulting α-tocopheroxyl radical to trap additional peroxyl radicals in biological membranes. Therefore, products of the latter reaction may be useful markers for the antioxidative activity of α-tocopherol in biological systems.

α-Tocopherol was reacted with peroxyl radicals of phosphatidylcholine at 37°C in liposomes (Yamauchi *et al.*, 1994). The phospholipid-peroxyl radicals were generated by the reaction of 1,2-dilinoleoyl-3-*sn*-phosphatidylcholine (DLPC) with a free radical initiator, 2,2′-azo*bis*(2,4-dimethylvaleronitrile) (AMVN), under air. The peroxyl radicals derived from DLPC reacted with α-tocopherol in lipid bilayers to yield products corresponding to those observed in the bulk phase of methyl linoleate (Yamauchi *et al.*, 1990a). The structure was a mixture of DLPC-derived 8a-(lipid-dioxy)-α-tocopherones that contained a DLPC-peroxy substituent in the 8a-position of α-tocopherol (Fig. 5). The adducts of α-tocopheroxyl radicals with phospholipid-peroxyl radicals were also obtained as the reaction products of α-tocopherol with 1-palmitoyl-2-linoleoyl-3-*sn*-phosphatidylcholine and 1-palmitoyl-2-arachidonoyl-3-*sn*-phosphatidylcholine (Yamauchi *et al.*, unpublished data).

The antioxidative reaction of α-tocopherol was studied in DLPC liposomal systems (Yamauchi *et al.*, 1996). Peroxidation of the DLPC liposomes containing 0.1 mol% α-tocopherol was started in the lipid phase by lipid-soluble AMVN or in the aqueous phase by water-soluble 2,2′-azo*bis*(2-amidinopropane) dihydrochloride (AAPH). In both liposomal oxidation systems, α-tocopherol suppressed the formation of DLPC hydroperoxides until all the α-tocopherol had been depleted. The reaction products of α-tocopherol were 8a-

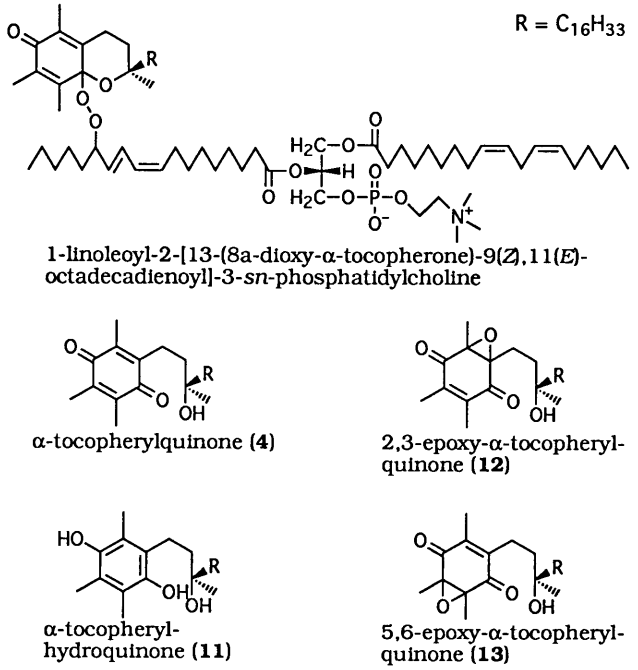

Fig. 5. Reaction products of α-tocopherol in liposomal and *in vivo* systems. 1-Linoleoyl-2-[13-(8a-dioxy-α-tocopherone)-9(Z),11(E)-octadecadienoyl]-3-*sn*-phosphatidylcholine is one of the addition product of α-tocopheroxyl radical with 1,2-dilinoleoyl-3-*sn*-phosphatidylcholine-peroxyl radicals.

(DLPC-dioxy)-α-tocopherones, α-tocopherylquinone (**4**), 2,3-epoxy-α-tocopherylquinone (**12**), and 5,6-epoxy-α-tocopherylquinone (**13**). The 8a-(DLPC-dioxy)-α-tocopherones were decomposed in the liposomes primarily by being hydrolyzed to produce α-tocopherylquinone (**4**). The formation of 8a-(DLPC-dioxy)-α-tocopherones and α-tocopherylquinone indicates that each α-tocopherol can trap two peroxyl radicals during the peroxidation of unsaturated phospholipid in liposomes (Burton & Ingold, 1981; Niki *et al.*, 1984). On the other hand, significant amounts of epoxy-α-tocopherylquinones (**11** and **12**) were detected in the liposomal peroxidation systems (Liebler *et al.*, 1991; Yamauchi *et al.*, 1996). Although the mechanism by which these epoxides were formed remains unknown, the oxidation of α-tocopherol to the epoxides results in the net consumption of two peroxyl radicals (Liebler & Burr, 1995).

Ham and Liebler (1995, 1997) have identified the oxidation products of α-tocopherol in rat liver mitochondria during the AAPH-initiated peroxidation or in a perfused rat liver model using *tert*-butylhydroperoxide as the initiator of lipid peroxidation. Their results indicate that antioxidative reactions of α-tocopherol in mitochondria and in the perfused liver yield the same products formed from by peroxyl radical trapping reactions in liposomes and chemical models. These include α-tocopherylquinone (**4**) and its reduction product α-tocopherylhydroquinone (**11**), as well as the epoxyquinones, 2,3-epoxy-α-tocopherylquinone (**12**) and 5,6-epoxy-α-tocopherylquinone (**13**). The studies with the perfused liver provided the first description of the antioxidant reactions of α-tocopherol in an intact organ system (Ham & Liebler, 1997). No evidence of the formation of 8a-(lipid-dioxy)-α-tocopherones was found in the intact organ system. Further studies on the identification of oxidation products of α-tocopherol on membranes are needed to elucidate the antioxidative activity of α-tocopherol in biological systems.

Reaction Products of γ- and δ-Tocopherols during the Autoxidation of Unsaturated Lipids

γ- and δ-Tocopherols are the main constituents of vitamin E in vegetable oils and act as useful antioxidants (Lea & Ward, 1959; Slover, 1971; Yuki & Ishikawa, 1976). Figure 6 shows the possible oxidation pathways for γ-tocopherol (**14**) in lipids or lipophilic solvents (Nilsson *et al.*, 1968; Yamauchi *et al.*, 1990b). γ-Tocopherol transfers a hydrogen atom to a peroxyl radical. The γ-tocopheroxyl radical produced is then resonance-stabilized and can then react irreversibly with a second peroxyl radical to form the covalent adducts, 8a-(alkyl-dioxy)-γ-tocopherones (**15**). The amounts of the adduct formed were relatively small compared with the adduct formed by the reaction of α-tocopherol (Yamauchi *et al.*, 1990b). The tocopheroxyl radicals are reacted by another route; bimolecular self-reaction. γ-Tocopheroxyl radicals can easily react with each other to form 5-(γ-tocopheroxy-5-yl)-γ-tocopherol (**16**) and 5-(γ-tocopheroxy)-γ-tocopherol (**17**) which are still able to act as antioxidants by virtue of their free hydroxy protons (Komoda & Harada, 1969; Fujitani & Ando, 1977). Therefore, these dimers further react with other peroxyl radicals to form some products, such as 8a-(lipid-dioxy)-5-(γ-tocopheroxy)-γ-tocopherone (**18**) (Yamauchi *et al.*, 1990b). In addition to these products, γ-tocored (**19**) was also produced during the lipid peroxidation (Komoda *et al.*, 1967; Yamauchi *et al.*, 1990b). γ-Tocored might be formed by the reaction of 5-(γ-tocopheroxy)-γ-tocopherol (**17**) with peroxyl radicals (Komoda & Harada, 1969).

The reaction products of δ-tocopherol (**20**) with peroxyl radicals were found to be 8a-(alkyl-dioxy)-δ-tocopherones (**21**), 5-(δ-tocopheroxy)-δ-tocopherol (**22**), 8a-(alkyl-dioxy)-5-(δ-tocopheroxy)-δ-tocopherones (**23**), 5-[5-(δ-tocopheroxy)-δ-tocopheroxy]-δ-tocopherol (**24**), 8a-(alkyl-dioxy)-5-[5-(δ-tocopheroxy)-δ-tocopheroxy]-δ-tocopherones (**25**), and δ-tocored (**26**) (Yamauchi *et al.*, 1990c). 5-(δ-Tocopheroxy-5-yl)-δ-tocopherol (**27**) has been reported to be formed in addition to 5-(δ-tocopheroxy)-δ-tocopherol (**22**) during the autoxidation of methyl linoleate and triglycerides (Ishikawa *et al.*, 1978; Fujitani & Ando, 1979). Figure 7 shows the possible pathways for the reaction of δ-tocopherol with peroxyl radicals (Yamauchi *et al.*, 1990c). The bimolecular self-reaction of the δ-tocopheroxyl radical is superior to that of the α-tocopheroxyl radical. The rate constants for the bimolecular couplings of α-, β-, γ-, and δ-tocopherols were obtained to be 3×10^3, 4×10^4, 4.5×10^4, and 1.5×10^5 M^{-1} s^{-1}, respectively (Barton *et al.*, 1985). Thus, not only δ-tocopherol but also its reducing dimer and trimer can scavenge peroxyl radicals to stop the autoxidation of edible oils.

The apparent superiority of γ- and δ-tocopherols compared to α-tocopherol in many *in vitro* systems is related to the fact that the former compounds are dimerizable to compounds that can still be effective as antioxidants. Tocopherols are present as a mixture in plant oils (Slover, 1971). The reaction products of the two kinds of co-existing to-

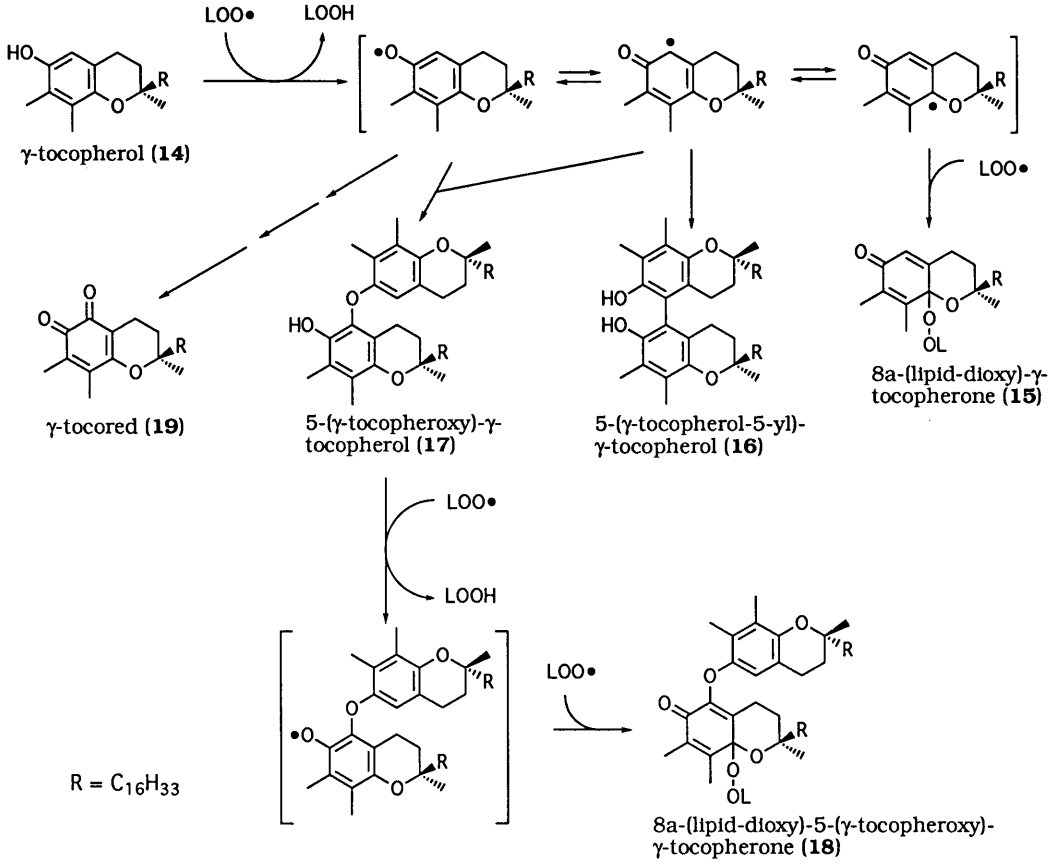

Fig. 6. Reaction products of γ-tocopherol. LOOH, lipid hydroperoxide; LOO•, lipid-peroxyl radical.

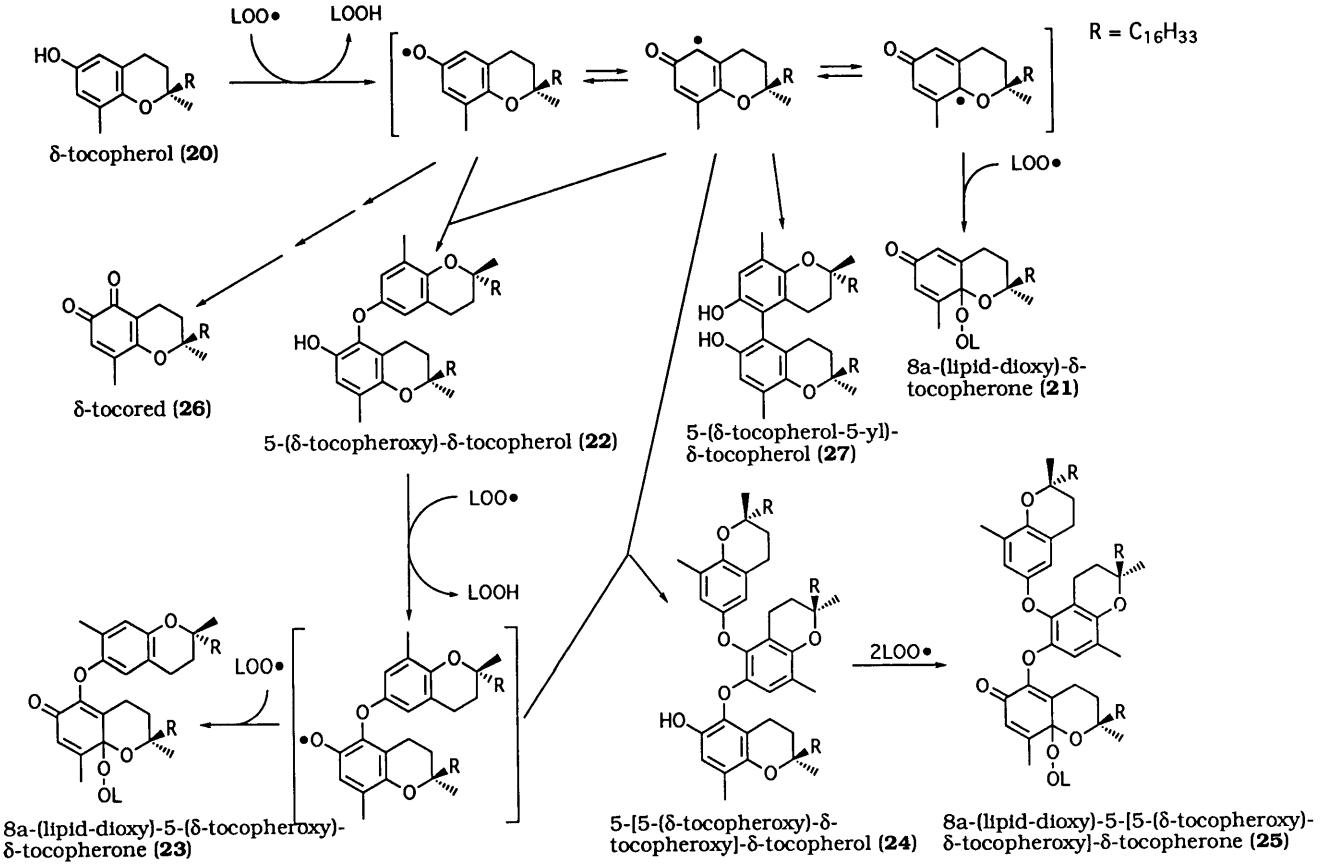

Fig. 7. Reaction products of δ-tocopherol. LOOH, lipid hydroperoxide; LOO•, lipid-peroxyl radical.

copherols, either α- plus γ-tocopherols or α- plus δ-tocopherol, in methyl linoleate indicated that the oxidation of α-tocopherol proceeded and was then followed by the reaction of γ-tocopherol and then δ-tocopherol after the consumption of α-tocopherol because of the apparent absence of mixed dimers of tocopherols (Ha & Igarashi, 1990). The products from the antioxidative reactions of γ- and δ-tocopherols have not been fully elucidated and need further studies.

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