Oxidation of Lipids. XVII. Crossover Effect of Tocopherols in the Spontaneous Oxidation of Methyl Linoleate†

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Tocopherols suppressed the spontaneous, thermal oxidation of methyl linoleate and produced a clear induction period. During the induction period, small amounts of *cis,trans*-hydroperoxides were formed but the formation of *trans,trans*-hydroperoxides was almost completely inhibited. Tocopherols were consumed and when they were depleted, the induction period was over and a fast oxidation proceeded to give more *trans,trans*-hydroperoxides than *cis,trans*-hydroperoxides. However, tocopherols, especially α-tocopherol, acted as prooxidants when their concentrations were extraordinarily high and the rate of initial radical formation was low. The reducing agent such as ascorbic acid diminished the prooxidant action of α-tocopherol. This prooxidant effect of tocopherols was ascribed to the spontaneous formation of tocopheroxyl radical followed by the hydrogen atom abstraction by the tocopheroxyl radical from the hydroperoxide to give a peroxyl radical.

Lipids are readily oxidized by molecular oxygen. There is an increasing number of evidence that shows the deleterious effects of the oxidation of lipids and its products in foods and in biological systems, and, accordingly, the inhibition of the oxidation of lipids has received much attention recently.[1-11] The aerobic organisms are protected against such oxygen toxicity by an array of defense systems: the preventive antioxidants such as glutathione peroxidase and catalase suppress or reduce the formation of active free radicals by decomposing without generation of free radicals lipid hydroperoxides and/or hydrogen peroxide which are precursors to free radicals, while the chain-breaking antioxidants such as vitamin E and vitamin C scavenge the radicals and interrupt the chain propagation. On the other hand, various kinds of natural and synthetic antioxidants are added to the edible oils and fats to suppress their oxidations. Tocopherols (vitamin E) are known as natural, potent chain-breaking antioxidants. In fact, it has been shown that tocopherols, especially α-tocopherol, scavenge the peroxyl radicals faster than any synthetic, phenolic antioxidants such as 2,6-di-*t*-butyl-4-methylphenol (BHT).[7-11] On the other hand, however, it has been also observed that α-tocopherol can act as a prooxidant under certain conditions.[12-23] For example, Olcott and Einset[13] found that the addition of α-tocopherol into the stabilized oil induced the oxidation during the induction period. Cillard and Cillard[15-17,19,20,22,23] have also observed that tocopherols can act as prooxidants depending on the conditions. More recently, Terao and Matsushita[21] reported the similar effect of α-tocopherol in the oxidation of methyl linoleate. A prooxidant effect of α-tocopherol quinone has been also reported.[24] The detailed mechanism for such a "crossover effect" of α-tocopherol is not well understood. In the course of our study on the oxidation of lipids and its inhibition, we have studied in the present work the prooxidant effect of tocopherols in the spontaneous oxidation of methyl linoleate. Methyl linoleate was chosen as a substrate since the mechanism of its oxidation is well established to give quantitatively four isomeric conjugated diene hydroperoxides, 13-hydroperoxy-9-*cis*,11-*trans*-, 13-hydroperoxy-9-*trans*,11-*trans*-, 9-hydroperoxy-10-*trans*,12-*cis*-, and 9-hydroperoxy-10-*trans*,12-*trans*-octadecadienoic acid methyl esters.[25-29] Thus, it is possible to follow the oxidation quantitatively by measuring either the oxygen uptake, substrate disappearance, hydroperoxide formation or conjugated diene formation.[29] This study confirms some of the previous observations, adds some new data and render a conclusion on the crossover effect of tocopherols.

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Experimental

Natural *R,R,R*-α-, *R,R,R*-β-, *R,R,R*-γ-, and *R,R,R*-δ-tocopherols were kindly supplied by Eisai Co., Ltd. Commercial ascorbic acid and its esters were used as received. Methyl linoleate purchased from Daigo Chemical Co. was purified by a silica-gel column before use. *t*-Butyl hydroperoxide kindly supplied by Nippon Oil and Fats Co. was distilled under reduced pressure, 42 °C/24 Torr (1Torr=133.322 Pa). Commercial 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as received.

Methyl linoleate and additives were dissolved in methyl decanoate and placed in the dark or under laboratory light at room temperature or at 37 °C in air. Sample size was 5 ml. Aliquots of the sample were taken out at regular intervals and analyzed as follows.

The methyl linoleate hydroperoxides were analyzed as their corresponding alcohol, after reduction of the product solution with triphenylphosphine, by high-pressure liquid chromatography (HPLC) using a silica-gel column and hexane/isopropyl alcohol/acetic acid (1000:10:1, v/v/v) as the eluent. The rate of consumption of tocopherols was followed by HPLC with a Finepak SIL 18 column. Methanol was delivered as an eluent at 1 ml min^{-1} and the eluent was monitored at 290 nm. ESR spectrum was

recorded on X-band JEOL FE1X spectrometer at room temperature.

Results

Tocopherols are active radical scavengers and efficiently suppress the oxidations of methyl linoleate initiated by a radical initiator.[7,11,29-31] Tocopherols are consumed by the reactions with peroxyl radicals and, when they are depleted, a fast oxidation proceeds.[30] It was found that polyunsaturated fatty acids such as linoleic acid and its esters were oxidized spontaneously even in the absence of any radical initiator. Figure 1 shows the results of spontaneous oxidation of methyl linoleate at room temperature under normal laboratory light in air in the absence and presence of α-tocopherol. In the absence of α-tocopherol, methyl linoleate was slowly oxidized without any noticeable induction period and the conjugated diene hydroperoxides accumulated with time. When 0.25 mM (1 M=1 mol dm^{-3}) of α-tocopherol was added, the oxidation was suppressed and little accumulation of hydroperoxides was observed in about 10 days. α-Tocopherol decreased at roughly constant rate and it was depleted in 14 days. The hydroperoxides were gradually formed as α-tocopherol disappeared. Figure 1 also shows the effect of initial amount of methyl linoleate hydroperoxide. As the initial concentration of methyl linoleate hydroperoxide was increased, the rate of consumption of α-tocopherol increased and the duration of induction period was shortened. In every case, fast oxidation proceeded when α-tocopherol disappeared.

Other tocopherols also suppressed the spontaneous oxidation of methyl linoleate similarly. When α-, β-, γ-, and δ-tocopherols were added simultaneously into methyl linoleate and the solution was stood at room temperature in air under laboratory light, tocopherols were consumed as shown in Fig. 2. As observed previously[30] in the oxidation of methyl linoleate initiated with a radical initiator, α-tocopherol was consumed first, β- and γ-tocopherols decreased after most of α-tocopherol disappeared, and finally δ-tocopherol was consumed. These results may be ascribed partly to the different reactivities of tocopherols ($\alpha>\beta>\gamma>\delta$) toward peroxyl radials[7,11,32] and also to the interactions between tocopheroxyl radical (oxyl radical from tocopherol) and tocopherol.[30] For example, α-tocopherol reacts with β-, γ-, and δ-

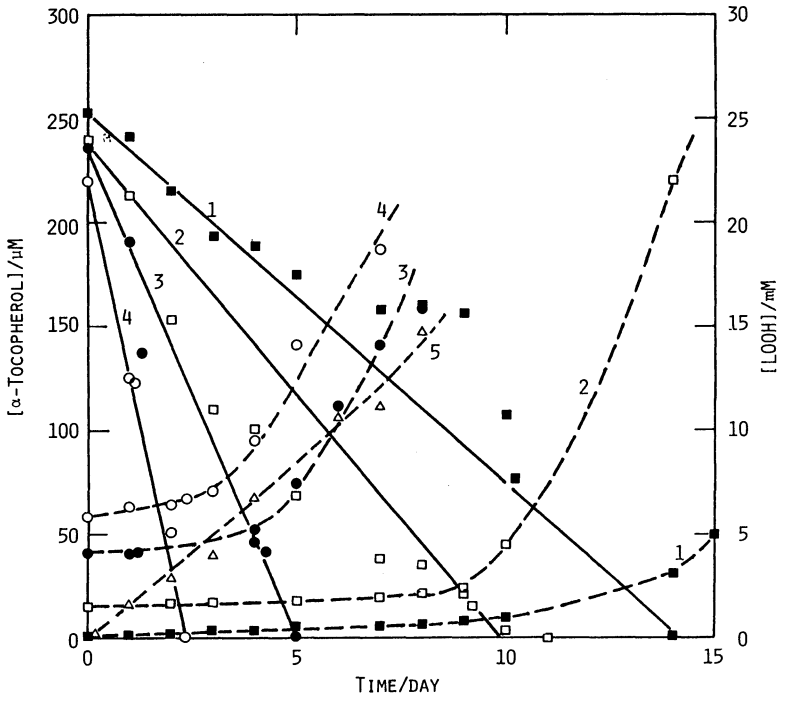


Fig. 1. Rate of accumulation of hydroperoxides (------) and α-tocopherol disappearance (———) in the spontaneous oxidation of methyl linoleate at room temperature under laboratory light.

Line	Mark	[α-Toc]/μM	[LOOH]$_0$/mM
1	■	251	0
2	□	241	1.22
3	●	249	3.95
4	○	229	5.92
5	△	0	0

tocopheroxyl radicals to give α-tocopheroxyl radical and corresponding tocopherols. Mukai has also observed that the reactivities toward phenoxyl radical decreased in the order of α->β-=γ->δ- tocopherols.[33]

Figure 3 shows the distribution of *cis,trans*- and *trans,trans*-hydroperoxides formed in the spontaneous oxidation of methyl linoleate in air under laboratory light in the presence of α-tocopherol. As described above, α-tocopherol suppressed the oxidation and when it was consumed completely, the oxidation proceeded rapidly. Interestingly, when α-tocopherol was present, only *cis,trans*-hydroperoxides were formed and little *trans,trans*-hydroperoxides were observed, whereas more *trans,trans*-hydroperoxides were formed

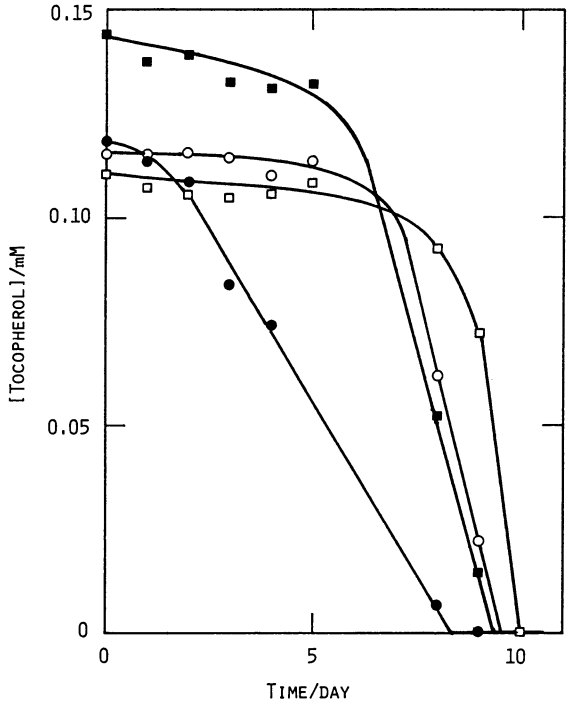


Fig. 2. Rate of disappearance of α-(●), β-(○), γ-(■), and δ-(□) tocopherols during spontaneous oxidation of methyl linoleate at room temperature under laboratory light.

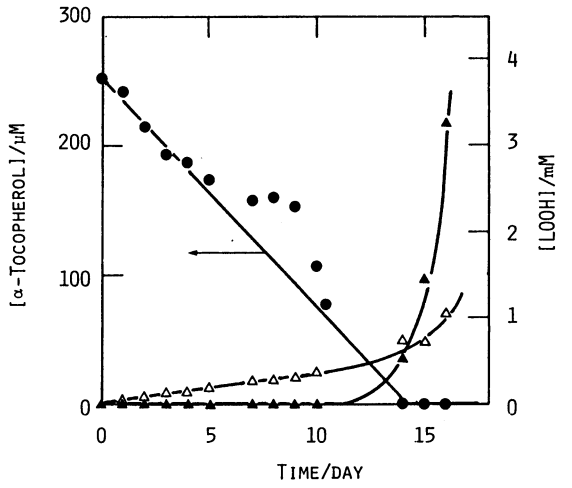


Fig. 3. Rate of hydroperoxide formation and α-tocopherol disappearance in the spontaneous oxidation of methyl linoleate at room temperature under laboratory light.
△: 13-Hydroperoxy-9-*cis*,11-*trans*-octadecadienoic acid methyl ester. ▲: 13-Hydroperoxyl-9-*trans*,11-*trans*-octadecadienoic acid methyl ester.

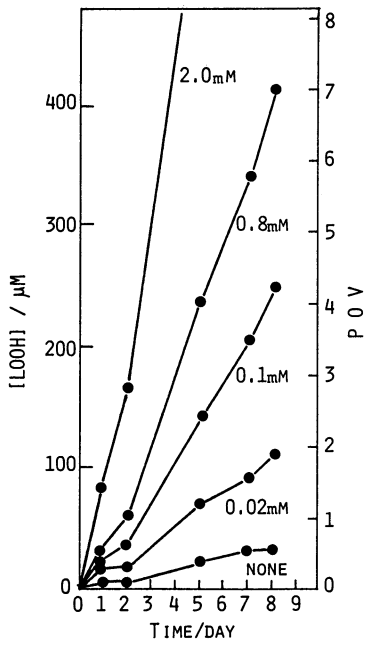


Fig. 4. Rate of spontaneous oxidation of methyl linoleate at 37 °C in the dark in the presence of α-tocopherol.

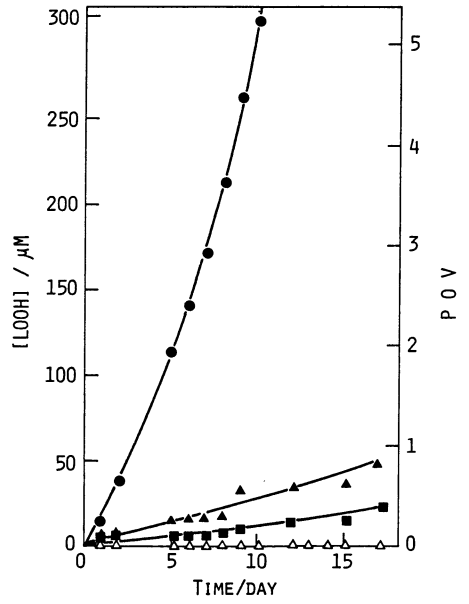


Fig. 5. Rate of accumulation of methyl linoleate hydroperoxide in the spontaneous oxidation of methyl linoleate at room temperature in the dark.
●: α-Toc 9.86 mM; ▲: β-Toc 10.5 mM; ■: γ-Toc 11.3 mM; △: none.

than *cis,trans*-ones after α-tocopherol was depleted.

Thus, α-tocopherol generally acts as an antioxidant and suppresses the spontaneous oxidation of methyl linoleate as well as the oxidation initiated with a radical initiator. However, when the rate of oxidation is quite slow or when the concentration of tocopherol is extraordinarily high, it may act as a prooxidant. When methyl linoleate was stood in air under laboratory light in the presence of 0.296, 2.43, and 18.3 mM α-tocopherol, the rate of hydroperoxide formation increased with increasing concentration of α-tocopherol (data not shown).

When methyl linoleate was stood at 37 °C in the dark, it was oxidized much slower than that stood under laboratory light. Under these conditions, α-tocopherol had a prooxidant effect even at relatively low concentrations. Figures 4 and 5 show the results observed when methyl linoleate was stood in the dark. The addition of tocopherols increased the initial rate of hydroperoxide accumulation with increasing α-tocopherol concentration (Fig. 4) and α-tocopherol had more profound effect than β- and γ-tocopherols (Fig. 5).

Discussion

α-Tocopherol acts as an antioxidant by scavenging chain-carrying peroxyl radical and breaking a chain propagation sequence. In fact, it is known that α-tocopherol scavenges peroxyl radicals much faster than the synthetic, hindered phenols often used commercially as antioxidants.[7,34,35] α-Tocopherol also suppresses the formation of *trans,trans*-hydroperoxide from methyl linoleate. The oxidation of methyl linoleate proceeds by a scheme shown in Fig. 6.[26,27] When the concentrations and/or activities of hydrogen donors are high, the peroxyl radical **1** rapidly abstracts a hydrogen atom to give *cis,trans*-hydroperoxide **2**, while the peroxyl radical **1** has more chance to release oxygen and yield the peroxyl radical **3** which eventually gives *trans,trans*-hydroperoxide **4** when the concentrations of active hydrogens are low. Thus, α-tocopherol acts as a strong hydrogen donor and gives *cis,trans*-hydroperoxide exclusively.[26,27,36-38]

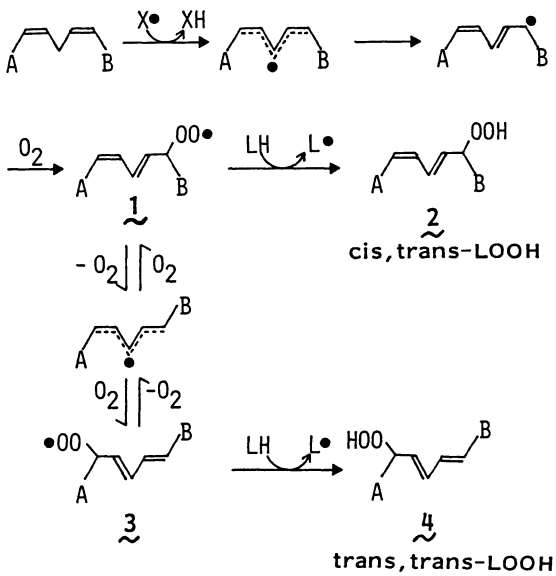
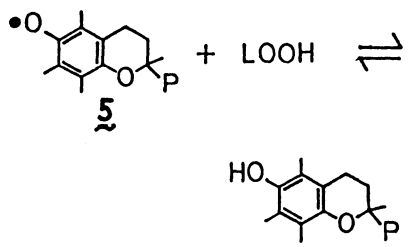


Fig. 6. Scheme for the oxidation of methyl linoleate.
A, B: $CH_3(CH_2)_4$-, $CH_3OC(O)(CH_2)_7$-.

However, as reported previously by other investigators[12-23] and as observed in this study, α-tocopherol can act as a prooxidant under certain circumstances, for example, when the rate of chain initiation is quite low and when the concentration of α-tocopherol is quite high. The prooxidant effect of α-tocopherol must stem from the hydrogen atom abstraction from hydroperoxide by α-tocopheroxyl radicals **5** (reaction 1),



(1)

where P is a phytyl side chain, $C_{16}H_{33}$. This reaction may proceed rapidly. In fact, as shown in Fig. 7, when *t*-butyl hydroperoxide was added to the benzene solution containing α-tocopheroxyl radical, tocopheroxyl radical disappeared quite rapidly. The rate constants for the hydrogen atom abstraction from

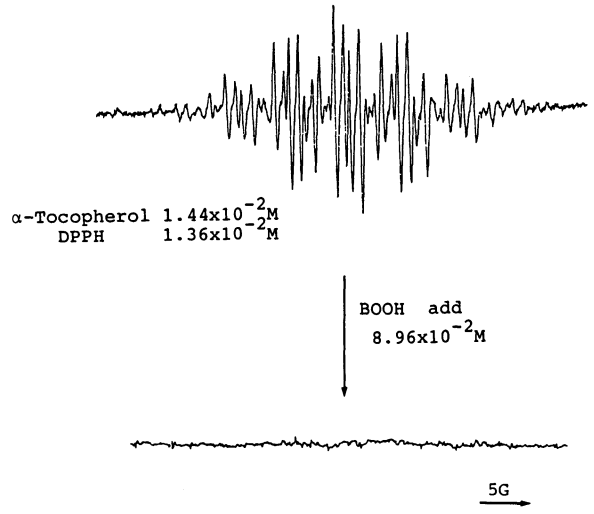


Fig. 7. ESR spectra observed when *t*-butyl hydroperoxide was added to the benzene solution containing α-tocopheroxyl radical.
A: ESR spectrum observed when 14.4 mM α-tocopherol was reacted with 13.6 mM DPPH in benzene. The spectrum was taken under vacuum. B: ESR spectrum observed under vacuum when 89.6 mM *t*-butyl hydroperoxide was added into the solution A.

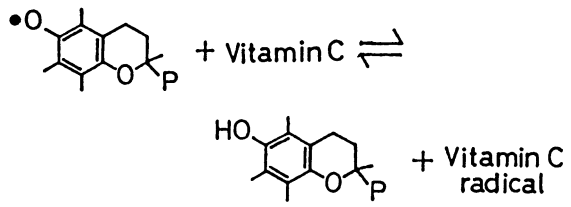
hydroperoxide by phenoxyl radicals have been reported.[39-41] Mukai et al.[42] reported quite recently the rate constant for the hydrogen atom abstraction from *t*-butyl hydroperoxide by 5,7-diisopropyl-6-chromanoxyl radical as 0.365 $M^{-1}s^{-1}$ at 25 °C. Considering the steric effect involved in the hydrogen atom transfer from hydroperoxide to a phenoxyl radical,[39,43,44] the hydrogen atom abstraction from lipid hydroperoxide by α-tocopheroxyl radical must be larger than that. The peroxyl radical formed in the reaction 1 must attack lipid and induce the chain oxidation. The direct attack of α-tocopheroxyl radical on the methyl linoleate can not be ruled out, although it must be much less favorable than the reaction 1.

As reported previously,[30,32,33] the stabilities of tocopheroxyl radicals decrease in the order of $\alpha>\beta=\gamma>\delta$, suggesting that α-tocopheroxyl radical is formed most easily among the four tocopheroxyl radicals. This must explain why α-tocopherol has the most profound prooxidant activity among the four tocopherols. Terao and Matsushita[21] found that BHT did not act as prooxidant for methyl linoleate. This must be because the phenoxyl radical derived from BHT is much less resonance-stabilized[32] and the spontaneous formation of the phenoxyl radical from BHT is quite slow.

In consistent with the above assumption, the prooxidant effect of α-tocopherol was diminished by a reducing agent such as ascorbic acid and its esters. As observed previously by Terao,[21] little prooxidant effect of α-tocopherol was observed in the presence of ascorbic acid esters such as 6-ascorbyl palmitate. Ascorbic acid added to the methyl linoleate as an ethanol solution also had a similar effect. It has been previously observed by pulse radiolysis[45] and ESR[46] studies that α-tocopheroxyl radical is reduced by ascorbic acid to regenerate α-tocopherol. Packer, Slater and Willson[45] obtained a value of 1.55×10^6 $M^{-1}s^{-1}$ as rate constant for the reaction 2. Mukai et al.[47] has recently obtained a rate constant of 549 $M^{-1}s^{-1}$ for the interaction between ascorbic acid and 5,7-diisopropyltocopheroxyl radical.

The crossover effect of α-tocopherol depends on the relative importance of the first step and the following competing reactions 2 and 3, which is determined by the ratio of the concentration of lipid peroxyl radical to that of lipid hydroperoxide. When this ratio is high as in the oxidations initiated with an added initiator, under light, or at high temperature, α-tocopherol functions as an antioxidant, while it acts as a prooxidant when this ratio is low. The higher the α-tocopherol concentration, the more α-tocopheroxyl radical must be formed spontaneously. Furthermore, α-tocopherol must give its radical most easily than any other tocopherols and synthetic phenols.

The prooxidant effect of vitamin E in vivo has not been reported. This may be because the concentration of vitamin E is usually low and/or because the vitamin E radical is reduced readily by, for example, vitamin C in biological systems.



In conclusion, α-tocopherol functions in general as a chain-breaking antioxidant, but it may also act as a prooxidant under certain conditions, for example, when its concentration is quite high or when the rate of oxidation (that is, the rate of chain initiation) is very low. It may be said that the antioxidant, like oxygen molecule, is also a double-edged sword.

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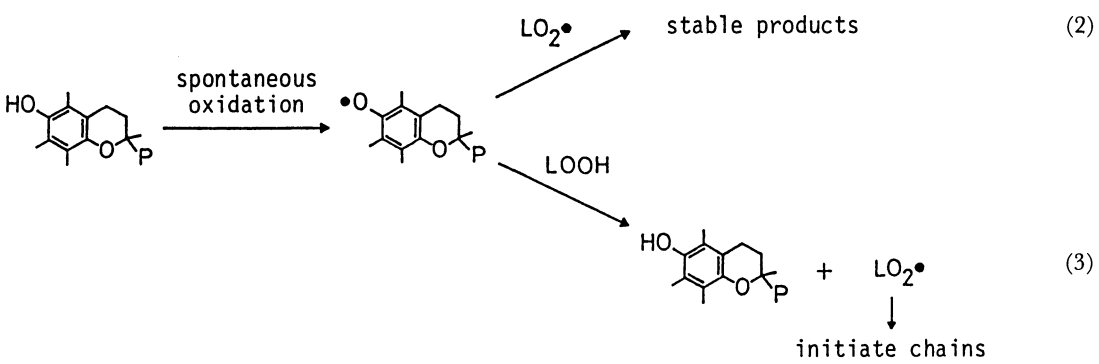
References

1) "Tocopherol, Oxygen, and Biomembranes," ed by C. de Duve and O. Hayaishi, Elsevier, Amsterdam (1978).
2) L. A. Witting, "Free Radicals in Biology, IV," ed by W. A. Pryor, Academic Press, (1980), pp. 295—319.
3) "Vitamin E. A Comprehensive Treatise," ed by L. J. Machlin, Marcel Dekker, New York (1980).
4) "Vitamin E: Biochemical, Hematological, and Clini-



cal Aspects," ed by B. Lubin and L. J. Machlin, Ann. N. Y. Acad. Sci., (1982), Vol. 393.
5) "Biology of Vitamin E," (Ciba Foundation Symp. 101), Pitman, London (1983).
6) "Clinical and Nutritional Aspects of Vitamin E," ed by O. Hayaishi and M. Mino, Elsevier, Amsterdam (1987).
7) G. W. Burton and K. U. Ingold, *Acc. Chem. Res.*, **19**, 194(1986).
8) P. B. McCay, *Ann. Rev. Nutr.*, **5**, 323 (1985).
9) G. W. Burton, D. O. Foster, B. Perly, T. F. Slater, I. C. P. Smith, and K. U. Ingold, *Phil. Trans. R. Soc. Lond.*, **B311**, 565 (1985).
10) A. Bendich, L. J. Machlin, O. Scandurra, G. W. Burton, and D. D. M. Wayner, *Adv. Free Rad. Biol. Med.*, **2**, 419 (1986).
11) E. Niki, *Chem. Phys. Lipids*, **44**, 227 (1987); *Ann. N. Y. Acad. Sci.*, **498**, 186 (1987).
12) W. O. Lundberg, "Autoxidation and Antioxidants, Vol. II," ed. by W. O. Lundberg, Interscience, New York (1962), pp. 451—476.
13) H. S. Olcott and E. Einset, *J. Am. Oil Chem. Soc.*, **35**, 159(1958).
14) C. Kanno, K. Yamauchi, and T. Tsugo, *Agr. Biol. Chem.*, **34**, 886 (1970).
15) J. Cillard and P. Cillard, *J. Am. Oil Chem. Soc.*, **57**, 39(1980).
16) J. Cillard, P. Cillard, and M. Cormier, *J. Am. Oil Chem. Soc.*, **57**, 252 (1980).
17) J. Cillard, P. Cillard, and M. Cormier, *J. Am. Oil Chem. Soc.*, **57**, 255 (1980).
18) H. Kamematsu, M. Aoyama, T. Maruyama, I. Niiya, and T. Matsumoto, *J. Jpn. Oil Chem. Soc.*, **33**, 241 (1984).
19) B. Bazin, J. Cillard, J. P. Koskas, and P. Cillard, *J. Am. Oil Chem. Soc.*, **61**, 1212(1984).
20) J. P. Koskas, J. Cillard, and P. Cillard, *J. Am. Oil Chem. Soc.*, **61**, 1466 (1984).
21) J. Terao and S. Matsushita, *Lipids*, **21**, 255(1986).
22) J. Cillard and P. Cillard, *J. Am. Oil Chem. Soc.*, **63**, 1165(1986).
23) S. R. Husain, J. Cillard, and P. Cillard, *J. Am. Oil Chem. Soc.*, **64**, 109 (1987).
24) J. A. Lindsey, H. Shang, H. Kaseki, N. Morisaki, T. Sato, and D. G. Comwell, *Lipids*, **20**, 151 (1985).
25) H. W. S. Chan and G. Levett, *Lipids*, **12**, 99 (1977).
26) N. A. Porter, B. A. Weber, H. Weener, and J. A. Khan, *J. Am. Chem. Soc.*, **102**, 5597 (1980).
27) N. A. Porter, *Acc. Chem. Res.*, **19**, 262 (1986); *Adv. Free Rad. Biol. Med.*, **2**, 283 (1986).
28) E. N. Frankel, *J. Am. Oil Chem. Soc.*, **61**, 1908 (1984); *Prog. Lipid Res.*, **23**, 197 (1985).
29) Y. Yamamoto, E. Niki, and Y. Kamiya, *Lipids*, **17**, 870 (1982).
30) E. Niki, J. Tsuchiya, Y. Yoshikawa, Y. Yamamoto, and Y. Kamiya, *Bull. Chem. Soc. Jpn.*, **59**, 497 (1986).
31) L. R. C. Barclay, S. J. Locke, J. M. MacNeil, G. W. Burton, and K. U. Ingold, *J. Am. Chem. Soc.*, **106**, 2479 (1984).
32) G. W. Burton, T. Doba, E. J. Gabe, L. Hughes, F. L. Lee, L. Prasad, and K. U. Ingold, *J. Am. Chem. Soc.*, **107**, 7053 (1985).
33) K. Mukai, Y. Watanabe, Y. Uemoto, and K. Ishizu, *Bull. Chem. Soc. Jpn.*, **59**, 3133 (1986).
34) G. W. Burton and K. U. Ingold, *J. Am. Chem. Soc.*, **103**, 6472 (1981).
35) E. Niki, R. Tanimura, and Y. Kamiya, *J. Jpn. Petrol. Inst.*, **27**, 21 (1984).
36) H. Weenen and N. A. Porter, *J. Am. Chem. Soc.*, **104**, 5216 (1982).
37) K. E. Peers, D. T. Coxon, and H. W. S. Chan, *J. Sci. Food Agric.*, **32**, 898 (1981).
38) K. E. Peers and D. T. Coxon, *Chem. Phys. Lipids*, **32**, 49 (1983).
39) J. R. Thomas, *J. Am. Chem. Soc.*, **86**, 4807 (1964).
40) L. R. Mahoney and M. A. DaRooge, *J. Am. Chem. Soc.*, **98**, 5619 (1967).
41) A. P. Griva, *Int. J. Chem. Kinet.*, **V**, 869 (1973).
42) K. Mukai, Y. Kohno, and K. Ishizu, *Biochem. Biophys. Res. Commun.*, **155**, 1046 (1988).
43) J. A. Howard and K. U. Ingold, *Can. J. Chem.*, **41**, 2800 (1963).
44) L. R. Mahoney, *Angew. Chem., Int. Ed. Engl.*, **8**, 547 (1969).
45) J. E. Packer, T. F. Slater, and R. L. Willson, *Nature (London)*, **278**, 737(1979).
46) E. Niki, J. Tsuchiya, R. Tanimura, and Y. Kamiya, *Chem. Lett.*, **1982**, 789.
47) K. Mukai, K. Fukuda, K. Ishizu, and Y. Kitamura, *Biochem. Biophys. Res. Commun.*, **146**, 134 (1987).