Free-Radical Scavenging Reactions of α-Tocopherol during the Autoxidation of Methyl Linoleate in Bulk Phase

Ryo Yamauchi,*,† Koji Kato,† and Yoshimitsu Ueno‡

Department of Food Science, Faculty of Agriculture, Gifu University, Gifu 501-11, Japan, and Laboratory of Food Chemistry, Tokai Women's Junior College, Gifu 504, Japan

Methyl linoleate containing α-tocopherol was autoxidized at 37 and 60 °C in bulk phase. The reaction products of α-tocopherol at 37 °C were 4a,5-epoxy-8a-hydroperoxy-α-tocopherones (**1**), 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 (**2**), a mixture of methyl 9-(α-tocopheroxy)-10(*E*),12(*Z*)-octadecadienoates and methyl 13-(α-tocopheroxy)-9(*Z*),11(*E*)-octadecadienoates (**3**), α-tocopherol spirodiene dimer (**4**), and α-tocopherol trimer (**5**). Compound **3** was the major product at 60 °C, and the *E,E*-isomers of **3** were produced in addition to the *E,Z*-isomers. When methyl linoleate containing a low concentration of α-tocopherol was autoxidized at 37 °C, α-tocopherol suppressed the formation of methyl linoleate hydroperoxides, and the main products of α-tocopherol were **1** and **2**. In contrast, α-tocopherol at a high concentration induced the accumulation of methyl linoleate hydroperoxides, and the reaction products were **1**–**5**. The autoxidation at 60 °C proceeded rapidly, and the main product of α-tocopherol was **1**. In addition, the accumulation of **3** was observed under air-insufficient conditions. The results indicate that the α-tocopheroxyl radical produced can react with both alkyl and alkylperoxyl radicals of unsaturated lipids during the autoxidation. Competing with these trapping reactions, **1** appears to be formed primarily by autoxidation reactions of α-tocopherol.

Keywords: *α-Tocopherol; methyl linoleate; lipid peroxidation; autoxidation*

INTRODUCTION

α-Tocopherol, an antioxidant in foods and living cells, inhibits autoxidation of lipids by trapping lipid-peroxyl radicals in two ways (Burton and Ingold, 1981; Niki *et al.*, 1984). First, lipid-peroxyl radicals are trapped by hydrogen atom transfer, giving hydroperoxides and α-tocopheroxyl radicals. Second, the resulting α-tocopheroxyl radicals react with other lipid-peroxyl radicals or each other to form nonradical products. To elucidate the mechanism of autoxidation inhibition by α-tocopherol, the reaction products of α-tocopherol with peroxyl radicals that generated in organic solvents from free radical initiators, such as 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN), have been investigated (Winterle *et al.*, 1984; Yamauchi *et al.*, 1989; Matsuo *et al.*, 1989; Liebler *et al.*, 1990, 1993). In our previous study, we isolated and characterized the primary products of α-tocopherol with peroxyl radicals of methyl linoleate formed by molecular oxygen and initiated with AMVN (Yamauchi *et al.*, 1990). The products were isomeric 8a-(lipid-peroxy)-α-tocopherones. When the lipid peroxidation was carried out under air-insufficient conditions, α-tocopherol could trap the lipid-alkyl radicals to form isomeric 6-*O*-(lipid-alkyl)-α-tocopherols (Yamauchi *et al.*, 1993). The products of α-tocopherol formed under relatively mild conditions of autoxidation are the spirodiene dimer and trimer of α-tocopherol (Csallany *et al.*, 1970; Yamauchi *et al.*, 1988). However, the reaction products of α-tocopherol during the autoxidation of unsaturated lipids are still unclear.

In the present study, the reaction products of α-tocopherol during the autoxidation of methyl linoleate were isolated and characterized. The autoxidation of methyl linoleate was carried out at 37 or 60 °C. The possible free-radical scavenging mechanism of α-tocopherol during the autoxidation of unsaturated lipids is discussed on the basis of the reaction products observed.

MATERIALS AND METHODS

Materials. *2R,4′R,8′R*-α-Tocopherol (type V) was purchased from Sigma Chemical Co. (St. Louis, MO) and purified by reversed-phase high-performance liquid chromatography (HPLC) before use. α-Tocopheryl acetate, used as an internal standard, was prepared by the acetylation of α-tocopherol with acetic anhydride in pyridine. Authentic samples of the oxidation products of α-tocopherol were prepared as described previously (Yamauchi *et al.*, 1988, 1990, 1993). The spirodiene dimer of α-tocopherol was synthesized following the procedure of Nelan and Robeson (1962). Methyl linoleate (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was purified and made peroxide-free by silica gel column chromatography just before use (Yamauchi *et al.*, 1984). All solvents were distilled in an all-glass still before use.

Apparatus. HPLC was performed with a Jasco Trirotar pump (Japan Spectroscopic Co., Tokyo, Japan) equipped with a Model GP-A40 gradient programmer. A Jasco Model 875-UV detector or a Model 820-FP spectrofluorometer was used as the detector. ^{1}H (270.17 MHz) nuclear magnetic resonance (NMR) spectra were recorded at 25 °C on a JEOL JNM-GX-270 FT NMR spectrometer using $CDCl_3$ as the solvent and tetramethylsilane as the internal standard. Mass spectra (MS) were obtained with a Shimadzu 9020DF instrument (Shimadzu Co., Kyoto, Japan). Samples were introduced by direct probe insertion, and electron impact (EI) MS samples were ionized with a 70-eV electron beam. Analyses in the chemical ionization (CI) mode were done with isobutane as the reagent gas. Circular dichroism (CD) measurements of the optical isomers in ethanol were performed with a Jasco J-600 spectropolarimeter.

* Author to whom correspondence should be addressed (fax 81-58-293-2930; e-mail yamautir@cc.gifu-u.ac.jp).
† Gifu University.
‡ Tokai Women's Junior College.

0021-8561/95/1443-1455$09.00/0

Reaction of α-Tocopherol and Methyl Linoleate. A mixture of methyl linoleate (10 g, 34 mmol) and α-tocopherol (0.10 g, 0.23 mmol) was placed in a Petri dish (8.6 cm in diameter, specific surface area of 5.7 cm^2/g of oil) and allowed to stand at 37 °C for 32 days in the dark. In another condition, a mixture of methyl linoleate (20 g, 68 mmol) and α-tocopherol (0.20 g, 0.46 mmol) was heated in a beaker (4.5 cm in diameter, specific surface area of 0.79 cm^2/g of oil) at 60 °C for 4 days. Each reaction mixture thus obtained was analyzed by reversed-phase HPLC using a μBondasphere 5 μm C_{18} 100 Å column (3.9 × 150 mm, Nihon Waters, Ltd., Tokyo, Japan) and a 15-min linear gradient of acetonitrile/methanol/water (10:88:2 v/v/v) to acetonitrile/2-propanol/hexane (30:40:30 v/v/v) at a flow rate of 1.0 mL/min. The eluent was monitored by measuring absorbance at 240 nm. The reaction products of α-tocopherol were isolated by preparative reversed-phase HPLC using a Wakosil 5C_{18} column (10 × 300 mm, Wako Pure Chemical Industries, Osaka, Japan) developed with methanol/ethanol (1:1 v/v) at a flow rate of 5.0 mL/min. The trimer in the column was eluted using 2-propanol as the solvent at the same flow rate. The products corresponding to 6-*O*-alkyl-α-tocopherols were further purified by normal-phase HPLC using a Wakosil 5Sil column (10 × 300 mm) and hexane/2-propanol (1000:1 v/v) at a flow rate of 4.0 mL/min. The isolated compounds were dissolved in ethanol or hexane and stored at −20 °C until analysis.

Time Course Experiment. Methyl linoleate containing α-tocopherol (0.1 or 0.5 mol %, based on methyl linoleate) was placed in a glass vial (1.5 cm in diameter) and incubated at 37 or 60 °C in the dark. The autoxidation was run on two different sample sizes, 0.50 and 3.0 g. The exchange of oxygen between air and oil in the 0.50-g sample was faster than that in the 3.0-g sample because of their different surface areas (the specific surface areas of the 0.50- and 3.0-g samples were 3.5 and 0.59 cm^2/g, respectively) (Yamauchi *et al.*, 1993). Thus, the 3.0-g sample might be under air-insufficient conditions compared with the 0.50-g sample. Periodically, aliquots of the sample were withdrawn, dissolved in ethanol, added to a definite amount of α-tocopheryl acetate (the internal standard), and injected into the HPLC. The amount of methyl linoleate hydroperoxides (MeL-OOH) was determined by reversed-phase HPLC with UV detection (Yamauchi *et al.*, 1992). α-Tocopherol was determined by reversed-phase HPLC with fluorescence detection (Yamauchi *et al.*, 1993). The reaction products of α-tocopherol were analyzed by reversed-phase HPLC using the same condition as described above. The amounts of the reaction products were calculated from the peak areas relative to the peak area of α-tocopheryl acetate by monitoring elution at 240 nm.

RESULTS

Reaction Products of α-Tocopherol at 37 °C. Methyl linoleate containing α-tocopherol was autoxidized at 37 °C for 32 days, and the reaction products of α-tocopherol were analyzed by reversed-phase HPLC (Figure 1). Peaks corresponding to α-tocopherol, the reaction products of α-tocopherol, **1**−**5**, and other unknown peaks appeared on the chromatogram. A peak eluting at 8.4 min showed UV (ethanol) λ_{max} at 236 nm, indicating the presence of conjugated diene in the molecule. This compound is thought to be methyl linoleate dimer containing an extra oxygen atom from the MS spectrum; CIMS *m*/*z* 603 (MH^+, 95%), 585 ($[M - OH]^+$, 64), 571 ($[M - OCH_3]^+$, 37), 503 (18), 489 (34), 403 (41), 309 ($OC_{17}H_{30}COOCH_3$, 77), 293 ($C_{17}H_{30}COOCH_3$, 100), 277 (52), and 185 (40). The compound has been reported as epoxy dimers of methyl linoleate (Garssen *et al.*, 1972). However, The structure of this compound was not further investigated. The peaks corresponding to the reaction products of α-tocopherol were collected by preparative reversed-phase HPLC. The structures of **1**−**5** were identified as subsequently described (Figure 2).

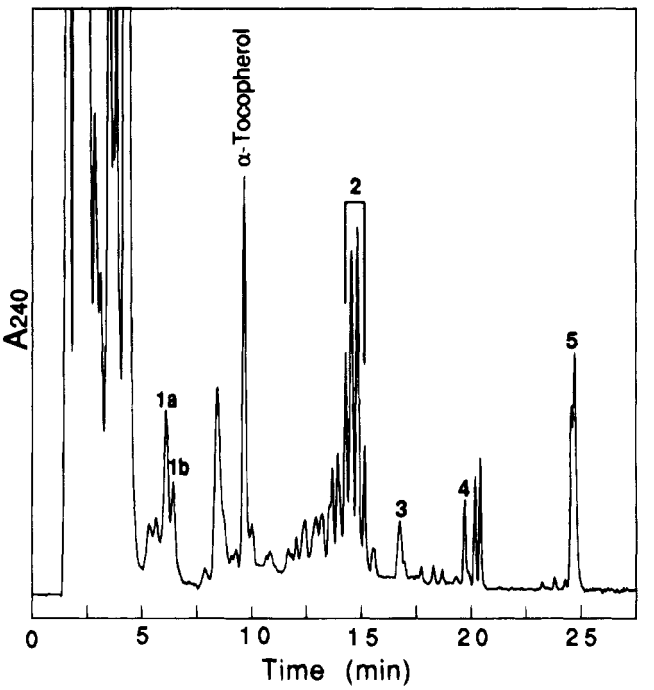

Figure 1. Reversed-phase HPLC of the products of the autoxidation of methyl linoleate and α-tocopherol at 37 °C for 32 days. HPLC was done with a μBondasphere 5 μm C_{18} column (3.9 × 150 mm) which was developed with a 15-min linear gradient of acetonitrile/methanol/water (10:88:2 v/v/v) to acetonitrile/2-propanol/hexane (30:40:30 v/v/v) at a flow rate of 1.0 mL/min. The eluent was monitored by measuring absorbance at 240 nm.

Compounds **1a** and **1b** were identified as diastereomers of 4a,5-epoxy-8a-hydroperoxy-α-tocopherone. The identities of the compounds were confirmed by UV and MS and by comparison of their HPLC behavior with that of authentic samples (Matsuo *et al.*, 1989). **1a**: UV (ethanol) λ_{max} 240 nm; CIMS *m*/*z* 479 (MH^+, 12%), 463 (35), 445 (71), 433 (24), 325 (100), and 139 (84). **1b**: UV (ethanol) λ_{max} 247 nm; CIMS *m*/*z* 479 (MH^+, 6%), 463 (39), 445 (93), 433 (12), 325 (100), and 139 (76). When **1a** and **1b** were analyzed by normal-phase HPLC using hexane/2-propanol (100:2 v/v) as the mobile phase, **1a** and **1b** were eluted in that order. However, the stereochemistry of **1a** and **1b** could not be determined from our data. Compound **2** contained four diastereomers of methyl 9-(8a-dioxy-α-tocopherone)-10(*E*),12(*Z*)-octadecadienoate (**2a**) and four diastereomers of methyl 13-(8a-dioxy-α-tocopherone)-9(*Z*),11(*E*)-octadecadienoate (**2b**) by UV, MS, and coelution with the authentic samples (Yamauchi *et al.*, 1990): UV (ethanol) λ_{max} 230 nm; CIMS *m*/*z* 771 (MH^+, 3%), 755 (24), 739 (14), 737 (10), 447 (46), 445 (41), 431 (33), 429 (22), 309 (63), 293 (100), and 165 (45). Compound **3** was identified as a mixture of methyl 9-(α-tocopheroxy)-10(*E*),12(*Z*)-octadecadienoate and methyl 13-(α-tocopheroxy)-9(*Z*),11(*E*)-octadecadienoate (Yamauchi *et al.*, 1993): UV (ethanol) λ_{max} 228 and 289 nm; CIMS *m*/*z* 457 (3%), 431 (73), 293 (100), and 165 (93). Compound **3** gave two peaks (**3a** and **3d**) corresponding to the 13- and 9-isomers by normal-phase HPLC using hexane/2-propanol (1000:1 v/v) as the mobile phase. The formation ratio of the 13- and 9-isomers was almost the same from the HPLC analysis (data not shown). Compound **4** was identified as the spirodiene dimer of α-tocopherol by comparison of the HPLC behavior with that of the authentic sample (Nelan and Robeson, 1962). Compounds eluting at 20.2 and 20.4 min had the same UV and MS spectra: UV

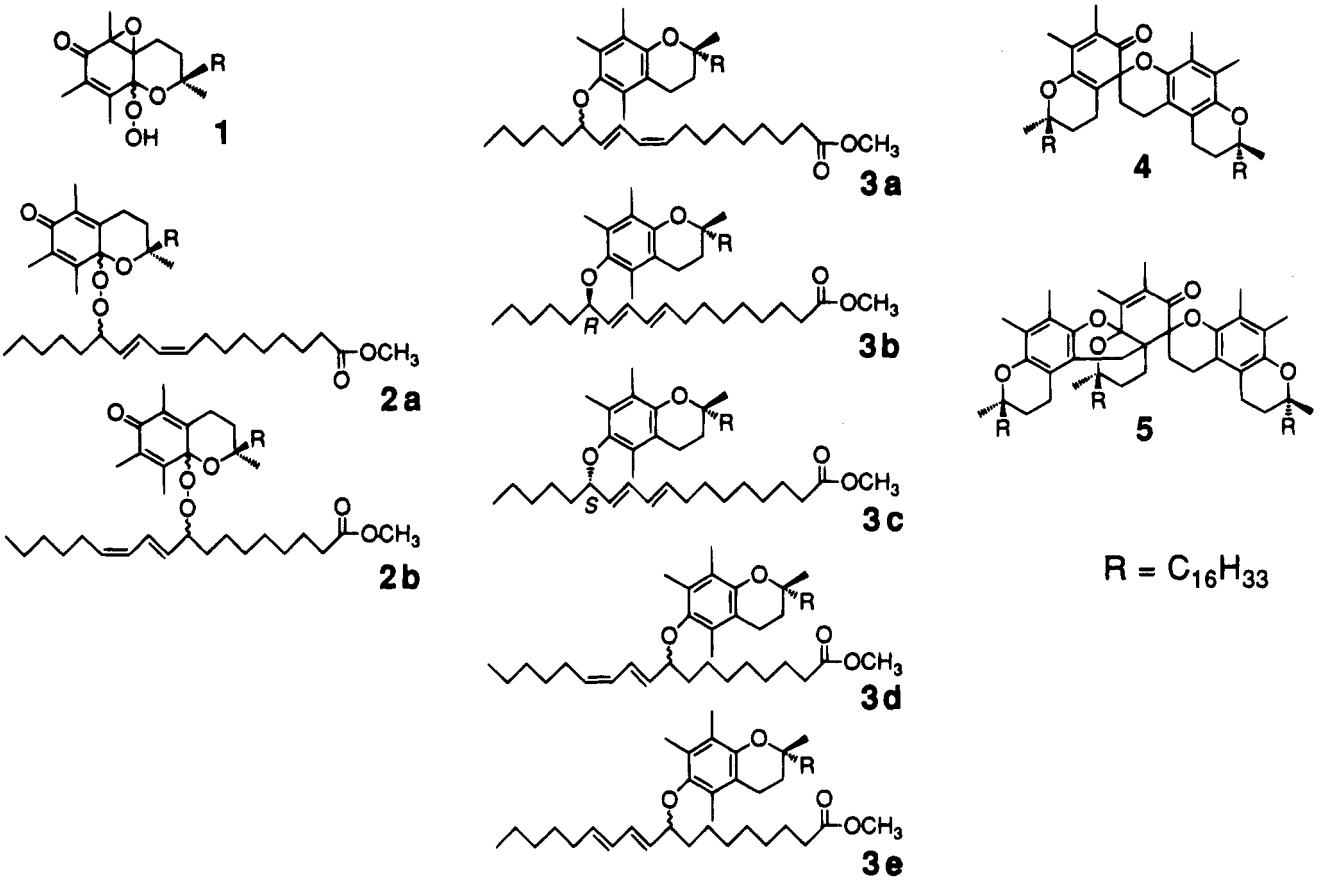

Figure 2. Structures of compounds **1**–**5**.

(hexane) λ_{max} 225 (shoulder), 282, and 286; CIMS *m*/*z* 873 (MH^+, 3%), 447 (100), 431 (44), 430 (30), 429 (49), and 165 (67). The MH^+ at *m*/*z* 873, which was 16 mass units higher than that for the spirodiene dimer of α-tocopherol, is indicative of α-tocopherol dimer containing an extra oxygen atom. However, we have not so far fully elucidated their structures. Compound **5** was identified as two stereoisomers of α-tocopherol trimer by comparison of the HPLC behavior with that of the authentic samples (Yamauchi *et al.*, 1988).

Reaction Products of α-Tocopherol at 60 °C. Methyl linoleate containing α-tocopherol was autoxidized at 60 °C for 4 days in the dark. The reaction products of α-tocopherol were analyzed by HPLC (Figure 3). One major peak corresponding to compound **3** and some minor peaks corresponding to **1**, **2**, **4**, **5**, α-tocopherol, methyl linoleate dimers, and unknown compounds appeared on the chromatogram. The identities of methyl linoleate dimers were confirmed by coelution with authentic samples (Yamauchi *et al.*, 1993). Compound **3** was further separated into four peaks (**3a**, **3b**, **3c**, and a mixture of **3d** and **3e**) by normal-phase HPLC. The structures of compounds **3a**–**3e** were identified as follows (Figure 2).

The spectral data were essentially identical with those of methyl (α-tocopheroxy)octadecadienoates (Yamauchi *et al.*, 1993). The positions of the double bonds in the fatty acid moieties were determined by the oxidative method described by Capella and Zorzut (1968). The trimethylsilyl derivatives of compounds **3a**–**3e** after hydroxylation of the olefinic bonds by oxidation with OsO_4 and subsequent reduction of the osmates with Na_2SO_3 were directly analyzed by MS (Yamauchi *et al.*, 1993). The position of the α-tocopheroxy group attached to the fatty acid moiety could be determined from the

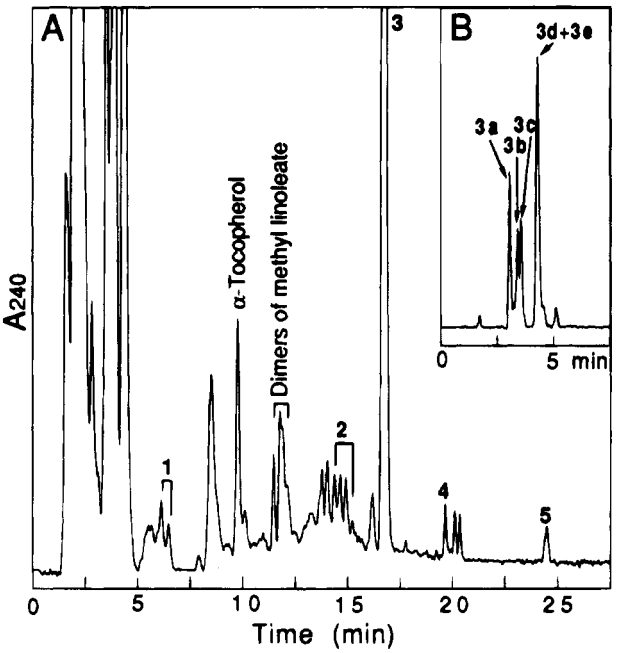

Figure 3. Reversed-phase HPLC of the products of the autoxidation of methyl linoleate and α-tocopherol at 60 °C for 4 days (A). The same HPLC condition was employed as in Figure 1. Peak 3 was collected and analyzed by normal-phase HPLC (B). Normal-phase HPLC was done with a μBondasphere 5 μm Si-100 Å column (3.9 × 150 mm) developed with hexane/2-propanol (1000:1 v/v) at flow rate of 1.0 mL/min.

fragment ion due to α-cleavage of the trimethylsilyloxy group of each derivative (*m*/*z* 173 for **3a**, **3b**, and **3c**; and *m*/*z* 259 for a mixture of **3d** and **3e**, respectively) (data not shown). Compounds **3b** and **3c** proved to be optically active. The CD spectrum of **3b** showed a

negative Cotton effect and that from **3c** showed a positive Cotton effect (data not shown). Therefore, the tocopheroxyl group of methyl 13-(α-tocopheroxy)octadecadienoates from **3b** is in the *R* configuration and that from **3c** is in the *S* configuration (Yamauchi *et al.*, 1990). On the other hand, compounds **3a**, **3d**, and **3e** were optically inactive, each consisting of enantiomeric mixtures.

The ^{1}H NMR spectra were consistent with those expected for methyl (α-tocopheroxy)octadecadienoates (Yamauchi *et al.*, 1993). The spectra are listed below using the following notation for the H atoms: H_m-*n*, where m denotes the moiety within the methyl (α-tocopheroxy)octadecadienoate molecule and can be l (linoleate) or t (tocopherol), and *n* denotes the C atom number to which the H atom is bonded. The numbering systems used are the standard numbering systems for the fatty acid chain and α-tocopherol. **3a**: ($CDCl_3$) δ 0.83−0.88 (m, 15H), 1.07−2.15 (m, 43H), 1.21 (s, 3H, H_t-2a), 2.06 and 2.08 (s, 6H, H_t-5a,7a), 2.12 and 2.13 (s, 3H, H_t-8b), 2.29 (t, *J* = 7.7 Hz, 2H, H_l-2), 2.54 (t, *J* = 6.4 Hz, 2H, H_t-4), 3.66 (s, 3H, OCH_3), 4.06 (dt, *J* = 5.1, 8.5 Hz, 1H, H_l-13), 5.38 (dt, *J* = 7.2 Hz, 1H, H_l-9), 5.67 (dd, *J* = 8.5, 14.9 Hz, 1H, H_l-12), 5.93 (t, *J* = 10.6 Hz, 1H, H_l-10), and 6.16 (dd, *J* = 11.0, 14.9 Hz, 1H, H_l-11). **3b**: ($CDCl_3$) δ 0.83−0.88 (m, 15H), 1.07−2.15 (m, 43H), 1.21 (s, 3H, H_t-2a), 2.06 (s, 6H, H_t-5a,7a), 2.12 (s, 3H, H_t-8b), 2.30 (t, *J* = 7.7 Hz, 2H, H_l-2), 2.54 (t, *J* = 6.8 Hz, 2H, H_t-4), 3.66 (s, 3H, OCH_3), 4.01 (dt, *J* = 5.1, 8.5 Hz, 1H, H_l-13), 5.55 (m, 2H, H_l-9,12), 5.85 (dd, *J* = 10.6, 14.9 Hz, 1H, H_l-10), and 5.96 (dd, *J* = 10.2, 15.3 Hz, 1H, H_l-11). **3c**: ($CDCl_3$) δ 0.83−0.88 (m, 15H), 1.07−2.15 (m, 43H), 1.21 (s, 3H, H_t-2a), 2.06 (s, 6H, H_t-5a,-7a), 2.12 (s, 3H, H_t-8b), 2.30 (t, *J* = 7.7 Hz, 2H, H_l-2), 2.54 (t, *J* = 6.8 Hz, 2H, H_t-4), 3.66 (s, 3H, OCH_3), 4.01 (dt, *J* = 5.1, 8.1 Hz, 1H, H_l-13), 5.57 (m, 2H, H_l-9,12), 5.86 (dd, *J* = 10.2, 14.9 Hz, 1H, H_l-10), and 5.97 (dd, *J* = 10.6, 14.9 Hz, 1H, H_l-11). Mixture of **3d** and **3e**: ($CDCl_3$) δ 0.83−0.88 (m, 15H), 1.07−2.15 (m, 43H), 1.21 and 1.22 (s, 3H, H_t-2a), 2.06 (s, 6H, H_t-5a,7a), 2.11 (s, 3H, H_t-8b), 2.29 (t, *J* = 7.7 Hz, 2H, H_l-2), 2.54 (t, *J* = 6.4 Hz, 2H, H_t-4), 3.65 (s, 3H, OCH_3), 4.03 (m, 1H, H_l-9), 5.39 (dt, *J* = 8.5, 11.5 Hz, $^1/_2$H, H_l-13, **3d**), 5.57 (m, 1H, H_l-10,13, **3e**), 5.65 (dd, *J* = 9.3, 15.3 Hz, $^1/_2$H, H_l-10, **3d**), 5.84 (dd, *J* = 10.2, 14.4 Hz, $^1/_2$H, H_l-12, **3e**), 5.91 (t, *J* = 10.6 Hz, $^1/_2$H, H_l-12, **3d**), 5.96 (dd, *J* = 10.6, 14.9 Hz, $^1/_2$H, H_l-11, **3e**), and 6.14 (dd, *J* = 10.6, 15.3 Hz, $^1/_2$H, H_l-11, **3d**).

Thus, the structures of **3a**−**3e** were identified as follows: **3a**, an enantiomeric mixture of methyl 13-(α-tocopheroxy)-9(*Z*),11(*E*)-octadecadienoate; yield 12.2 mg; UV (ethanol) λ_{max} 227 (ε 28 100) and 289 nm (2510); IR (film) ν_{max} 2950, 1745, 1460, 1415, 1380, 1250, 1165, 1085, 985, and 950 cm^{-1}; EIMS *m*/*z* 431 (40%), 293 (8), 205 (12), and 165 (100); CIMS *m*/*z* 458 (23%), 431 (90), 293 (100), and 165 (31); $[\alpha]^{25}_D$ +7 (*c* 0.22, ethanol). **3b**, methyl 13(*R*)-(α-tocopheroxy)-9(*E*),11(*E*)-octadecadienoate; yield 6.8 mg; UV (ethanol) λ_{max} 227 (ε 31 600) and 289 (2500) nm; IR (film) ν_{max} 2950, 1745, 1460, 1415, 1380, 1250, 1165, 1085, and 985 cm^{-1}; EIMS *m*/*z* 431 (38%), 293 (9), 205 (11), and 165 (100); CIMS *m*/*z* 458 (26%), 431 (96), 293 (100), and 165 (36); $[\alpha]^{25}_D$ −18 (*c* 0.36, ethanol). **3c**, methyl 13(*S*)-(α-tocopheroxy)-9(*E*),11(*E*)-octadecadienoate; yield 6.8 mg; UV (ethanol) λ_{max} 227 (ε 30 200) and 289 (2430) nm; IR (film) ν_{max} 2950, 1745, 1460, 1415, 1380, 1250, 1165, 1085, and 985 cm^{-1}; EIMS *m*/*z* 431 (29%), 293 (7), 205 (10), 165 (86), and 43 (100); CIMS *m*/*z* 458 (27%), 431 (100), 293 (77), and 165 (30); $[\alpha]^{25}_D$ +29 (*c* 0.36, ethanol). Mixture of **3d** and **3e**, an enantiomeric mixture of methyl 9-(α-tocopheroxy)-10(*E*),12(*Z*)-octadecadienoate (**3d**) and an enantiomeric mixture of methyl 9-(α-tocopheroxy)-10-(*E*),12(*E*)-octadecadienoate (**3e**); yield 20.5 mg; UV (ethanol) λ_{max} 227 (ε 29 000) and 289 (2500) nm; IR (film) ν_{max} 2950, 1745, 1460, 1415, 1380, 1250, 1165, 1085, 985, and 950 cm^{-1}; EIMS *m*/*z* 431 (30%), 293 (17), 205 (10), 165 (84), and 43 (100); CIMS *m*/*z* 458 (24%), 431 (87), 293 (100), and 165 (29); $[\alpha]^{25}_D$ +1 (*c* 0.41, ethanol). The formation ratio of compounds **3a**−**3c** and a mixture of **3d** and **3e** was about 2:1:1:4 on the basis of each peak area of the HPLC chromatogram. Thus, almost equal amounts of the four stereoisomers, the 13-*E*,*Z*-, 13-*E*,*E*-, 9-*E*,*Z*-, and 9-*E*,*E*-isomers, were formed during the reaction.

Reaction of α-Tocopherol during the Autoxidation of Methyl Linoleate. Methyl linoleate containing two different concentrations of α-tocopherol (0.1 and 0.5 mol %, based on methyl linoleate) was autoxidized in bulk phase at 37 or 60 °C in the dark. The reaction was run on two different sample sizes (0.50 and 3.0 g) in the glass vial. The exchange of oxygen between air and oil in the 0.50-g sample may be faster than that in the 3.0-g sample because of their different surface areas (specific surface areas of the 0.50- and 3.0-g samples were 3.5 and 0.59 cm^2/g, respectively) (Yamauchi *et al.*, 1993).

Figure 4 shows the results of the formation of MeL-OOH, α-tocopherol decay, and the formation of the reaction products, 4a,5-epoxy-8a-hydroperoxy-α-tocopherones (**1**), 8a-(alkylperoxy)-α-tocopherones (**2**), 6-*O*-alkyl-α-tocopherols (**3**), spirodiene dimer of α-tocopherol (**4**), and α-tocopherol trimer (**5**), during the autoxidation of methyl linoleate at 37 °C. When methyl linoleate containing 0.1 mol % α-tocopherol was oxidized, α-tocopherol could suppress the formation of MeL-OOH in both sample sizes of 0.50 and 3.0 g (Figure 4A). The main products first formed were **2** and then **1** was accumulated. Only trace amounts of **4** were formed during the reaction. Methyl linoleate containing 0.5 mol % of α-tocopherol showed a linear accumulation of MeL-OOH with depletion of the α-tocopherol (Figure 4B). The main products of α-tocopherol were **1**, **2**, **4**, and **5** in the 0.5-g sample. Besides these products, **3** was formed in the 3.0-g sample.

When the autoxidation was carried out at 60 °C, the reaction proceeded rapidly (Figure 5). Figure 5A shows the results of methyl linoleate containing 0.1 mol % α-tocopherol. α-Tocopherol could suppress the formation of MeL-OOH in the 0.50-g sample. The main product of α-tocopherol in the 0.5-g sample was **1**, and those in the 3.0-g sample were **1** and **3**, respectively. Only trace amounts of the other reaction products appeared in the reaction mixture. When methyl linoleate was oxidized in the presence of 0.5 mol % α-tocopherol, MeL-OOH accumulated, and the loss of α-tocopherol and the formation of the reaction products were largely influenced by the sample sizes (Figure 5B). In the 0.5-g sample, α-tocopherol disappeared by 4 days and only **1** accumulated in the reaction mixture. In the 3.0-g sample, on the other hand, the α-tocopherol disappeared by 9 days and very large amounts of **3** accumulated in the reaction mixture. Table 1 compares the relative product yields of α-tocopherol during the autoxidation of methyl linoleate at 37 and 60 °C. The reaction products of α-tocopherol detected in the present

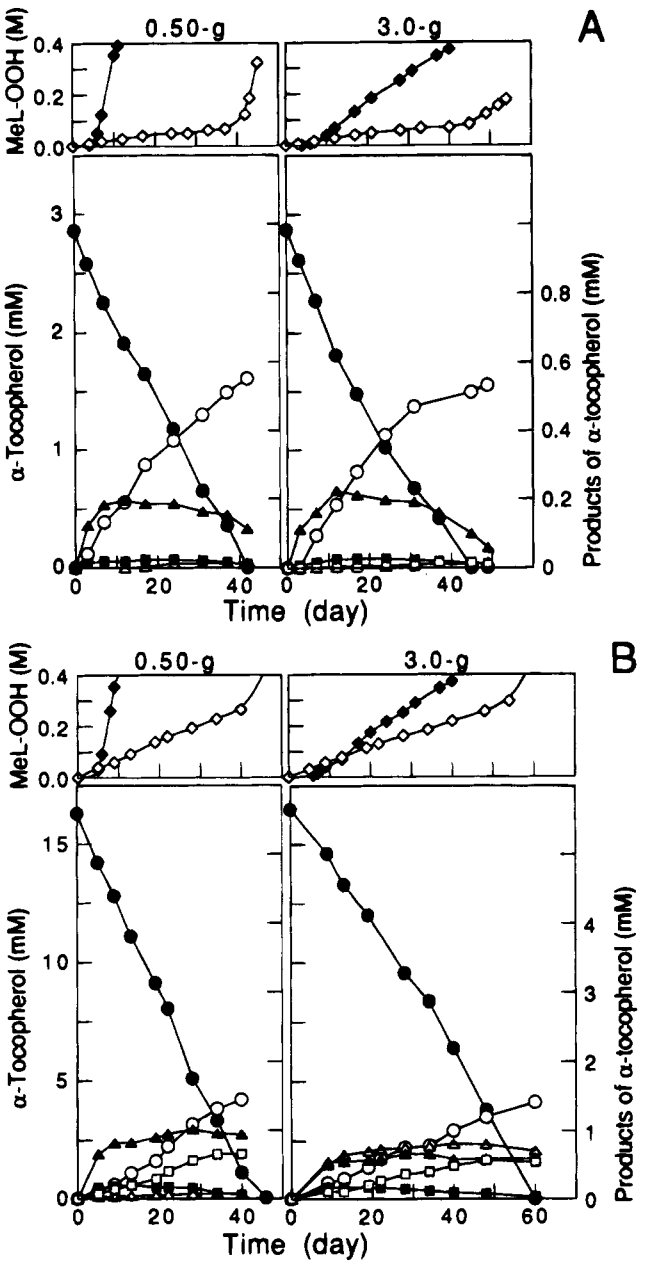

Figure 4. Reaction of α-tocopherol during the autoxidation of methyl linoleate at 37 °C. Methyl linoleate was autoxidized in the presence of (A) 0.1 or (B) 0.5 mol % α-tocopherol. The reaction was carried out in two sample sizes in the glass vial: 0.50 and 3.0 g. MeL-OOH, (◆) without or (◇) with α-tocopherol; (●) α-tocopherol; and the reaction products of α-tocopherol, (○) compound **1**, (▲) compound **2**, (△) compound **3**, (■) compound **4**, and (□) compound **5**.

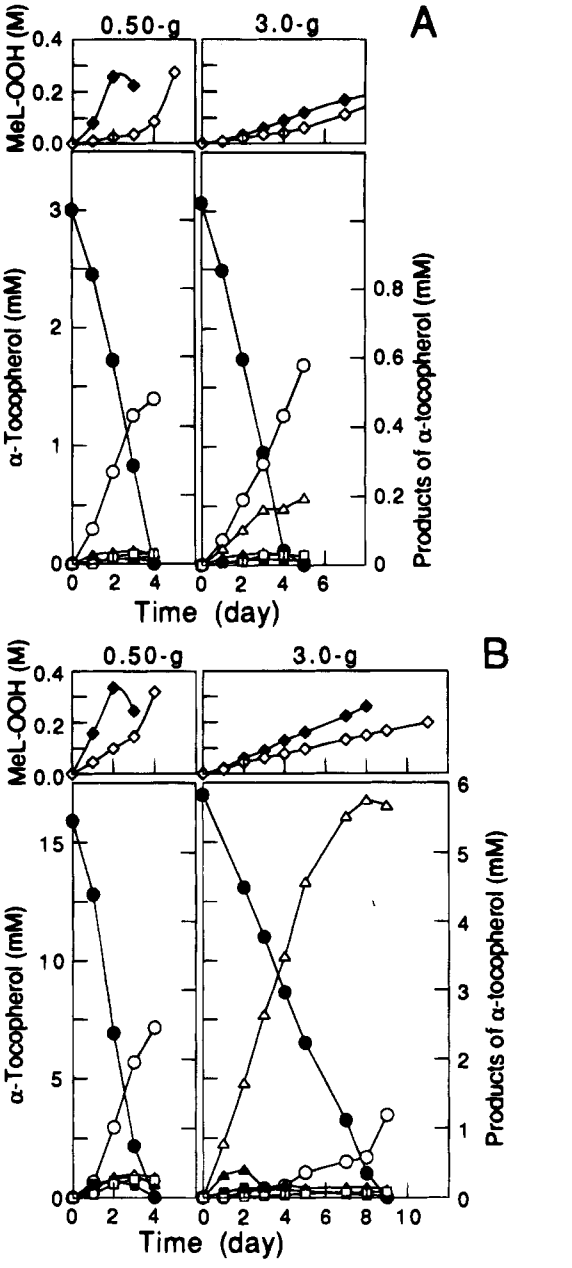

Figure 5. Reaction of α-tocopherol during the autoxidation of methyl linoleate at 60 °C. Methyl linoleate was autoxidized in the presence of (A) 0.1 or (B) 0.5 mol % α-tocopherol. The reaction was carried out in two sample sizes: 0.50 and 3.0 g. MeL-OOH, (◆) without or (◇) with α-tocopherol; (●) α-tocopherol; and the reaction products of α-tocopherol, (○) compound **1**, (▲) compound **2**, (△) compound **3**, (■) compound **4**, and (□) compound **5**.

study account for 30–50% of the consumed α-tocopherol and the rest is unknown products.

DISCUSSION

α-Tocopherol reacts with peroxyl radicals to produce hydroperoxides and α-tocopheroxyl radical, which traps a second peroxyl radical to produce a stable product (Burton and Ingold, 1981; Niki *et al.*, 1984). We have already reported the isolation and characterization of 8a-(alkylperoxy)-α-tocopherones and 6-*O*-alkyl-α-tocopherols as the products of α-tocopheroxyl radical with methyl linoleate–peroxyl and methyl linoleate–alkyl radicals during the AMVN-induced peroxidation in bulk phase (Yamauchi *et al.*, 1990, 1993). In the present study, the 8a-(alkylperoxy)-α-tocopherones (**2**) could be detected as the reaction products of α-tocopherol during the autoxidation of methyl linoleate. The 8a-(alkylperoxy)-α-tocopherones seems to be thermolabile. When methyl myristate containing 0.1 mol % of the 8a-(alkylperoxy)-α-tocopherones was stored at 37 or 60 °C, the remaining 8a-(alkylperoxy)-α-tocopherones after 24 h were 70% and 10% of the starting compound (data not shown). Thus, the produced 8a-(alkylperoxy)-α-tocopherones might immediately be decomposed at 60 °C, and only trace amounts of these compounds were detected in the reaction mixture. The 6-*O*-alkyl-α-tocopherols (**3**), which have been reported to be the reaction products of α-tocopherol with methyl linoleate–alkyl radicals (Yamauchi *et al.*, 1993), were also detected as the reaction products under the air-insufficient conditions. In addition to these free-radical trapping

Table 1. Product Distributions from α-Tocopherol during the Autoxidation of Methyl Linoleate at 37 and 60 °C[a]

reaction system			yield,[c] %					
TH[b] in oil, %	sample size, g	incubation time, days	TH	1	2	3	4	5
Autoxidation at 37 °C								
0.1	0.5	12	66.8	6.6	7.0	nd[d]	1.4	nd
0.1	3.0	12	63.1	6.3	7.7	nd	1.4	nd
0.5	0.5	19	60.7	3.4	5.5	0.3	2.1	5.3
0.5	3.0	28	57.9	4.6	4.0	4.6	1.8	6.6
Autoxidation at 60 °C								
0.1	0.5	2	57.3	9.0	1.0	0.3	1.3	2.0
0.1	3.0	2	56.7	6.1	1.0	3.3	1.3	1.0
0.5	0.5	2	43.4	6.4	1.6	1.9	3.1	3.8
0.5	3.0	4	50.6	1.0	1.1	20.5	1.3	0.7

[a] These data are from the experiments described in Figures 4 and 5. [b] TH, α-tocopherol. [c] Mol % to each theoretical yield based on the starting material. [d] nd, not detectable.

products, 4a,5-epoxy-8a-hydroperoxy-α-tocopherone (**1**) was accumulated in all reaction samples tested in this study. This compound has been reported as the autoxidation product of α-tocopherol (Matsuo *et al.*, 1989; Liebler *et al.*, 1990). No α-tocopherylquinone, which has been considered to be as the reaction product of α-tocopherol with free radicals (Gruger and Tappel, 1970), could be detected during the autoxidation of methyl linoleate. The formation of α-tocopherylquinone is undoubtedly due to hydrolysis of the 8a-(lipid-peroxy)-α-tocopherones. The 8a-(lipid-peroxy)-α-tocopherones are unstable in acid (Yamauchi *et al.*, 1989), and the chromatographic separation or the acidic condition may result in the formation of α-tocopherylquinone.

The autoxidation of methyl linoleate (LH) and inhibition by α-tocopherol (TH) in homogeneous solution at sufficient oxygen pressures proceeds by the following reaction sequence (Frankel, 1980; Porter, 1986; Chan, 1987):

initiation:

$$LH \rightarrow L^{\bullet} \quad (1)$$

propagation:

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \quad (2)$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \quad (3)$$

termination:

$$2LOO^{\bullet} \rightarrow \text{stable products} \quad (4)$$

$$LOO^{\bullet} + TH \rightarrow LOOH + T^{\bullet} \quad (5)$$

$$LOO^{\bullet} + T^{\bullet} \rightarrow \text{stable products} \quad (6)$$

$$2T^{\bullet} \text{ or } 3T^{\bullet} \rightarrow \text{dimer or trimer} \quad (7)$$

In the presence of α-tocopherol, reaction 5 results in the formation of hydroperoxide (LOOH) and α-tocopheroxyl radical (T•), which reacts rapidly and irreversibly with a second peroxyl radical (LOO•) to form stable products (reaction 6) (Burton and Ingold, 1986). Our results indicate that the reaction of peroxyl radicals with a low concentration of α-tocopherol under air-sufficient conditions proceeds by reaction 6 to give the stable products and terminated the autoxidation (Figure 4A). α-Tocopherol at high concentrations, on the other hand, acts as a prooxidant during the autoxidation of polyunsaturated fatty acids (Cillard *et al.*, 1980; Terao and Matsushita, 1986). This prooxidant effect of α-tocopherol leads to an increase of the level of hydroperoxides with conjugated diene structure. The prooxidant effect of α-tocopherol has been proposed to be induced by the hydrogen abstraction between the tocopheroxyl radical and unsaturated lipids (the reverse of reaction 5; Terao and Matsushita, 1986). Therefore, MeL-OOH accumulated in the reaction mixture containing a high concentration of α-tocopherol (Figures 4B and 5B). Alternatively, there may be other routes by which some of the tocopheroxyl radicals react, *e.g.*, a bimolecular self-reaction (reaction 7). Although reaction 7 is very slow (Burton *et al.*, 1985), dimer and trimer could be formed in a reaction mixture containing a large amount of α-tocopherol (Figure 4B). The second product-forming pathway yields epoxyhydroperoxy-α-tocopherones (epoxy-TOOH) by peroxyl radical dependent epoxidation of the tocopheroxyl radical, followed by oxygen addition to the 8a-position and hydrogen abstraction (Liebler *et al.*, 1990):

$$T^{\bullet} + LOO^{\bullet} \rightarrow \text{epoxy-}T^{\bullet} + LO^{\bullet} \quad (8)$$

$$\text{epoxy-}T^{\bullet} + O_2 \rightarrow \text{epoxy-}TOO^{\bullet} \quad (9)$$

$$\text{epoxy-}TOO^{\bullet} + LH \rightarrow \text{epoxy-}TOOH + L^{\bullet} \quad (10)$$

This pathway can consume α-tocopherol by autoxidation if α-tocopherol acts as the H• donor in reaction 10. Oxidation of α-tocopherol to 4a,5-epoxy-8a-hydroperoxy-α-tocopherones (**1**) therefore results in no net trapping of peroxyl radicals, and α-tocopherol oxidation by this pathway would yield no antioxidant effect. Compound **1** was somewhat stable and accumulated in the all reaction systems (Figures 4 and 5).

All of the alkyl radicals produced are expected to react very rapidly with oxygen (reaction 2). When the oxygen pressure is lowered, on the other hand, the alkyl radicals (L•) produced by reaction 3 can react with α-tocopherol to form 6-*O*-alkyl-α-tocopherols (**3**) (Yamauchi *et al.*, 1993):

$$L^{\bullet} + T^{\bullet} \rightarrow \text{stable products} \quad (11)$$

Reaction 11 would only yield compound **3** with the *Z,E* diene system. The formation of **3a** and **3d** at 37 °C in the 3.0-g sample suggests that reaction 11 occurs at a high concentration of α-tocopherol under air-insufficient conditions. In addition, radical elimination of the peroxyl radicals may result in the formation of L• and

oxygen, and reaction 12, the reverse of reaction 2, can take place during autoxidation (Porter *et al.*, 1981):

$$LOO^{\bullet} \rightarrow L^{\bullet} + O_2 \quad (12)$$

The formation of L• is substantiated by the finding that the isomerization of MeL-OOH is accompanied by the exchange of the oxygen atoms of the hydroperoxy group with atmospheric oxygen (Chan *et al.*, 1978). For the peroxyl radicals of methyl linoleate, the alkyl radical generated by reaction 12 is a resonance-hybrid pentadiene radical. Thus, a total of eight 6-*O*-alkyl-α-tocopherol isomers, the optical (*R* and *S*), geometrical (*Z,E* and *E,E* dienes), and positional (9 and 13) isomers, could appear in the reaction mixture at 60 °C (Figure 3). Moreover, peaks corresponding to methyl linoleate dimers, which have been reported as the anaerobic reaction products of MeL-OOH (Morita and Tokita, 1984), were observed in the air-insufficient conditions at 60 °C (Figure 3). These results suggest that the alkyl radicals trapped by α-tocopheroxyl radicals at high temperature are produced by β-scission of methyl linoleate–peroxyl radicals. The formation of 6-*O*-alkyl-α-tocopherols was observed in the larger sample size of reaction mixture (Figures 4 and 5). Therefore, the supply of oxygen may be dependent on the sample size in the reaction vessel during the autoxidation of unsaturated lipids.

ACKNOWLEDGMENT

We thank A. Umemura for technical assistance.

LITERATURE CITED

Burton, G. W.; Ingold, K. U. Autoxidation of biological molecules. 1. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. *J. Am. Chem. Soc.* **1981**, *103*, 6472–6477.

Burton, G. W.; Ingold, K. U. Vitamin E: Application of the principles of physical organic chemistry to the exploration of its structure and function. *Acc. Chem. Res.* **1986**, *19*, 194–201.

Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasaed, L.; Ingold, K. U. Autoxidation of biological molecules. 4. Maximizing the antioxidant activity of phenols. *J. Am. Chem. Soc.* **1985**, *107*, 7053–7065.

Capella, P.; Zorzut, C. M. Determination of double bond position in monounsaturated fatty acid esters by mass spectrometry of their trimethylsilyloxy derivatives. *Anal. Chem.* **1968**, *40*, 1458–1463.

Chan, H. W.-S. The mechanism of autoxidation. In *Autoxidation of Unsaturated Lipids*; Chan, H. W.-S., Ed.; Academic Press: London, 1987; pp 1–16.

Chan, H, W.-S.; Levett, G.; Matthew, J. A. Thermal isomerisation of methyl linoleate hydroperoxides. Evidence of molecular oxygen as a leaving group in a radical rearrangement. *J. Chem. Soc., Chem. Commun.* **1978**, 756–757.

Cillard, J.; Cillard, P.; Cormier, M; Girre, L. α-Tocopherol prooxidant effect in aqueous media. Increased autoxidation rate of linoleic acid. *J. Am. Oil Chem. Soc.* **1980**, *57*, 252–255.

Csallany, A. S.; Chiu, M.; Draper, H. H. Oxidation products of α-tocopherol formed in autoxidizing methyl linoleate. *Lipids* **1970**, *5*, 63–70.

Frankel, E. N. Lipid oxidation. *Prog. Lipid Res.* **1980**, *19*, 1–22.

Garssen, G. J.; Vliegenthart, J. F. G.; Boldingh, J. The origin and structures of dimeric fatty acids from the anaerobic reaction between soya-bean lipoxygenase, linoleic acid and its hydroperoxide. *Biochem. J.* **1972**, *130*, 435–442.

Gruger, E. H., Jr.; Tappel, A. L. Reactions of biological antioxidants: 1. Fe(III)-catalyzed reactions of lipid hydroperoxides with α-tocopherol. *Lipids* **1970**, *5*, 326–331.

Liebler, D. C.; Baker, P. F.; Kaysen, K. L. Oxidation of vitamin E: Evidence for competing autoxidation and peroxyl radical trapping reactions of the tocopheroxyl radical. *J. Am. Chem. Soc.* **1990**, *112*, 6995–7000.

Liebler, D. C.; Burr, J. A.; Matsumoto, S.; Matsuo, M. Reactions of the vitamin E model compound 2,2,5,7,8-pentamethylchroman-6-ol with peroxyl radicals. *Chem. Res. Toxicol.* **1993**, *6*, 351–355.

Matsuo, M.; Matsumoto, S.; Iitaka, Y.; Niki, E. Radical-scavenging reactions of vitamin E and its model compound, 2,2,5,7,8-pentamethylchroman-6-ol, in a *tert*-butylperoxyl radical-generating system. *J. Am. Chem. Soc.* **1989**, *111*, 7179–7185.

Morita, M.; Tokita, M. Methyl linoleate dimer and methyl 8-phenyloctanoate in the copper-catalyzed decomposition products of methyl linoleate hydroperoxides. *Agric. Biol. Chem.* **1984**, *48*, 2567–2568.

Nelan, D. R.; Robeson, C. D. The oxidation products from α-tocopherol and potassium ferricyanide and its reaction with ascorbic and hydrochloric acids. *J. Am. Chem. Soc.* **1962**, *84*, 2963–2965.

Niki, E.; Saito, T.; Kawakami, A.; Kamiya, Y. Inhibition of oxidation of methyl linoleate in solution by vitamin E and vitamin C. *J. Biol. Chem.* **1984**, *259*, 4177–4182.

Porter, N. A. Mechanisms for the autoxidation of polyunsaturated Lipids. *Acc. Chem. Res.* **1986**, *19*, 262–268.

Porter, N. A.; Lehman, L. S.; Weber, B. A.; Smith, K. J. Unified mechanism of polyunsaturated fatty acid autoxidation. Competition of peroxyl radical hydrogen atom abstraction, β-scission, and cyclization. *J. Am. Chem. Soc.* **1981**, *103*, 6447–6455.

Terao, J.; Matsushita, S. The peroxidizing effect of α-tocopherol on autoxidation of methyl linoleate in bulk phase. *Lipids* **1986**, *21*, 255–260.

Yamauchi, R.; Goto, Y.; Kato, K.; Ueno, Y. Prooxidant effect of dihydroxyacetone and reducing sugars on the autoxidation of methyl linoleate in emulsions. *Agric. Biol. Chem.* **1984**, *48*, 843–848.

Yamauchi, R.; Kato, K.; Ueno, Y. Formation of trimers of α-tocopherol and its model compound, 2,2,5,7,8-pentamethylchroman-6-ol, in autoxidizing methyl linoleate. *Lipids* **1988**, *23*, 779–783.

Yamauchi, R.; Matsui, T.; Kato, K.; Ueno, Y. Reaction of α-tocopherol with 2,2′-azobis(2,4-dimethylvaleronitrile) in benzene. *Agric. Biol. Chem.* **1989**, *53*, 3257–3262.

Yamauchi, R.; Matsui, T.; Kato, K.; Ueno, Y. Reaction products of α-tocopherol with methyl linoleate-peroxyl radicals. *Lipids* **1990**, *25*, 152–158.

Yamauchi, R.; Miyake, N.; Kato, K.; Ueno, Y. Peroxyl-radical reaction of retinyl acetate in solution. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 1529–1532.

Yamauchi, R.; Miyake, N.; Kato, K.; Ueno, Y. Reaction of α-tocopherol with alkyl and alkylperoxyl radicals of methyl linoleate. *Lipids* **1993**, *28*, 201–206.

Winterle, J.; Dulin, D.; Mill, T. Products and stoichiometry of reaction of vitamin E with alkylperoxy radicals. *J. Org. Chem.* **1984**, *49*, 491–495.

Received for review October 21, 1994. Revised manuscript received March 1, 1995. Accepted March 21, 1995.[⊗] This work was supported in part by a grant from the Asahi Breweries Foundation, Tokyo, Japan.

JF940595F

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1995.