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Oxidation Products from the Mixture of α-Tocopherol and γ-Tocopherol during Autoxidation of Methyl Linoleate

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Abstract

The oxidation products from the mixture of α-tocopherol(α-Toc) and γ-tocopherol(γ-Toc) during autoxidation of methyl linoleate were isolated and identified. The structures of the oxidation products were characterized by UV, IR, ^{1}H and ^{13}CNMR and mass spectrometry. 5-[2-(α-tocopherol-5'-yl)ethyl]-α-tocopherylquinone, 5-[2-(α-tocopherol-5'-yl)ethyl]-8a-hydroxy-α-tocopherone and O-[8-(5-ethoxymethyl-7-methyltocol)methyl]-α-tocopherol were obtained from the mixture of α-Toc and γ-Toc as the oxidation products derived from α-Toc. γ-Toc diphenyl ether dimer(γ-TED) and γ-Toc biphenyl dimer(γ-TBD) were identified from the mixture as the oxidation products derived from γ-Toc. However oxidation product composed of both α-Toc and γ-Toc was detected in oxidation products of the mixture. These results support the facts that at first oxidation of α-Toc proceeds during autoxidation of lipids and then γ-Toc decomposes after approximate consumption of α-Toc.

Introduction

Tocopherols(Tocs) included in lipid act as H-atom donor to lipid peroxide radical. They function to break the chain reaction by peroxide radical, at the same time changing themselves to Tocs radical. It is known that tocopheroxy radical is oxidized further to produce tocopherylquinone, dimer or trimer. Tocs dimer is produced by recombination of each Tocs radical and they change to unknown decompositin products[1,2]. As above, Tocs decompose to several oxidation products during lipid autoxidation as a result of its antioxidative action. There have been many reports on the oxidation products of Tocs produced using several oxidants such as chemical oxidants or radical generator[3~8]. As the oxidation products of α-Toc, α-tocopherylquinone[9,10], α-tocopherone[11,12], α-Toc dimer and trimer[3,4,10,13,14], and formyl compound[6] have been known. γ-Tocopherylquinone [10], γ-tocopherone[12], γ-TED and γ-TBD[15,16], and formyl compound[6] are reported as the oxidation products of γ-Toc. Nelan *et al.*[20] obtained a dimer composed of both α-Toc and α-tocopherylquinone by decomposition of α-Toc spirodimer with HCl. Recently Suarna *et al.*[21,22] investigated a new oxidation product, 5-ethoxymethyl-7, 8-dimethyltocol, which was produced by oxidation of α-Toc with t-butyl hydroperoxide in chloroform. Fujitani *et al.* [23] reported α-Toc ethane dimer(α-TED), γ-TED and γ-TBD by the autoxidation of active oxygen method. All of these reports were studied on the oxidation products in the individual system of Tocs. However, little work has been presented on the oxidation products in the mixture of α-Toc and γ-Toc. In previous study[17], the antioxidative activities of equimolar mixtures of α-Toc and γ-Toc were determined in stripped corn oil during

the autoxidation. It was shown that α-Toc was consumed faster than γ-Toc, and γ-Toc were consumed after α-Toc had been exhausted. It is very important to identify the oxidation products derived from Tocs in order to clarify the behavior of each Tocs during the lipid autoxidation and to establish the mechanism of the antioxidative action of Tocs. In this paper, we examined the oxidation products to investigate the antioxidative actions and mechanism in the mixture system of Tocs during the lipid autoxidation.

Materials and Methods

Materials

α-Toc, γ-Toc and δ-Toc having purity more than 99% were kindly donated by Eisai Co.(Tokyo, Japan) and used without further purification. Methyl linoleate(99% purity) purchased from Tokyo Kasei Co.(Tokyo, Japan) was used without further treatment.

Autoxidation and isolation of oxidation products

Both Tocs solutions containing 0.5% α-Toc and γ-Toc in methyl linoleate were oxidized by the active oxygen method(AOM)[24]. Twenty milliliters of each solution were placed in each tube(24×200 mm) of an AOM apparatus(Kuramochi Kagaku Co., Tokyo, Japan), maintained at 97.8±0.1℃. Air was bubbled into the solutions at a constant rate of 2.33mℓ/sec. After autoxidation for 10hrs each solution was saponified and then unsaponifiable matters were fractionated twice by preparative HPLC. The first preparative HPLC was performed with a Shimadzu LC-3A apparatus equipped with a Shimadzu SPD-2A UV detector. A Nucleosil 50-5(8.0×300㎜)column was used, and main elution was performed with Hexane/Diisopropylether(90 : 10, v/v), at flow rate of 1.0mℓ/min. The effluent was monitored at 295nm and collected into each fraction. Both chromatograms of preparative HPLC are shown in Fig. 1. Each fractionated product was further purified by the second preparative HPLC using Hexane/Diisopropylether 98 : 2 and 80 : 20 (v/v) as eluents. And then the purity of these isolated products was confirmed by TLC. Thin-layer plates(thickness, 0.25mm) of Kieselgel HF 254 (Merck) were developed with Hexane/Diethyl ether(90 : 10, v/v). The chromatograms were examined with ultraviolet light and Emmerie-Engels (E-E) reagent.

Spectroscopy

Ultraviolet(UV) spectra were recorded with a Shimadzu UV-210 double-beam spectrophotometer. Infrared(IR) spectra were measured with a Shimadzu IR-435 spectrometer by the thin film method. Proton(^{1}H) and carbon(^{13}C)nuclear magnetic resonance(NMR) spectra were recorded on a JEOL JNM-GX 270FT NMR spectrometer using $CDCl_3$ as the solvent and tetra-methylsilane as an internal standard. Mass spectra were recorded with a JMS-DX 300 mass spectrometer using a direct sample-introducing method at an ionization energy of 70eV.

Preparation of authentic Tocs dimers

α-Toc spirodimer was obtained by the oxidation of α-Toc with alkaline $K_3Fe(CN)_6$[3] and α-Toc ethane dimer was produced by treatment with pyrogallol[18]. γ-TED and γ-TBD were prepared by the oxidation of γ-Toc with p-benzoquinone[19]. The structures of these dimers were confirmed by spectroscopy.

Results

The reaction products in the mixture of α-Toc and γ-Toc in methyl linoleate were analyzed by

preparative HPLC(Fig. 1), and products A1-A9 were observed on the HPLC chromatogram. Products A1 were considered to be methyl linoleate because visualized as blue spots under UV light and were stained purple by E-E reagent. Products with circle mark, from A2 to A5, A8 and A9 were shown as reducing compound on TLC. The other products were shown as the mixtures on TLC. Thus products A2, A3, A4, A5, A8 and A9 were given further isolation and purification based on changing composition ratio of eluents by preparative HPLC. Namely product A2 was purified by Hexane/Diisopropylether 98 : 2. From product A3 to A5, A8 and A9 were purified by Hexane/Diisopropylether 80 : 20. And then the purification of these products was confirmed by TLC. The structures of these products were confirmed from their spectral data and identified as follow :

A2 : MS*m/z* 830(M^+), 603, 416 ; IR(film) ν3550 cm^{-1}(OH) ; UV(hexane)λ 297nm ;
TLC Rf(Hexane : Diethylether 90 : 10) 0.75.

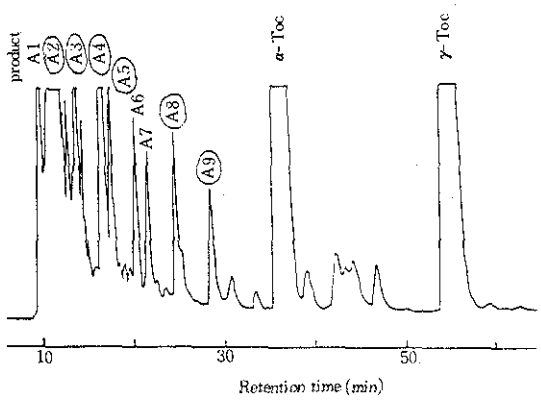

Fig. 1. Preparative HPLC chromatograms of oxidation products from the mixture of α-Toc and γ-Toc.
HPLC was done with Nucleosil 50-5(8.0× 300mm) column developed with Hexane /Diethylether(90 : 10, v/v) at a flow rate of 1.0mℓ/min. The eluent was monitored by an absorbance at 295nm.

Product A2 corresponded spectrometrically with the dimer of γ-Toc. In the ^{1}HNMR spectrum(Fig.

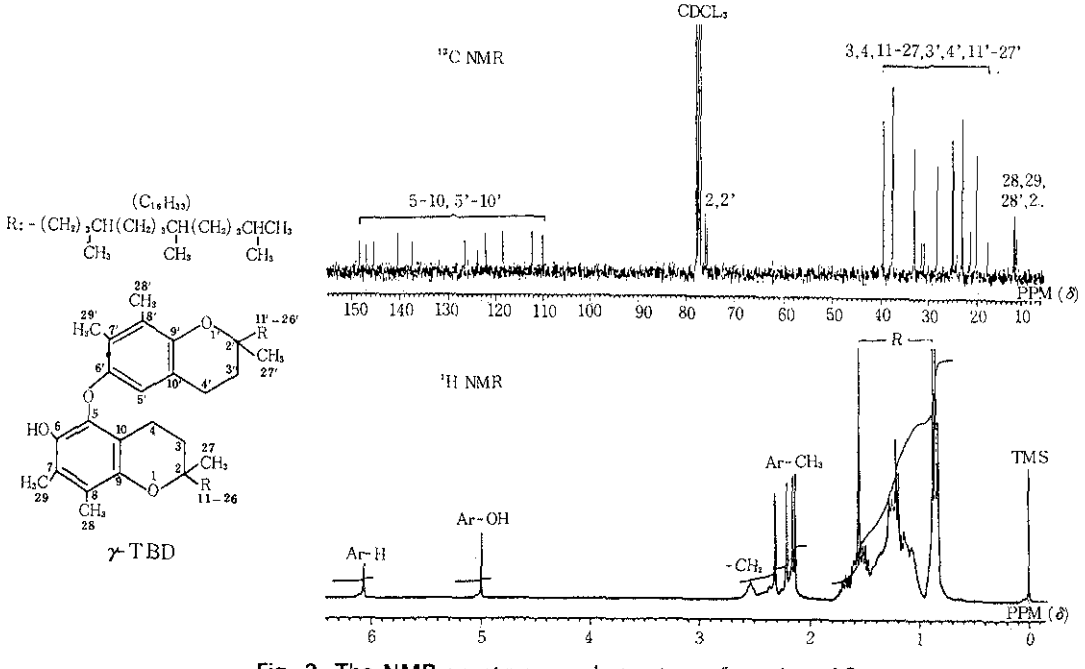

Fig. 2. The NMR spectrums and structure of product A2
(Product A2 is the Chromatogram shown in Fig 1.)

2) singlet peaks due to an aromatic proton(1H, s) and an aromatic OH group(1H, s) were present at 6.06 and 4.99ppm, respectively. Aromatic methyl protons were present at 2.31, 2.20 and 2.15ppm. In the ^{13}CNMR spectrum(Fig. 2) signals due to aromatic carbons were evident in a low magnetic field(148.4ppm, 147.1, 145.5, 140.4, 137.5, 126.5, 123.9, 122.2(d), 118.6, 112.4, 110.2), and 2-2 position carbons were present at 76.0 and 75.5ppm. From these results, product A2 was identified as 5-(γ-tocopheroxy)-γ-tocopherol(γ-Toc diphenyl ether dimer, γ-TED),

A3 : MS m/z 830(M^+) ; IR(film) ν3500cm^{-1} ; UV (hexane) λ304nm ;
TLC Rf(Hexane : Diethylether 90 : 10)0.72.

A4 : MS m/z 830(M^+) ; IR(film) ν3550cm^{-1} ; UV (hexane) λ305nm ;
TLC Rf(Hexane : Diethylether 90 : 10)0.5

In the ^{1}HNMR spectrum(Fig. 3) singlet peaks due to aromatic OH groups(2H, s) were present at 4.40ppm. As the peaks at 2.17 and 2.19ppm due to aromatic methyl protons(6H, s) were singlet, these two substances were considered to have a symmetrical structure. The methylene protons at the 4-position in the chroma ring were shifted to a higher field and overlapped with aromatic methyl protons. This shift also explained the 5,5'-coupling structure owing to the anisotropy of another benzene nucleus. The ^{13}CNMR was consistent with that of authentic γ-Toc. The data of products A3 and A4 were also expected for dimers of γ-Toc (mol. wt. 830 from their mass spectra). In particular, it was possible to establish the geometric relationship between A3 and A4 by interpretation of the spectra. From these results, products A3 and A4 were identified as 5-(γ-tocopherol-5'-yl)-γ-to-

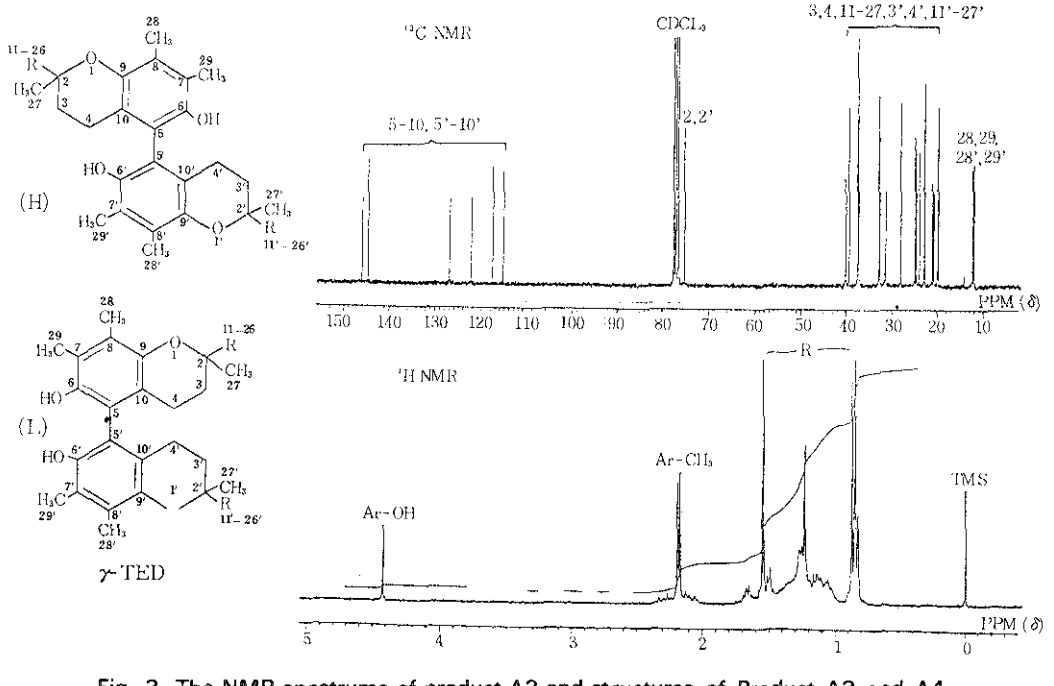

Fig. 3. The NMR spectrums of product A3 and structures of Product A3 and A4.
(Product A3 and A4 are the chromatograms shown in Fig. 1.)
(H) : Product A3 (L) : Product A4
*The NMR spectrums of product A4 was the same with product A3.

copherol(γ-Toc biphenyl dimer, γ-TBD) and were considered to be mutual atropisomers. The spectral data of products A2, A3 and A4 were corresponded with the authentic dimer and the literature value[15,16].

A5 and A9 : MSm/z874(M^+),631, 430 ; IR(film) ν3500cm^{-1}(OH), 1670cm^{+1}(C=O) ; UV(hexane) λ294nm ;
TLC Rf(Hexane : Diethylether 90 : 10)0.4(A5), 0.3(A9) ;
^{1}HNMR($CDCl_3$)δ3.86ppm, (2H,s, OH), 2.15(6H,s,CH_3), 2.08(6H, s, CH_3), 1.98(4H,s,CH_3) ;
^{13}CNMR($CDCl_3$)δ202.8ppm, 153.2, 145.2, 141.8, 128.2, 123.1, 121.9, 115.3, 114.9, 99.3, 79.0, 74.3, 42.8, 40.6, 40.3, 38.9, 37.0(t), 32.2(t), 30.3, 27.5, 26.9, 24.0(t), 23.0, 22.2(d), 19.1(t), 14.1, 11.6, 11.1(d).

Product A5 and A9 corresponded spectrometrically with dimers derived from α-Toc. From the above results, it was proposed that it has dimeric structure and α-tocopheryl quinone(α-TQ) was easily converted to 8a-hydroxy-α-tocopherone(α-TP) as shown in Fig. 4. Thus product A5 was identified as 5-[2-(α-tocopherol-5'-yl)ethyl]-α-tocopheryl quinone(α-Toc-α-TQ dimer) and product A9 was identified as 5-[2-(α-tocopherol-5'-yl) ethyl]-8a-hydroxy-α-tocopherone(α-Toc-α-TP dimer).

A8 : MS m/z 902(M^+), 847, 618, 474, 428 ; IR (film) ν3400cm^{-1}(OH) ; UV(hexane) λ305, 208nm ;
TLC Rf(Hexane : Diethylether 90 : 10)0.38 ;
^{1}HNMR($CDCl_3$)δ7.62ppm, (1H,s,OH), 4.70(2H,s,CH_2-O), 3.61(3),g,CH_3CH_2-O), 2.19(6H,s, CH_3), 2.17(3H,s,CH_3), 2.10(3H,s,CH_3) ;

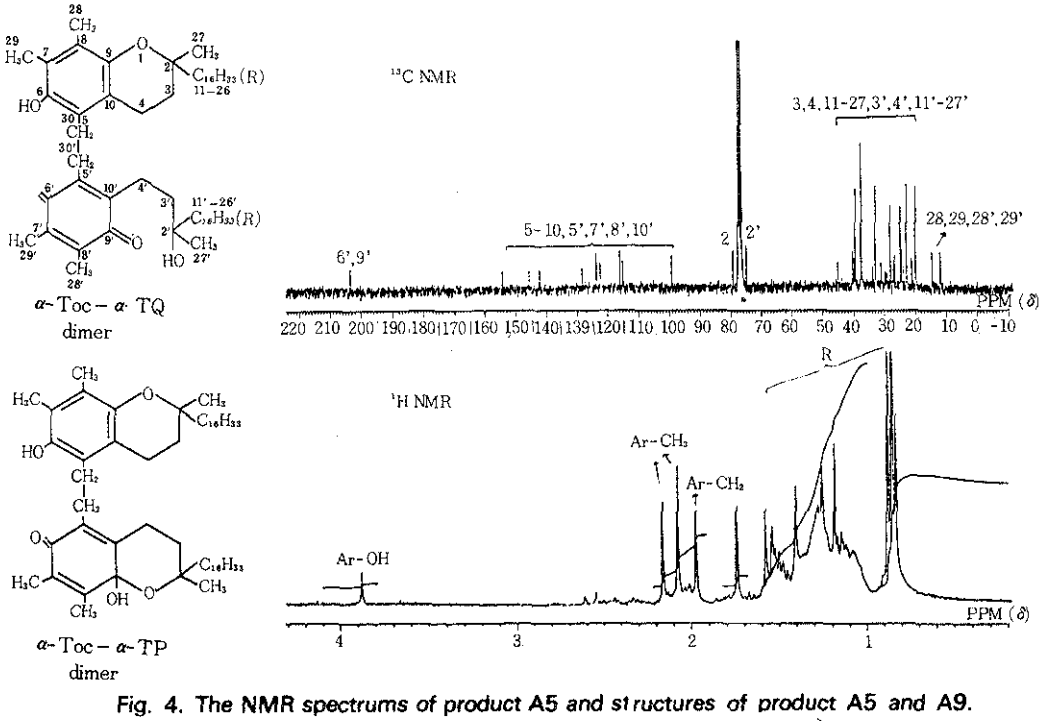

Fig. 4. The NMR spectrums of product A5 and structures of product A5 and A9.
(Product A5 and A9 are the chromatograms shown in Fig. 1.)
α-Toc-α-TQ : Product A5 α-Toc-α-TQ : Product A9
*The NMR spectrum of product A9 was the same with product A5.

^{13}CNMR($CDCl_3$)δ148.3ppm, 145.5, 126.5, 124.8, 117.0, 116.0, 75.3, 68.9, 67.1, 40.6, 40.2, 38.3 (d), 31.8, 30.6, 28.8, 25.7, 25.3, 24.6, 23.6(d), 21.9, 20.8(t), 16.0, 15.0, 12.8, 12.6

Product A8 was proposed new dimeric structure as shown in Fig. 5 and was identified as O-[8-(5-ethoxymethyl-7-methyltocol) methyl]-α-tocopherol(α-Toc-α-ET dimer). It was considered that product A8 was obtained as the saponification of this experiment was done with ethanol.

Discussion

The antioxidative activity of Toc in oils was based on chain breaking of radical reaction according to act as H-atom transfer. Thus, phenoxy and phenyl radicals were produced from Tocs, and successively Tocs dimers were formed by recombination of these radicals. It is considered that dimers produced by oxidation of Tocs have different structures according to the differences in the oxidation method or the reaction conditions adopted. In this study, oxidation products from the mixture of α-Toc and γ-Toc in methyl linoleate under the AOM condition were isolated and identified. The oxidation products in the mixture of α-Toc and γ-Toc were γ-TED, γTBD(H) and γTBD(L). Futhermore, as the oxidation products of α-Toc produced were two dimers : one is composed of α-Toc and αtocopheryl quinone and the other is composed of αToc and 5-ethoxy-methyl-7, 8-dimethyltocol. No dimers were composed of both α-Toc and γ-Toc. From above results, the oxidation mechanism in the mixture system of Tocs during the autoxidation was estimated as shown in Fig. 6. In the mixture of α-Toc and γ-Toc, α-Toc was consumed in the initial stage.

And γ-Toc radical regenerated from γ-Toc by attack of oxygen radical is reduced to γ-Toc by the action of α-Toc. Burton[25,26], Niki[27] and Leh-

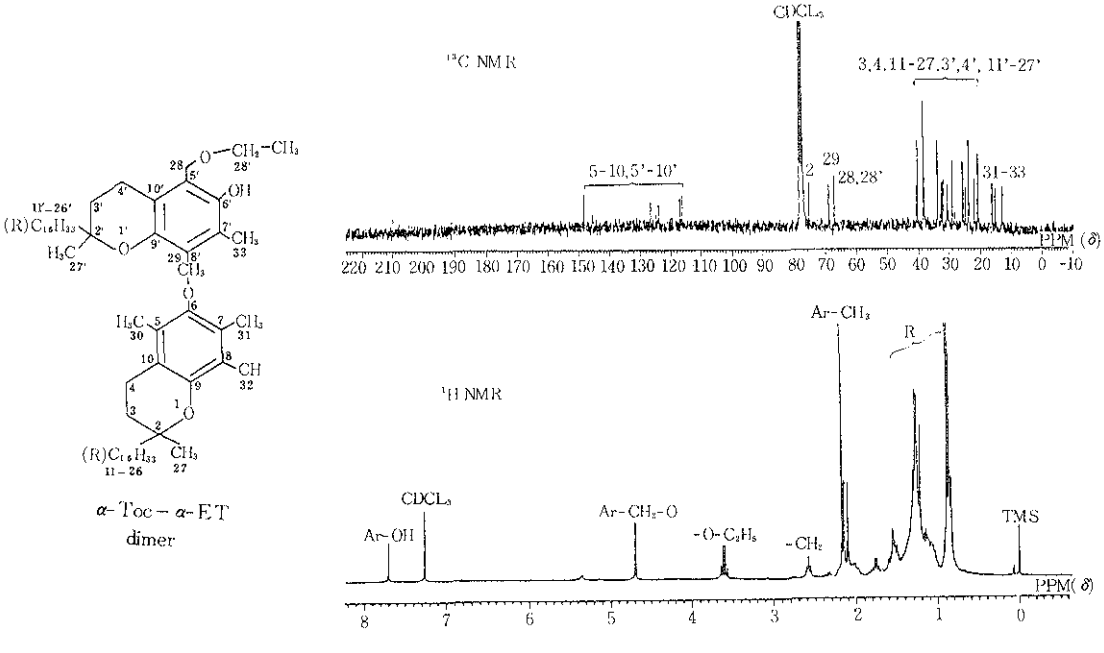

Fig. 5. The NMR spectrums and structure of product A8.
(Product A8 is the chromatogram shown in Fig. 1)

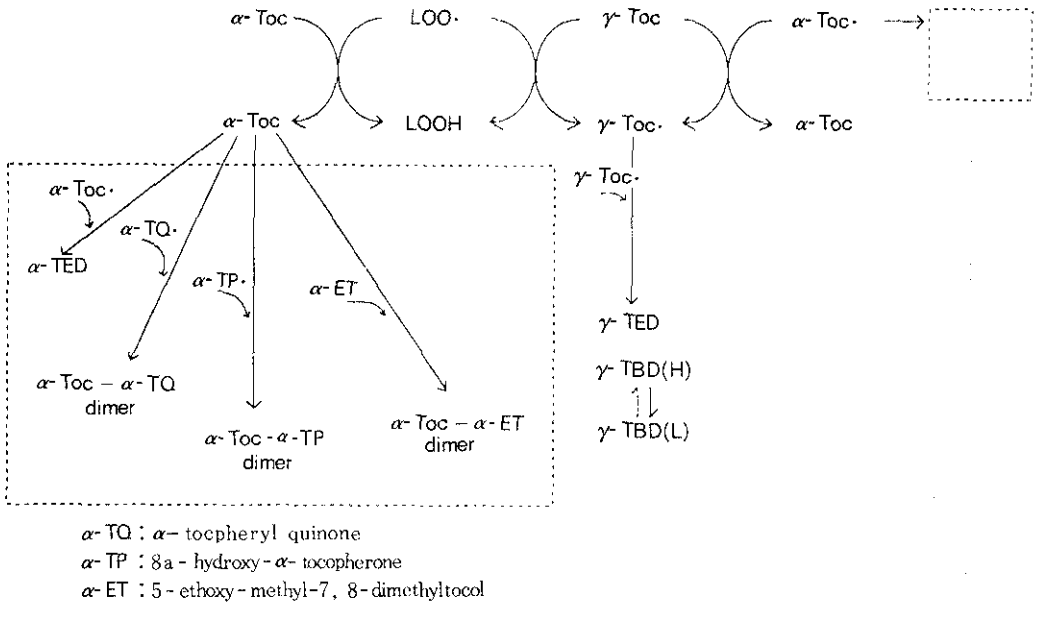

Fig. 6. Possible mechanism of oxidation of Tocs in autoxidizing of methyl linoleate.

mann[28] studied for the mutual action of Tocs also. They have reported that α-Toc was acted as H-atom transfer to γ-Toc radical since α-Toc has a high reactiv toward peroxyl radical[25-27] and effer of protecting γ-Toc. The oxidation products of α-Toc is produced in the earlier stage. Successively, γ-Toc is started to be decomposed after α-Toc is entirely exhausted, and the oxidation products of γ-Toc accumulate. In conclusion, our results indicate that the autoxidation mechanism in the mixture system of Tocs was further confirmed by the identification of oxidation products.

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Methyl Linoleate의 자동산화에 따른 α-Tocopherol과 γ-Tocopherol혼합계의 산화생성물

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요 약

Methyl linoleate에 α-Toc와 γ-Toc을 혼합첨가해서 자동산화시킨 후 그 산화생성물을 검토하였다. 산화물의 同定은 UV, IR, NMR 및 mass spectro에 의해 측정하였다. 생성된 산화물로서 α-Toc로 부터는 5-[2-(α-tocopherol-5'-yl) ethyl]-α-tocopheryl quinone과 5-[2-(α-tocopherol-5'-yl)ethyl-8a-hydroxy-α'-tocopherone, O-[8-(5-ethoxymethyl-7-methyltocol)methyl]-α-tocopherol의 지금까지 보고된 바 없는 새로운 이량체가 검출되었다. 또한 γ-Toc로부터는 γ-Toc biphenyl ether dimer와 γ-Toc diphenyl dimer가 생성되었다. 그러나 α-Toc과 γ-Toc의 양자를 포함하는 산화물은 생성되지 않았다. 본 실험의 산화조건하에서는 α-Toc과 γ-Toc의 산화물로서 이량체만 검출되었다. α-Toc과 γ-Toc의 상호작용의 mechanism이 본 연구의 산화생성물의 同定에 의해 확인되어졌다. 즉, α-Toc과 γ-Toc의 혼합첨가계에서는 먼저 α-Toc의 산화분해가 일어나면서 γ-Toc의 산화가 시작된다. 그러나 γ-Toc에서 생긴 γ-Toc radical는 α-Toc에 의해 γ-Toc로 재생되므로 α-Toc의 산화물이 먼저 생성되고 α-Toc가 소모된 후 γ-Toc의 산화가 일어나서 γ-Toc의 이량체가 생성된다고 볼 수 있다.