February, 1986] Bull. Chem. Soc. Jpn., 59, 497—501 (1986)

Oxidation of Lipids. XIII. Antioxidant Activities of α-, β-, γ-, and δ-Tocopherols

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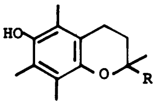
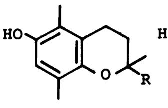
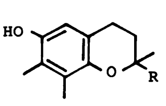
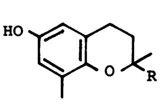
(Received September 18, 1985)

The antioxidant activities of α-, β-, γ-, and δ-tocopherols were studied. They were all effective as antioxidant in the oxidations of methyl linoleate in homogeneous solution. When one of the four tocopherols was used alone, they were consumed at the same rate and they suppressed the oxidation for the same period. However, the stabilities of chromanyloxyl radicals derived from the corresponding tocopherols varied considerably and decreased in the order $\alpha > \beta \cong \gamma > \delta$ -chromanyloxyl radicals. α-Tocopherol reacts quite rapidly with β-, γ-, and δ-chromanyloxyl radicals to give α-chromanyloxyl radical and β-, γ-, and δ-tocopherols respectively. Accordingly, when the four tocopherols were used together, α-tocopherol was consumed first, then β- and γ-tocopherols were consumed, and δ-tocopherol began to decrease after most of α-, β-, and γ-tocopherols were depleted.

The non-enzymatic, random peroxidations of biological molecules and foods by molecular oxygen have received much attention recently in connection with their pathological, toxicological, clinical, and nutritional effects.[1–12] Tocopherols (vitamin E) have been accepted to function as potent antioxidant by scavenging peroxyl radicals and interrupting free radical chain reactions. In a series of our study on the oxidation of lipids and its inhibition, we have studied in this work on the relative antioxidant activities of α-, β-, γ-, and δ-tocopherols.

R: $C_{16}H_{33}$-

α-Tocopherol β-Tocopherol γ-Tocopherol δ-Tocopherol

Experimental

Natural *d*-α-, *d*-β-, *d*-γ-, and *d*-δ-tocopherols provided by Eisai Co. Ltd. were used as received. Commercial methyl linoleate was purified by silica-gel column before use. Dimyristoyl phosphatidylcholine (PC) was used as received from Sigma Chemicals. 2,2′-Azobis(2,4-dimethylvaleronitrile) (AMVN) was used as a radical initiator in order to generate initiating free radicals at a constant and known rate. The rate of chain initiation was determined by the conventional method[13–15] as reported previously[16,17] using 2,6-di-*t*-butyl-4-methylphenol as an antioxidant. Commercial galvinoxyl and 2,2-diphenyl-1-picrylhydrazyl were used as received.

Liposome was prepared as reported previously[18,19] and subjected to sonication with a Branson Sonifier Model 185 to obtain a unilamellar vesicles. The oxidation was carried out at 37 °C under air in a Pyrex glass ampoule immersed in a water bath. The reaction mixture was agitated with a magnetic stirrer. The rate of oxygen uptake was followed with a pressure transducer and the rate of consumption of tocopherols was followed with high performance liquid chromatography at 294 nm. Electron spin resonance (ESR) spectra were recorded on a X-band JEOL FE1X spectrometer at room temperature.

Results and Discussion

The rate of spontaneous oxidation of methyl linoleate was quite small, but the oxidation proceeded smoothly in the presence of AMVN without any noticeable induction period and a constant rate of oxygen uptake was observed. When tocopherol was added to the reaction mixture, the oxidation was suppressed markedly and a clear induction period was observed. Figures 1 and 2 show the results of oxidation of methyl linoleate initiated with AMVN in the presence of α-tocopherol and γ-tocopherol respectively. The dotted line in Fig. 1 shows the oxygen uptake in the absence of tocopherol. Figures 1 and 2 show that α-tocopherol and γ-tocopherol were consumed linearly with time and when all of tocopherols were depleted, the induction period was over and a fast oxidation took place. It is noteworthy that the rate of oxidation after the induction period is similar to that in the absence of tocopherol. Similar results

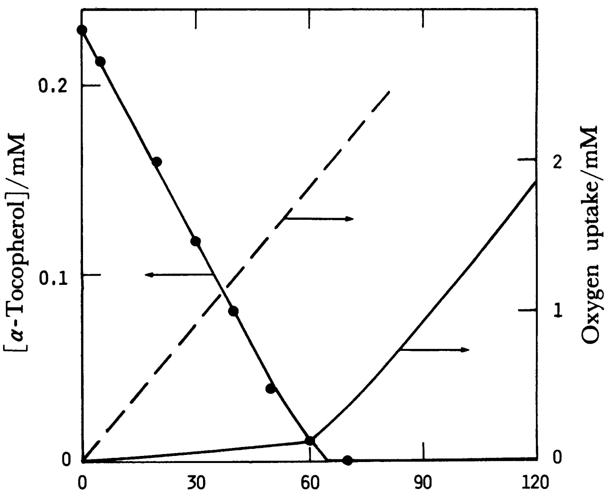


Fig. 1. Rates of oxygen uptake and α-tocopherol decay in the oxidation of 144 mM methyl linoleate in benzene under air at 37 °C initiated with 30.0 mM AMVN. The dotted line show the oxygen uptake in the absence of α-tocopherol.

Table 1. Inhibition of Oxidation of Methyl Linoleate (LH) by Tocopherols (TOC) in Benzene at 37 °C Under Air

[LH] / mM	[AMVN] / mM	TOC_1 / mM	TOC_2 / mM	t_{inh} / s	R_p / nM/s	t_{inh}[AMVN] / [TOC]	$10^5 R_p$ / [LH][AMVN]$^{1/2}$
144	19.3	0		0	790		3.95
144	30.0	α 0.230		3680	950	4.80×10^5	3.81
137	28.8	β 0.262		4510	868	4.96×10^5	3.73
137	28.9	γ 0.234		3820	870	4.72×10^5	3.74
137	28.9	δ 0.271		4820	837	5.14×10^5	3.60
144	30.3	α 0.230	β 0.274	8170	1130	4.91×10^5	4.51
144	30.4	α 0.230	γ 0.245	7530	914	4.82×10^5	3.64
144	30.5	α 0.230	δ 0.284	8700	918	5.16×10^5	3.65
144	30.4	γ 0.245	δ 0.284	9190	899	5.28×10^5	3.58
148	31.1	β 0.282	γ 0.176	7430	1040	5.05×10^5	3.98
140	29.6	β 0.300	γ 0.328	11120	915	5.24×10^5	3.80
144	30.4	β 0.307	δ 0.307	10220	823	5.06×10^5	3.28

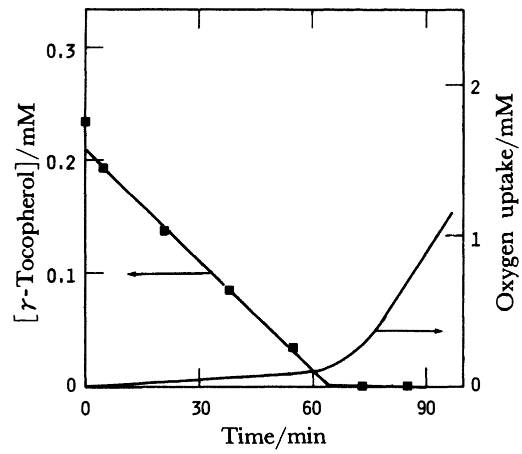


Fig. 2. Rates of oxygen uptake and γ-tocopherol disappearance in the oxidation of 137 mM methyl linoleate in benzene under air at 37 °C initiated with 28.9 mM AMVN.

have been observed for β-and δ-tocopherols. The pertinent results are summarized in Table 1.

The oxidation of methyl linoleate (LH) initiated with AMVN (A–N=N–A) and inhibited by tocopherol (IH) proceeds by the following reaction mechanisms,[14,16–18,20]

$$\text{A–N=N–A} \longrightarrow [\text{A}\cdot\text{N}_2\cdot\text{A}] \longrightarrow (1-e)\text{A–A} \quad (1)$$

$$\searrow 2e\text{A}\cdot, \quad (2)$$

$$\text{A}\cdot + \text{O}_2 \longrightarrow \text{AO}_2\cdot, \quad (3)$$

$$\text{AO}_2\cdot + \text{LH} \xrightarrow{\text{O}_2} \text{AOOH} + \text{LO}_2\cdot, \quad (4)$$

Propagation:

$$\text{LO}_2\cdot + \text{LH} \xrightarrow{k_p} \text{LOOH} + \text{L}\cdot, \quad (5)$$

$$\text{L}\cdot + \text{O}_2 \longrightarrow \text{LO}_2\cdot \quad (6)$$

Termination;

$$\text{LO}_2\cdot + \text{IH} \xrightarrow{k_{inh}} \text{LOOH} + \text{I}\cdot, \quad (7)$$

$$(n-1)\text{LO}_2\cdot + \text{I}\cdot \longrightarrow \text{stable products}, \quad (8)$$

$$\text{LO}_2\cdot + \text{LO}_2\cdot \xrightarrow{k_t} \text{stable products}, \quad (9)$$

where e is the efficiency of free radical generation from AMVN, n is the stoichiometric number of peroxyl radicals trapped by each antioxidant, and k_p, k_{inh}, and k_t are the rate constants for reactions 5, 7, and 9 respectively.

The rate of chain initiation, R_i, is given by Eq. 10,

$$R_i = 2ek_d[\text{AMVN}], \quad (10)$$

where k_d is the rate constant for unimolecular decomposition of AMVN. The induction period, t_{inh}, is given by Eq. 11.

$$t_{inh} = n[\text{IH}]/R_i = n[\text{IH}]/2ek_d[\text{AMVN}] \quad (11)$$

Table 1 also shows the results of oxidation of methyl linoleate inhibited by the combination of two types of tocopherols. Figure 3 shows the plot of induction period as a function of [IH]/R_i. Table 1 and Fig. 3 show that the induction period is proportional to the total concentration of tocopherols independent of their types and inversrly proportional to the rate of chain initiation or the concentration of AMVN as expected from Eq. 11.[21]

The rate of oxidation in the absence of antioxidant or after the induction period is given by Eq. 12.

$$R_p = k_p[\text{LH}](R_i/2k_t)^{1/2} \quad (12)$$

The data in the last column of Table 1 show that Eq. 12 holds for all tocopherols.

Figure 4 shows the rates of disappearance of α-tocopherol and γ-tocopherol during the oxidation of methyl linoleate in benzene in the presence of both α- and γ-tocopherols. Figure 5 shows the similar results

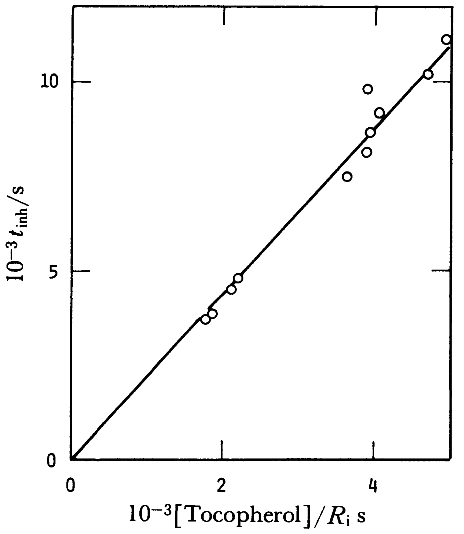


Fig. 3. Plot of induction period as a function of [tocopherol]/R_i for the oxidations of methyl linoleate in benzene under air at 37 °C initiated with AMVN.

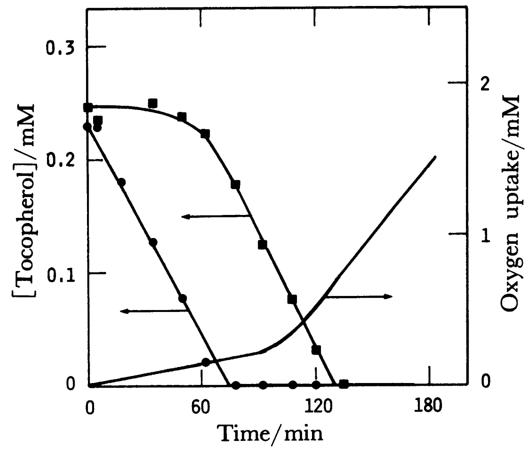


Fig. 4. Rates of oxygen uptake and disappearance of α-(●) and γ-(■) tocopherols in the oxidation of 144 mM methyl linoleate in benzene under air at 37 °C initiated with 30.4 mM AMVN.

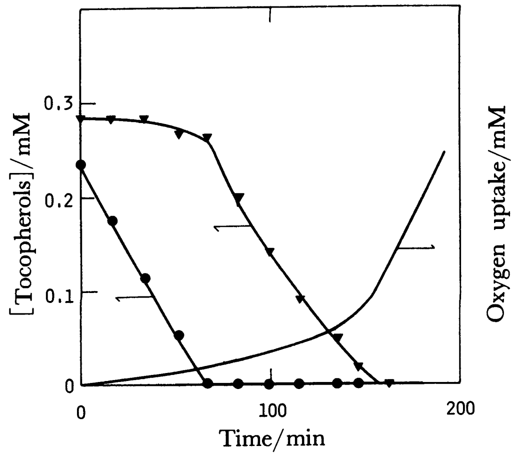


Fig. 5. Oxygen uptake and disappearance of α-(●) and δ-(▼) tocopherols during the oxidation of 144 mM methyl linoleate in benzene initiated with 30.5 mM AMVN inhibited by 0.230 mM α-tocopherol and 0.284 mM δ-tocopherol.

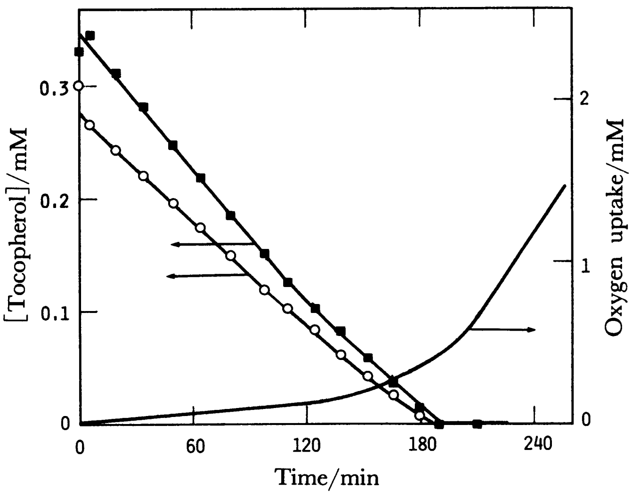


Fig. 6. Rates of oxygen uptake and β-(○) and γ-(■) tocopherols disappearance in the oxidation of 140 mM methyl linoleate in benzene under air at 37 °C initiated with 29.6 mM AMVN.

observed in the oxidation of methyl linoleate inhibited by both α- and δ-tocopherols. These results show that only α-tocopherol disappeared first linearly while γ- or δ-tocopherol remained almost unchanged at first and then they disappeared after α-tocopherol was depleted. When all tocopherols disappeared, the induction period was over and a fast oxidation took place. On the other hand, Fig. 6 shows that β- and γ-tocopherols disappeared at a similar rate when both of them were present together.

Figure 7 shows the rate of disappearance of four tocopherols during the oxidation of methyl linoleate in benzene. It can be seen that α-tocopherol disappeared first, then β- and γ-tocopherols disappeared at a similar rate, and finally δ-tocopherol followed. Interestingly, β- and γ-tocopherols were not consumed when appreciable amount of α-tocopherol was present, while most of δ-tocopherol remained until α-, β-, and γ-tocopherols were depleted.

The above results suggest that four tocopherols behave similarly as antioxidant when only one of them is present alone but also that the stabilities of tocopherols and/or chromanyloxyl radicals derived therefrom differ considerably.

The stabilities of chromanyloxyl radicals were studied by ESR. It has been observed that each tocopherol has its own specific ESR spectrum.[23–26] Chromanyloxyl radicals were generated as previously reported[26,27] by the interaction of tocopherol with galvinoxyl radical. The ESR spectrum of galvinoxyl radical disappeared instantaneously when it was reacted with α-tocopherol, and α-chromanyloxyl

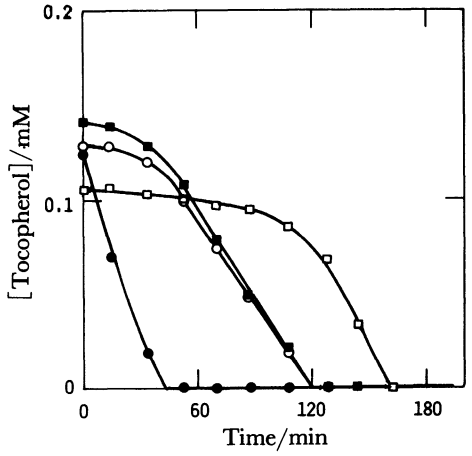


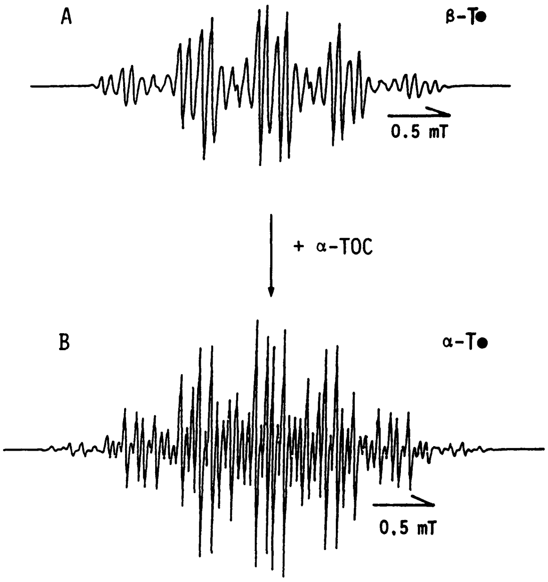
Fig. 7. Rates of disappearance of tocopherols during the oxidation of 144 mM methyl linoleate in benzene under air at 37 °C initiated with 30.1 mM AMVN. Initial concentrations of tocopherols are: α(●)=0.124 mM, β(○)=0.130 mM, γ(■)=0.141 mM, γ(□)=0.105 mM.

radical was observed. β-, γ-, and δ-Tocopherols also reacted with galvinoxyl similarly. The rates of interactions between galvinoxyl and tocopherols in homogeneous solution were too fast to follow by a conventional method.

The rate of interactions between galvinoxyl radical incorporated into dimyristoyl PC liposomes and tocopherol incorporated into different dimyristoyl PC liposomes was followed by ESR. Galvinoxyl radical disappeared much more slowly than in homogeneous system and the pseudo first order rate constant was obtained as 10.1×10^{-4}, 5.44×10^{-4}, 5.00×10^{-4}, and 4.76×10^{-4} s^{-1} at 30 °C for α-, β-, γ-, and δ-tocopherols respectively. Burton and Ingold[14] measured the reactivities of four tocopherols toward polystyrenylperoxyl radical and observed that the reactivities decreased in the same order of $\alpha>\beta\cong\gamma>\delta$ -tocopherols.

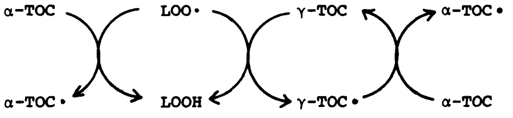
The interactions of tocopherol with chromanyloxyl radicals were also studied. The chromanyloxyl radical was prepared by the interaction of the corresponding tocopherol with 2,2-dipheyl-1-picrylhydrazyl. When α-tocopherol was prepared at first and then β-, γ-, or δ-tocopherol was added, no change in ESR spectrum was observed. On the other hand, when β-chromanyloxyl radical was first prepared and then α-tocopherol was added to the solution, β-chromanyloxyl radical disappeared quite rapidly and it was replaced by α-chromanyloxyl radical as shown in Fig. 8. Similar results were observed for the interactions of γ- and δ-chromanyloxyl radicals with α-tocopherol.

The above results suggest that both the reactivities of tocopherols toward peroxyl and phenoxyl radicals and stabilities of chromanyloxyl radicals decrease in the order of $\alpha>\beta\cong\gamma>\delta$. When, for example, both α- and γ-tocopherols are present, α-tocopherol scavenges the peroxyl radical predominantly, while γ-tocopherol scavenges peroxyl radicals more slowly but γ-chromanyloxyl radical formed by the interaction of γ-tocopherol with peroxyl radical must react with α-tocopherol rapidly to give γ-tocopherol and α-chromanyloxyl radical. Accordingly, only α-tocopherol must be consumed at first, while γ-tocopherol remains almost unchanged.



$$\beta\text{-T}\bullet + \alpha\text{-TOC} \longrightarrow \beta\text{-TOC} + \alpha\text{-T}\bullet$$

Fig. 8. Change of ESR spectrum observed when β-chromanyloxyl radical was interacted with α-tocopherol.
A: ESR spectrum of β-chromanyloxyl radical, B: ESR spectrum observed when α-tocopherol was added to the solution containing β-chromanyloxyl radical.



This study was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.

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21) It may be noteworthy that the stoichiometric numbers *n* for the hindered phenols with alkyl substituents on both ortho positions are close to 2, whereas those for non-hindered phenols are smaller than 2.[22] The results of Table 1 and Fig. 3 suggest that the values of *n* for α-, β-, γ-, and δ-tocopherols are same within experimental error under the present reaction conditions. It has been found previously[17] that α-tocopherol and 2,6-di-*t*-butyl-4-methylphenol have same *n* values. These results may be attributed to the high concentration of peroxyl radicals in the present oxidation initiated with AMVN. If the concentration of peroxyl radical is smaller and the chromanoxyl radical has longer lifetime, the *n* values for β-, γ-, and δ-tocopherols may be smaller than that for α-tocopherol.

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