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Oxidation of Lipids. XIV. Inhibition of Oxidation of Methyl Linoleate by Fatty Acid Esters of L-Ascorbic Acid

Mareyuki TAKAHASHI, Etsuo NIKI,* Akira KAWAKAMI, Akira KUMASAKA, Yorihiro YAMAMOTO, Yoshio KAMIYA, and Kunihiko TANAKA†

Department of Reaction Chemistry, Faculty of Engineering, The University of Tokyo, Hongo, Tokyo 113
†Sumitomo Chemical Co., Ltd., 3-1-98 Kasugade-naka, Konohana-ku, Osaka 554

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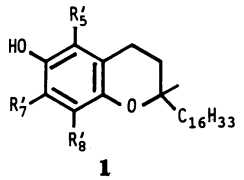
Various kinds of fatty acid esters of L-ascorbic acid were synthesized and their role as chain-breaking antioxidant was studied in the oxidation of methyl linoleate in homogeneous solution. Fatty acid esters of ascorbic acid at either 6- or both 5- and 6-positions were effective as antioxidant and suppressed the oxidation of methyl linoleate, whereas those having ester group at 2-position did not act as antioxidant. 5,6-Erythorbyl dipalmitate was also effective as an antioxidant. Ascorbic acid esters inhibited the oxidation cooperatively with α- and δ-tocopherols as observed for free ascorbic acid. The antioxidant activity was correlated with the reactivity toward galvinoxyl radical.

The oxidations of lipids by molecular oxygen in biological systems and foods have received much attention recently in connection with their pathological, toxicological, and nutritional effects.[1-12] Tocopherols **1** (vitamin E)[13-16] and L-ascorbic acid **2** (vitamin C)[17-27] have been known to function as oil-soluble and water-soluble antioxidants, respectively,both in vivo and in vitro. In spite of much work on ascorbic acid and also the commercial production in large quantities, the chemistry of ascorbic acid has not been established in a manner that is scientifically satisfactory. The chemistry of ascorbic acid is in fact complicated partly because ascorbic acid can function as a radical scavenger, but it may also act as a prooxidant in combination with metal ions such as iron and copper.[28]



α-Tocopherol: $R_5'=R_7'=R_8'=CH_3$

δ-Tocopherol: $R_5'=R_7'=H$, $R_8'=CH_3$

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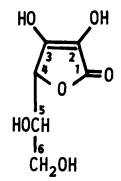

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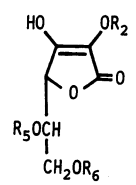

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We have extended our study on the oxidation of lipids and its inhibition to study the role of ascorbic acid esters as chain breaking antioxidant in the oxidation of methyl linoleate in homogeneous solution. Various fatty acid esters of L-ascorbic acid **3** with different carbon numbers and positions have been prepared and examined. Erythorbic acid (D-isoascorbic acid) ester was also prepared and studied. Methyl linoleate was chosen as a substrate, since its oxidation has been shown to give four conjugated diene hydroperoxides quantitatively by a straight-forward mechanism.[29-31]

Table 1. Ascorbic Acid Esters **3** Used in This Study

Abbreviation	R_2	R_5	R_6
AsA	H	H	H
5,6-C_3-AsA	H	COC_2H_5	COC_2H_5
5,6-C_8-AsA	H	COC_7H_{15}	COC_7H_{15}
2,5,6-C_8-AsA	COC_7H_{15}	COC_7H_{15}	COC_7H_{15}
5,6-C_{14}-AsA	H	$COC_{13}H_{27}$	$COC_{13}H_{27}$
6-C_{16}-AsA	H	H	$COC_{15}H_{31}$
2,6-C_{16}-AsA	$COC_{15}H_{31}$	H	$COC_{15}H_{31}$
5,6-C_{16}-AsA	H	$COC_{15}H_{31}$	$COC_{15}H_{31}$
2,5,6-C_{16}-AsA	$COC_{15}H_{31}$	$COC_{15}H_{31}$	$COC_{15}H_{31}$
6-C_{18}-AsA	H	H	$COC_{17}H_{35}$
5,6-C_{18}-AsA	H	$COC_{17}H_{35}$	$COC_{17}H_{35}$
5,6-C_{22}-AsA	H	$COC_{21}H_{43}$	$COC_{21}H_{43}$
5,6-C_{16}-EA[a]	H	$COC_{15}H_{31}$	$COC_{15}H_{31}$

a) 5,6-Erythorbyl dipalmitate.

Experimental

Commercial methyl linoleate was purified by silica-gel column before use. Natural d-α-tocopherol and d-δ-tocopherol were provided by Eisai Co., Ltd. Various kinds of fatty acid esters of L-ascorbic acid were synthesized by the esterification of ascorbic acid at 2-, 5-, and 6-positions with corresponding fatty acids, followed by deacylation at 2-position in alcohol. Table 1 shows the ascorbic acid esters used in this study. 5,6-Erythorbyl dipalmitate was also synthesized similarly and studied. 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. Commercial galvinoxyl was used as received.

The oxidation was carried out at 37 °C under air in a glass ampoule immersed in a water bath. The rate of oxidation was measured in an automatic recording gas absorption apparatus by following the rate of oxygen uptake using a pressure transducer. The rate of free radical generation, that is, the rate of chain initiation was measured by conventional method from the induction period observed by the addition of a chain breaking antioxidant.[16] 2,6-Di-*t*-butyl-4-methylphenol (BMP) or α-tocopherol was used as the antioxidant. Commercial BMP was recrystallized from methanol before use. The rate of consumption of tocopherols was followed at 294 nm with a high-performance liquid chromatography (HPLC) by directly injecting 50 μl aliquots of the reaction mixture. The analytical system consisted of

JASCO liquid chromatograph equipped with a Finepak SIL-CN, 4.6×250 mm column, and samples were eluted with hexane–isopropyl alcohol–acetic acid (1000:15:1, v/v/v) at a flow rate of 1 ml min^{-1}. The rate of disappearance of ascorbic acid ersters was also measured with HPLC at 245 nm using 4.6×250 mm Finepak SIL-C_{18-5} column and metanol–water (85/15, v/v) containing 0.05% formic acid as an eluent (1 ml min^{-1}). The electron spin resonance (ESR) spectrum was recorded on X-band JEOL FE1X spectrometer.

Results and Discussion

Inhibition of Oxidation of Methyl Linoleate by Ascorbic Acid Esters. The rate of spontaneous autoxidation of methyl linoleate at ambient temperature is quite small, but it is oxidized at a constant rate without induction period in the presence of radical initiator AMVN. As shown in Fig. 1, when the fatty acid ester of ascorbic acid at either 6- or both 5-and 6-positions is added to the solution, the rate of oxidation was markedly suppressed and produced a clear induction period. During this induction period, the ascorbic acid ester was consumed linearly with time as shown in Fig. 2 and when it was depleted, the rate of oxidation increased to the same level as that without ascorbic acid ester. This is quite similar to the oxidation in the presence of α-tocoperhol and it clearly shows that ascorbic acid ester functions as a chain breaking antioxidant.

On the other hand, the ascorbic acid esters having ester group at 2-position did not show any antioxidant activity. 5,6-Erythorbyl dipalmitate was also effective in suppressing the oxidation.

Inhibition of Oxidation of Methyl Linoleate in Solution by the Combination of Ascorbic Acid Ester and Tocopherol. The pertinent results of the oxidations of methyl linoleate in chloroform initiated with AMVN and inhibited by both α-tocopherol and ascorbic acid ester are shown in Table 2. From the result of oxidation inhibited by α-tocopherol alone the constant ek_d was calculated as 2.87×10^{-6} s^{-1},

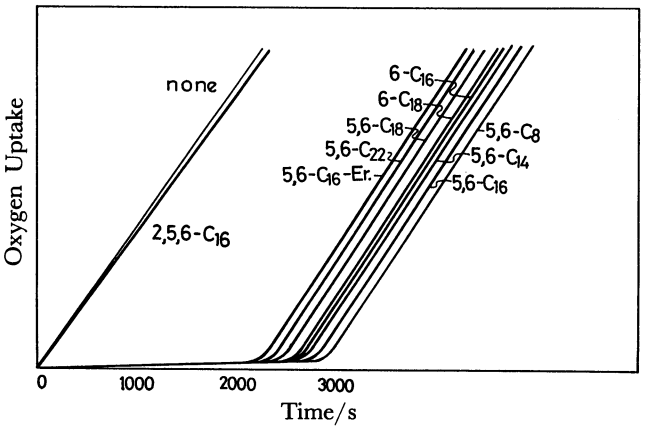


Fig. 1. Inhibition of oxidation of 145 mM methyl linoleate in solution by fatty acid esters of ascorbic acid at 37°C under air initiated with AMVN.

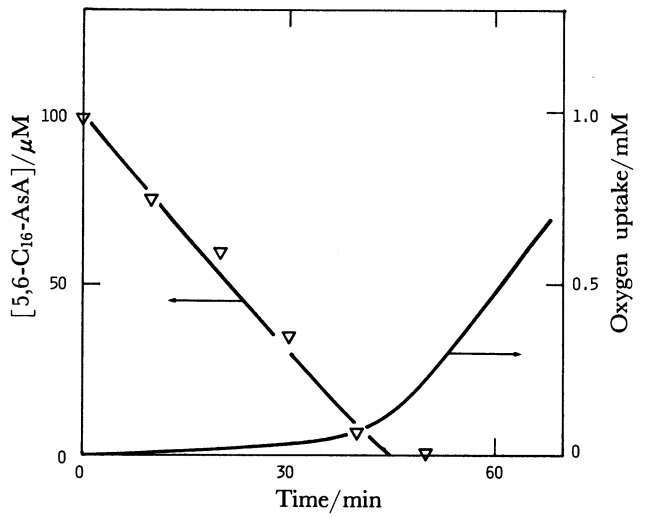


Fig. 2. Rates of oxygen uptake and consumption of 5,6-ascorbyl distearate in the oxidation of 159 mM methyl linoleate in benzene initiated with 10.1 mM AMVN at 37°C under air.

Table 2. Oxidation of Methyl Linoleate (LH) in Chloroform at 37°C Inhibited by α-Tocopherol and Ascorbic Acid Esters

LH	AMVN	α-Toc	AsAR		10^6R_i[a]	t_{inh}	n[b]	10^6R_p[c]	kcl[d]	$k_p/(2k_t)^{1/2}$[e]
mM	mM	μM	mM		M/s	s		M/s		$(Ms)^{-1/2}$
144	19.4	0			0.111	0		1.02	9.0	0.020
141	19.5	76.5			0.112	1365	2.0[f]	0.92	8.2	0.019
119	16.1	64.4	2,5,6-C_8	0.290	0.0924	1500	2.15	0.74	8.0	0.020
119	15.8	64.4	2,5,6-C_8	0.301	0.0907	1275	1.80	0.66	7.3	0.018
119	15.9	64.4	2,6-C_{16}	0.259	0.0913	1425	2.02	0.71	7.8	0.020
119	16.0	64.4	2,5,6-C_{16}	0.179	0.0918	1470	2.10	0.76	8.3	0.021
119	16.3	64.4	2,5,6-C_{16}	0.181	0.0936	1485	2.16	0.70	7.5	0.019
119	16.0	64.4	5,6-C_3	0.574	0.0918	>8500		n.d.[g]		
119	16.1	64.4	5,6-C_{16}	0.244	0.0924	5100	7.32	0.71	7.7	0.020
119	15.9	64.4	6-C_{18}	0.363	0.0913	7515	10.7	0.69	7.6	0.019

a) Rate of chain initiation calculated from Eq. 2. b) Apparent stoichiometric number for α-tocopherol calculated from Eq. 5. c) Rate of oxygen uptake after the induction period. d) Kinetic chain length, R_p/R_i. e) Oxidizability calculated from Eq. 7. f) Assumed. g) Not determined.

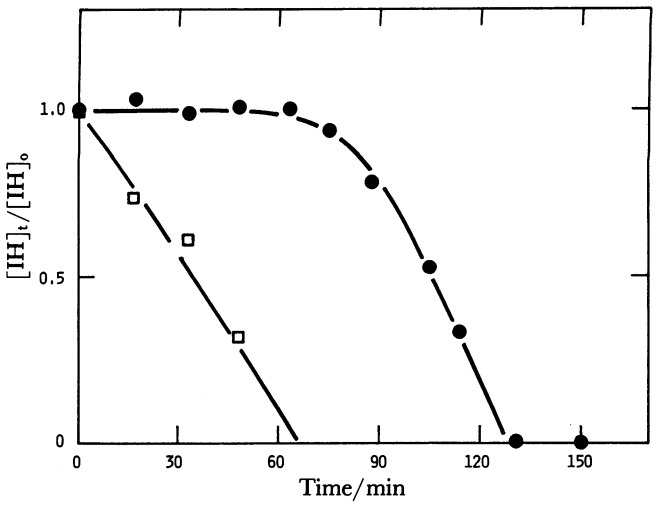


Fig. 3. Rate of consumption of antioxidant (IH) during the oxidation of 135 mM methyl linolate at 37°C. [AMVN]=18.1 mM, [α-tocoherol (●)]=50 mM, [5,6-C_{18}-AsA (□)]=500 mM.

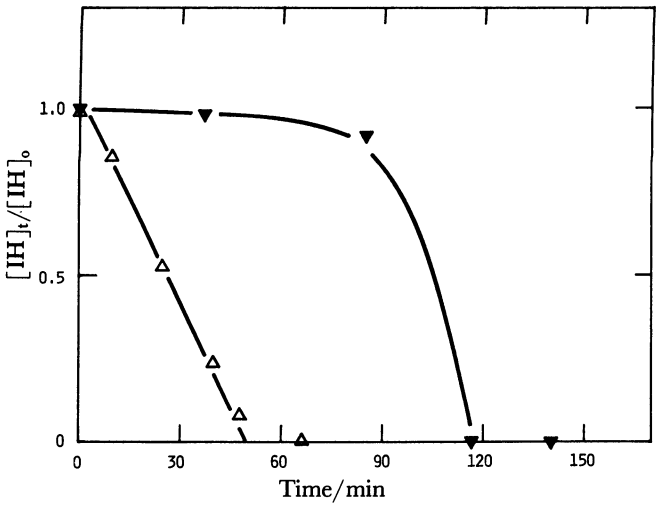


Fig. 4. Rate of consumption of antioxidant (IH) during the oxidation of 146 mM methyl linoleate under air at 37°C. [AMVN]=19.5 mM, [δ-tocopherol (▼)]=50 mM, [6-C_{18}-AsA (△)]=500 mM.

where A is $(CH_3)_2CHCH_2C(CH_3)CN-$ and e and k_d are the efficiency of free radical production and first-order rate constant for the decomposition of AMVN, respectively. The rate of chain initiation in other runs was calculated from Eq. 2 (M=mol dm^{-3}).

$$A\text{-}N{=}N\text{-}A \longrightarrow [A\cdot\ N_2\cdot\ A] \begin{cases} \nearrow 2eA\cdot & (1) \\ \searrow (1-e)A-A & (1') \end{cases}$$

$$R_i = 2ek_d[AMVN] = 5.74 \times 10^{-6}[AMVN]\ M\ s^{-1} \quad (2)$$

Both tocopherol and ascorbic acid esters (IH) scavenge peroxyl radicals by donating hydrogen atom (Eq. 3). The resulting radical, I·, may further trap peroxyl radicals (Eq. 4). The stoichiometric number of peroxyl radicals trapped by each antioxidant, n, is calculated from Eq. 5.

$$LO_2\cdot + IH \longrightarrow LOOH + I\cdot \quad (3)$$

$$(n-1)LO_2\cdot + I\cdot \longrightarrow \text{stable products} \quad (4)$$

$$n = R_i\cdot t_{inh}/[IH] \quad (5)$$

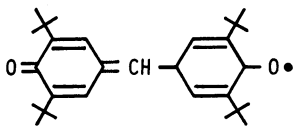
As Table 2 shows, the value of n in the absence of ascorbic acid is close to 2.0. That the apparent n values calculated from Eq. 5 are close to 2 in the presence of ascorbic acid esters which have ester group at 2-position suggests that these ascorbic acid esters do not function as radical scavenger. On the other hand, the apparent n values in the presence of 5,6-ascorbyl dipropionate, 5,6-ascorbyl dipalmitate, and 6-ascorbyl stearate are much higher than 2, suggesting that these ascorbic acid esters also function as hydrogen donor.

Figures 3 and 4 show that rate of consumption of tocopherol and ascorbic acid ester during the oxidation. Figure 3 shows that 5,6-ascorbyl distearate disappeared first linearly whereas α-tocopherol remained almost constant and it began to decrease after ascorbic acid ester was depleted. Similarly, as shown in Fig. 4, ascorbic acid ester was consumed first and δ-tocopherol was consumed after the ascorbic acid ester was depleted in the combination of δ-tocopherol and 6-ascorbyl stearate. Tocopherol (TH) must scavenge peroxyl radicals more rapidly than ascorbic acid ester (AH),[26] but the tocopheryl radical (T·) formed must react with ascorbic acid ester rapidly to regenerate tocopherol as observed in the combination of tocopherol and free ascorbic acid.[26,33,34]

$$LO_2\cdot \begin{cases} \xrightarrow{TH} LOOH + T\cdot & (6) \\ \xrightarrow{AH} LOOH + A\cdot & (7) \end{cases}$$

$$T^{\cdot} + AH \longrightarrow TH + A\cdot \quad (8)$$

The ESR study supports the above conclusion. Galvinoxyl **4** is a stable phenoxyl radical, but it reacts with active hydrogens. For example, galvinoxyl reacts with good hydrogen donors such as tocopherols, ascorbic acid, cysteine, and glutathione.[35] As Fig. 5 shows, the ESR spectrum of galvinoxyl changed instantaneously to that of α-tocopheryloxyl radical when α-tocopherol was added to the benzene solution of galvinoxyl at 25°C. When 5,6-ascorbyl dipalmitate was added to the above solution, the ESR spectrum of α-tocopheryloxyl radical (Fig. 5, B) disappeared rapidly. On the other hand, when 2,5,6-ascorbyl tripalmitate was added instead of 5,6-ascorbyl dipalmitate, no change was observed for the ESR spectrum of α-tocopheryloxyl radical.



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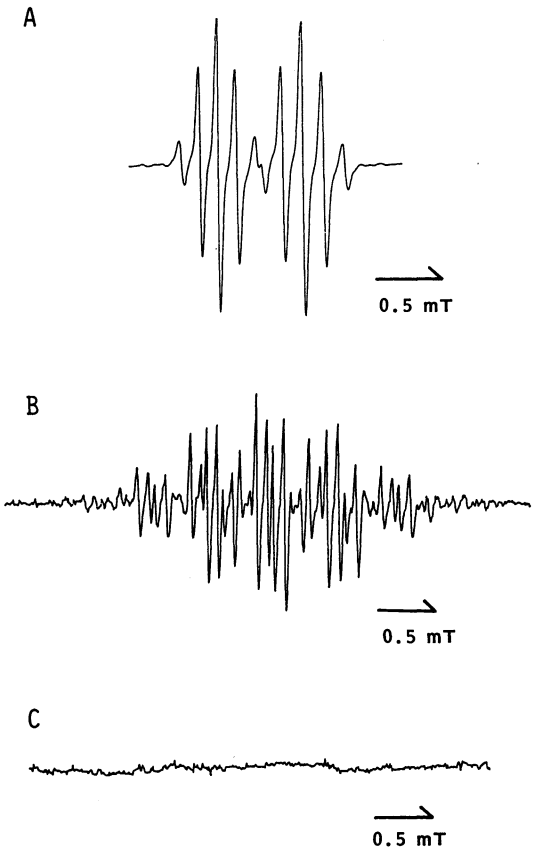

Fig. 5. Interaction of α-tocopheryloxyl radical with 5,6-ascorbyl dipalmitate in benzene at 25°C under vacuum.
A: ESR spectrum of galvinoxyl radical in benzene. B: ESR spectrum observed when α-tocopherol was added to solution A. C: ESR spectrum observed when 5,6-ascorbyl dipalmitate was added to the solution B.

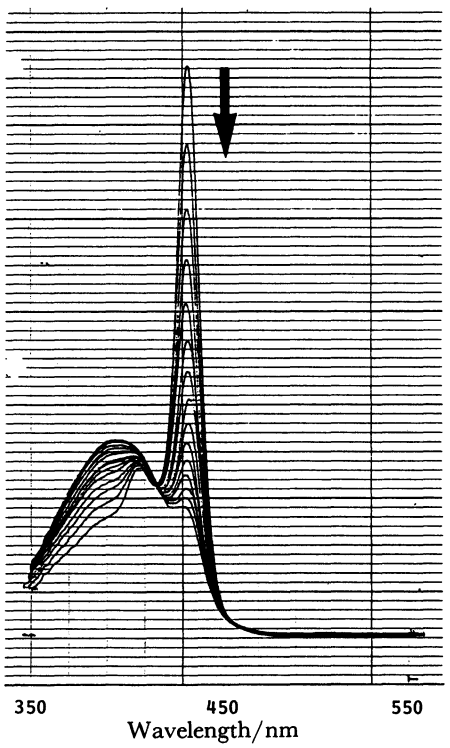

Fig. 6. Interaction of 20.8μM galvinoxyl with 28.9μM 5,6-ascorbyl dipropionate in chloroform at room temperature. The spectra were recorded every 2 min since 1 min after the mixing of galvinoxyl and 5,6-ascorbyl dipropionate.

When both tocopherol and ascorbic acid ester were depleted, the induction period was over and the rapid oxidation took place. Table 2 also shows the rate of oxygen uptake after the induction period. Under these conditions, the kinetic chain length is not very long, but nevertheless it is much larger than 1, suggesting that methyl linoleate is oxidized by a free radical chain mechanism. The oxidizability, $k_p/(2k_t)^{1/2}$, calculated from Eq. 9 is 0.020, where k_p and k_t are the rate constants for chain propagation and termination, respectively.

$$R_p = \frac{k_p}{(2k_t)^{1/2}}[LH]R_i^{1/2} \quad (9)$$

Interactions of Ascorbic Acid Esters with Galvinoxyl. The reactivity of hydrogen donor toward galvinoxyl gives the relative activity as a chain breaking antioxidant. Figure 6 shows the example of the result of interaction between galvinoxyl and ascorbic acid esters. It was found that all the ascorbic acid esters at either 6- or both 5- and 6-positions shown in Table 1 reacted with galvinoxyl at similar rate, whereas those substituted at 2-position did not interact with galvinoxyl.

The above results show that fatty acid esters of ascorbic acid and erythorbic acid at either 6- or both 5-and 6-positions act as chain-breaking antioxidant but that they are no more active as oxygen radical scavenger when ascorbic acid is esterified at 2-position. In other words, ascorbic acid esters function as antioxidant by donating the hydrogen atom at 2-position to oxygen radicals and interrupting free radical chain reaction. It may be worth noting, however, that even the ascorbic acid esters at 2-position may be effective as antioxidant in vivo when they are hydrolyzed by esterase.

Ascorbic acid derivatives have been previously prepared by several groups,[17,21,35] but many of the esters listed in Table 1 are novel compounds. Commercially available esters of ascorbic acid such as 6-ascorbyl palmitate and 6-ascorbyl stearate have solubility problem, but the fatty acid esters at both 5- and 6-positions of ascorbic acid improve the solubilities.

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