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Oxidation of Lipids. V. Oxidation of Methyl Linoleate in Aqueous Dispersion

Yorihiro YAMAMOTO, Shunichi HAGA, Etsuo NIKI,* and Yoshio KAMIYA
Department of Reaction Chemistry, Faculty of Engineering, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113
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The rate and products of oxidation of methyl linoleate dispersed in water by Triton X-100 were measured at 50 °C. The oxidation was initiated by water- or oil-soluble azo-initiators. The oxidation proceeded smoothly by both types of initiators without any noticeable induction period and a constant rate of oxygen uptake was observed. Conjugated diene hydroperoxides were formed almost quantitatively. The oxidizability for methyl linoleate in aqueous dispersion was obtained as $k_p/(2k_t)^{1/2}=0.032(\text{s}\cdot\text{mol/L-oil})^{-1/2}$ where k_p and k_t are the rate constants for propagation and termination reactions, respectively. This value was similar to that observed in the homogeneous oxidation in chlorobenzene. L-Ascorbic acid inhibited the oxidation initiated by water-soluble initiator but it could not inhibit the oxidation initiated by oil-soluble initiator. 2,6-Di-*t*-butyl-4-methylphenol inhibited the oxidation initiated by both initiators. On the other hand, α-tocopherol was effective for the oxidation initiated by oil-soluble initiator and it incompletely inhibited the oxidation initiated by water-soluble initiator.

The oxidation of polyunsaturated fatty acids has received renewed attention recently in connection with the deterioration of foods and oils and with the peroxidation in biological systems.[1-6] In the course of our study on the oxidation of lipids and its inhibition,[7-10] we have extended the work to the oxidation of methyl linoleate in water dispersion. Methyl linoleate was chosen as a substrate since its oxidation proceeds by a straightforward mechanism to give four isomeric hydroperoxides quantitatively at the initial stage.[7,9,11-20] The azo compounds were used as a radical initiator in order to obtain a constant rate of chain initiation.

Experimental

Material. Methyl linoleate was obtained from Sigma Chemical Co. and used as received. Prior to the oxidation, little conjugated diene hydroperoxides were observed by HPLC analysis (see later text). Ethyl palmitate was purchased from Tokyo Kasei Kogyo Co. and used as received. 2,2′-Azobis(2,4-dimethylpentanenitrile) (AMVN) and 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH) used as a radical initiator were obtained from Wako Pure Chemical Industries. L-Ascorbic acid and Triton X-100 were their highest grade available. Commercial 2,6-di-*t*-butyl-4-methylphenol (BMP) was recrystallized from methanol. Natural D-α-tocopherol was kindly supplied from Eisai Co.

Procedure. Appropriate amounts of methyl linoleate, ethyl palmitate, and AMVN (and, if necessary, benzene solution of BMP and α-tocopherol) were taken into a 30 ml Pyrex glass ampoule and dissolved into benzene. The ampoule was connected to the water aspirator and benzene was removed at room temperature. The removal of benzene was checked by the weight. Five ml of 0.01 M (1 M=1 mol dm^{-3}) aqueous Triton X-100 (when necessary, L-ascorbic acid was dissolved in this solution) was added into the ampoule and shaken vigorously on Vortex mixer for two minutes. The oxidation initiated by a water-soluble initiator, AAPH, dissolved in an aqueous Triton X-100 was carried out by a similar procedure.

The ampoule was degassed and then oxygen was introduced into the vessel. The oxidation was carried out at 50 °C under atmospheric pressure of oxygen for the desired time. The rate of oxygen uptake was followed by pressure decrease using a pressure transducer connected to the vessel.

The oxidation was ceased by cooling the ampoule, then methanol was added into the ampoule in order to homogenize the aqueous dispersion. The consumption of methyl linoleate was measured by GLC. An aliquot of methanol solution was evacuated using the evaporator and the reside was dissolved in hexane. This hexane solution was analyzed by HPLC to determine the amounts of hydroperoxides formed, using a silica-gel column and hexane/isopropyl alcohol/acetic acid (1000:10:1, v/v/v) as the eluent. The extinction coefficient was taken from the literature.[12]

Results and Discussion

Oxidation of Methyl Linoleate (LH) Micelle Initiated by Oil-soluble Initiator. Table 1 shows the results of AMVN-initiated oxidation of LH in an aqueous dispersion at 50 °C. As shown in Fig. 1 (Run 2), the oxidation of LH proceeded smoothly without any noticeable induction period and a constant rate of oxygen uptake was observed.

The oxidation of LH initiated by oil-soluble initiator (IN_2I) proceeds by the mechanism[9] shown below:

$$\text{initiation} \quad (IN_2I)_{oil} \longrightarrow 2e\text{I}\cdot + N_2 + (1-e)\text{I-I}, \quad (1)$$

$$\text{I}\cdot + O_2 \xrightarrow{\text{LH, } O_2} \text{IOOH} + LO_2\cdot, \quad (2)$$

$$\text{propagation} \quad LO_2\cdot + \text{LH} \xrightarrow{k_p} \text{LOOH} + \text{L}\cdot, \quad (3)$$

$$\text{L}\cdot + O_2 \rightleftharpoons LO_2\cdot, \quad (4)$$

$$\text{termination} \quad LO_2\cdot + LO_2\cdot \xrightarrow{k_t} \text{nonradical products.} \quad (5)$$

The rate of oxygen uptake ($-dO_2/dt$) is given by

$$-dO_2/dt = R_i^{1/2}[\text{LH}]k_p/(2k_t)^{1/2}, \quad (6)$$

where k_p, k_t, and R_i are the rate constants for Reactions 3 and 5, and rate of initiation, respectively. The rate of initiation was measured by the Eq. 7 from the induction period (t_{inh}) observed in the presence of inhibitor (InhH). The constant n is the number of peroxyl radicals trapped by one inhibitor and it was assumed to be 2.[21]

$$R_i = n[\text{InhH}]/t_{inh}. \quad (7)$$

Table 1. Oxidation of methyl linoleate (LH) dispersed in 5 ml 0.01 M aqueous Triton X-100 at 50 °C initiated by AMVN

Run No.	1	2	3	4	5	6	7	8	9	10
LH (mmol)	0.606	0.867	1.09	1.45	1.46	1.49	1.71	1.77	2.37	2.93
[LH]/(mol/L-oil)	0.605	1.10	1.14	1.48	1.49	1.50	1.60	1.80	2.41	3.02
Ethyl palmitate (ml)	0.8	0.5	0.6	0.5	0.5	0.5	0.5	0.4	0.2	0
[AMVN]/(mol/L-oil)	0.208	0.230	0.219	0.024	0.101	0.066	0.170	0.206	0.202	0.067
$(-dO_2/dt)\times10^5$/(mol/L-oil/s)	3.43	6.14	6.77	2.60	4.74	4.21	7.04	8.30	9.14	7.82
$R_i\times10^6$/(mol/L-oil/s)	2.45	2.76	2.57	0.28	1.18	0.78	2.00	2.42	2.39	0.81
Kinetic chain length	13.5	22.2	25.8	92.4	39.6	53.4	34.7	33.8	37.8	96.2
$(k_p/(2k_t)^{1/2})\times10^3$/(s·mol/L-oil)$^{-1/2}$	36.2	33.9	37.0	33.2	29.2	31.8	31.1	29.6	24.6	28.7
cis,trans-LOOH/*trans,trans*-LOOH	0.34		0.48	0.59	0.54	0.51		0.50	0.64	0.82

Table 2. Inhibition of oxidation of methyl linoleate (LH) dispersed in 5 ml 0.01 M aqueous Triton X-100 at 50 °C initiated by AMVN[a)]

Run No.	2	11	12	13	14
LH (mmol)	0.867	0.894	0.859	0.841	0.837
[LH]/(mol/L-oil)	1.10	1.05	1.10	1.08	1.08
Ethyl palmitate (ml)	0.5	0.5	0.5	0.5	0.5
[AMVN]/(mol/L-oil)	0.230	0.233	0.235	0.233	0.237
BMP (μmol)		2.54			
α-Tocopherol (μmol)			2.30		2.30
L-Ascorbic acid (μmol)				75.1	75.1
t_{inh}/min	0	39	35	0	41
$(-dO_2/dt)\times10^5$/(mol/L-oil/s)	6.14	(1.27)5.93	(0.56)5.39	6.49	(0.86)6.52
$R_i\times10^6$/(mol/L-oil/s)	2.76	2.75	2.76	2.74	2.79
Kinetic chain length	22.2	(3.6)21.2	(1.0)19.0	23.3	(2.1)22.8
$(k_p/(2k_t)^{1/2})\times10^3$/(s·mol/L-oil)$^{-1/2}$	33.9	32.8	29.5	36.3	36.1

a) The numbers in the parentheses are the rate of oxidation and kinetic chain length during the induction period.

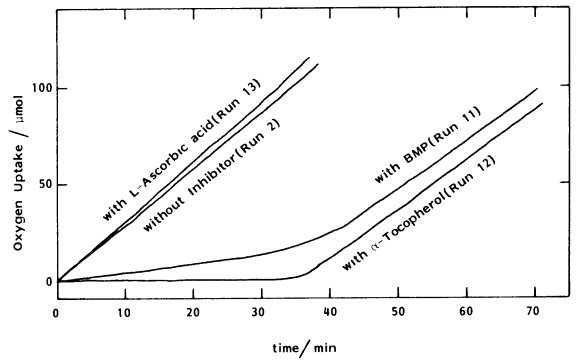

Fig. 1. Rate of oxygen uptake in the AMVN-initiated oxidation of methyl linoleate dispersed in aqueous Triton X-100 at 50 °C.

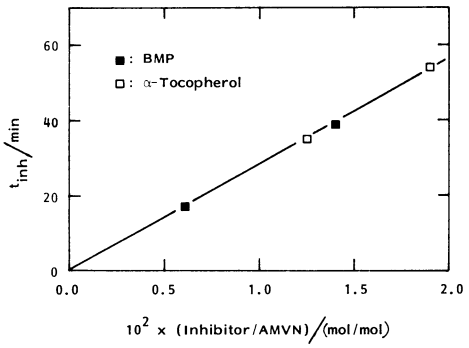

Fig. 2. Plot of induction period(t_{inh}) against the ratio of inhibitor to AMVN concentration in the AMVN-initiated oxidation of methyl linoleate in aqueous dispersion at 50 °C.

Figure 1 shows that the induction period was observed in the presence of BMP or α-tocopherol (Runs 11 and 12). The induction period is produced because BMP or α-tocopherol scavenges the chain carrying peroxyl radicals by Reactions 8 and 9, and suppresses

$$LO_2\cdot + InhH \xrightarrow{k_{inh}} LOOH + Inh\cdot, \quad (8)$$

$$LO_2\cdot + Inh\cdot \longrightarrow Inh\text{-}OOL, \quad (9)$$

the oxidation. As the inhibitor consumed, the rate of oxidation increases and when all the inhibitor is exhausted the oxidation proceeds at the same rate as that in the absence of an inhibitor; that is, the lines for Runs 11 and 12 after the induction period are parallel with the line for Run 2.

Table 2 summarizes the effect of inhibitors on the AMVN-initiated oxidation of LH in an aqueous dispersion. The rate of oxidation during the induction period was smaller when α-tocopherol was used as the inhibitor than when BMP was used. This is the reason why α-tocopherol traps the chain carrying peroxyl radicals faster than BMP.[8,21)]

Figure 2 shows the induction period changes as a

function of the ratio of inhibitor to AMVN. A good linear correlation was observed and the slope for α-tocopherol was identical with that for BMP, suggesting the same stoichiometric number n for both inhibitors.

Equation 6 suggests that the rate of oxygen uptake is the first order with respect to the concentration of the substrate. Figure 3 shows the plot of $-dO_2/dt$ as a function of [LH] $R_i^{1/2}$ and a linear correlation was obtained. This also supports that the oxidation proceeds by the Reactions 1 to 5. The slope gives the oxidizability, $k_p/(2k_t)^{1/2}$, as 0.032 (s·mol/L-oil)$^{-1/2}$.

Oxidation of Methyl Linoleate(LH) Micelle Initiated by Water-soluble Initiator. A constant rate of oxygen uptake was observed in the oxidation of LH micelle initiated by AAPH as shown in Fig. 4 (Run 23), which indicates that the rate of radical formation in the oil region of micelle was constant nevertheless radicals were initially formed in the aqueous phase.[22]

Table 3 summarizes the effect of Triton X-100 on the AAPH-initiated oxidation of LH in an aqueous dispersion. When Triton X-100 was not added and LH was floating on the water-air interface, little oxygen uptake was observed (Run 15). The rate of oxygen uptake increased with an increase in the concentration of Triton X-100 (Runs 16—19). However, an appreciable rate of oxygen uptake was observed in the oxidation of 0.1 M aqueous Triton X-100 alone (Run 20), therefore the concentration of Triton X-100 was kept constant at 0.01 M.

Table 4 summarizes some representative results of the AAPH-initiated oxidation of LH dispersed in 5 ml of 0.01 M aqueous Triton X-100 at 50 °C. The rate of initiation (R_i) was calculated from the induction period (t_{inh}) produced in the presence of BMP (see later results). The oxidizability, $k_p/(2k_t)^{1/2}$, was calculated from equa-

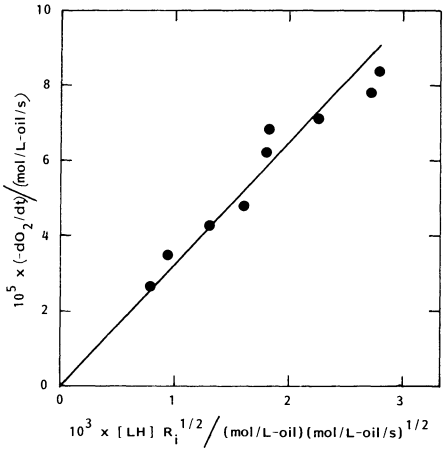

Fig. 3. Plot of the rate of AMVN-initiated oxidation of methyl linoleate (LH) in aqueous dispersion at 50 °C *vs.* [LH] $R_i^{1/2}$.

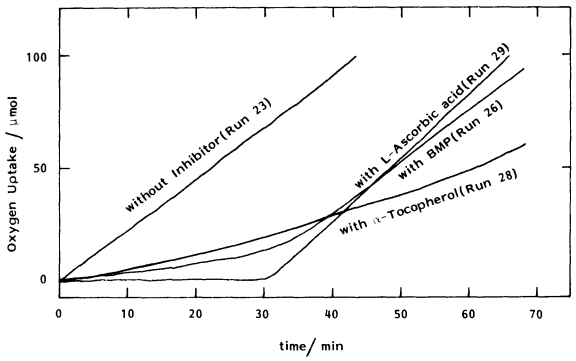

Fig. 4. Plot of oxygen uptake in the AAPH-initiated oxidation of methyl linoleate dispersed in aqueous Triton X-100 at 50 °C.

Table 3. Effect of Triton X-100 on the AAPH-initiated oxidation of methyl linoleate (LH) in 5 ml water at 50 °C

Run No.	15	16	17	18	19	20
LH (mmol)	0.873	0.971	0.903	0.858	0.893	0
[LH]/(mol/L-oil)	3.02	3.02	3.02	3.02	3.02	
AAPH (μmol)	80.8	91.5	90.8	90.5	88.9	94.5
Triton X-100 (mmol/L-soln[a])	0	1.7	10	50	100	100
$(-dO_2/dt) \times 10^6$/(mol/L-soln/s)	0	1.50	7.17	5.87	12.3	0.99

a) Concentration in total aqueous solution.

Table 4. AAPH-intiated oxidation of methyl linoleate (LH) dispersed in 5 ml 0.01 M aqueous Triton X-100 at 50 °C

Run No.	21	22	23	24	17	25
LH (mmol)	0.490	0.832	0.843	0.890	0.903	1.403
[LH]/(mol/L-oil)	3.02	3.02	3.02	3.02	3.02	3.02
AAPH (μmol)	90.4	93.0	91.9	91.9	90.8	97.0
[AAPH]/(mmol/L-soln)	17.5	17.6	17.4	17.4	17.1	17.8
$(-dO_2/dt) \times 10^4$/(mol/L-oil/s)	2.23	1.43	1.36	1.32	1.27	0.94
$R_i \times 10^6$/(mol/L-oil/s)	3.40	2.06	2.01	1.91	1.85	1.28
Kinetic chain length	65.0	68.8	67.2	68.5	68.2	72.9
$(k_p/(2k_t)^{1/2}) \times 10^3$/(s·mol/L-oil)$^{-1/2}$	40.0	32.9	31.8	31.6	30.9	27.5

Table 5. Inhibition of AAPH-initiated oxidation of methyl linoleate (LH) dispersed in 5 ml 0.01 M aqueous Triton X-100 at 50 °C[a)]

Run No.	23	26	27	28	29	30
LH (mmol)	0.843	0.854	0.891	0.828	0.853	0.866
[LH]/(mol/L-oil)	3.02	3.02	3.02	3.02	3.02	3.02
AAPH (μmol)	91.9	96.7	91.5	91.1	92.6	95.6
[AAPH]/(mmol/L-soln)	17.4	18.3	17.3	17.3	17.5	18.1
BMP (μmol)		0.50	1.01			
α-Tocopherol (μmol)				0.50		
L-Ascorbic acid (μmol)					2.01	3.04
t_{inh}/min	0	30.5	61.5	?	30	45
$(-dO_2/dt) \times 10^4$/(mol/L-oil/s)	1.36	(0.14) 1.41	(0.17) 1.40		(0) 1.48	(0) 1.48
$R_i \times 10^6$/(mol/L-oil/s)	2.01	2.10	1.91	2.03	2.00	2.04
Kinetic chain length	67.2	(5.8) 66.6	(7.8) 72.5		(0) 73.5	(0) 72.0
$(k_p/(2k_t)^{1/2}) \times 10^3$/(s·mol/L-oil)$^{-1/2}$	31.8	32.2	33.4		34.6	34.3

a) The numbers in the parentheses are the rate of oxidation and kinetic chain length during the induction period.

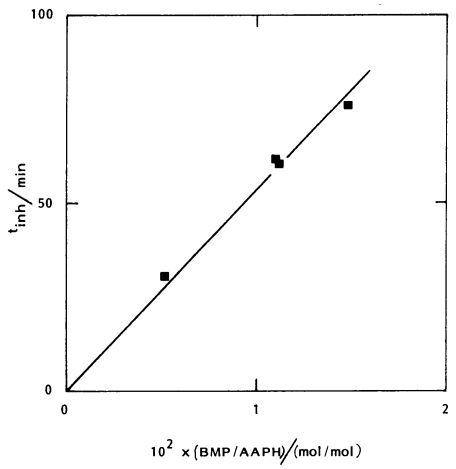

Fig. 5. Plot of induction period(t_{inh}) against the ratio of BMP to AAPH concentration in the AAPH-initiated oxidation of methyl linoleate in aqueous dispersion at 50 °C.

Table 6. Products of the oxidation of methyl linoleate (LH) dispersed in aqueous Triton X-100 at 50 °C

Run No.	4	21	17	25
LH (μmol)	1451	490	903	1403
Initiator	AMVN	AAPH	AAPH	AAPH
Time/min	40	30	50	60
ΔO_2/μmol	61	65	114	157
ΔLH/μmol		51	80	135
Hydroperoxides analyzed by HPLC (μmol)				
13-*cis,trans*-LOOH	11.8	13.4	24.6	32.6
13-*trans,trans*-LOOH	19.2	16.7	29.0	40.4
9-*trans,cis*-LOOH	10.9	11.8	21.7	30.0
9-*trans,trans*-LOOH	19.3	16.0	28.0	38.3
Total LOOH	61	58	103	141
Kinetic chain length	92.4	65.0	68.2	72.9
ΔO_2/initial LH	0.042	0.13	0.13	0.11

tion 6 as 0.032 (s·mol/L-oil)$^{-1/2}$ on the average of Runs 17, 22, 23, and 24. This value agreed well with that obtained in the AMVN-initiated oxidation of LH dispersed in an aqueous Triton X-100. These values were also in good agreement with the oxidizability obtained in the oxidation of LH in chlorobenzene at 50 °C; 0.033 $(M \cdot s)^{-1/2}$.[23)]

The effect of inhibitors on the AAPH-initiated oxidation of LH in an aqueous dispersion is summarized in Table 5 and Fig. 4. Water-soluble inhibitor, L-ascorbic acid, suppressed the oxidation (Runs 29 and 30). BMP also inhibited the oxidation (Runs 26 and 27) and we can calculated the concentration of radical produced in the oil region of micelle in an unit time (R_i) from the induction period (T_{inh}) by Eq. 7. Figure 5 shows the plot of t_{inh} against the ratio of BMP to AAPH and a linear correlation was obtained. On the other hand, α-tocopherol inhibited the oxidation incompletely as shown in Fig. 4.

Products. Table 6 summarizes the oxygen uptake, substrate reacted, and the formation of hydroperoxides in the oxidation of methyl linoleate (LH) in an aqueous dispersion at 50 °C. As observed in the oxidation of LH in an homogeneous solution, the major products were for isomeric conjugated diene hydroperoxides: (9*Z*,11*E*)-13-hydroperoxy-, (9*E*,11*E*)-13-hydroperoxy-, (10*E*,12*Z*)-9-hydroperoxy-, and (10*E*,12*E*)-9-hydroperoxyoctadecadienoic acid methyl esters. The amounts of oxygen uptake, substrate disappearance, and the formation of hydroperoxides agreed fairy well with each other. Figure 6 shows the dependence of *cis,trans*-LOOH/*trans,trans*-LOOH ratio on the concentration of substrate in the oil phase. The intersections and the slopes were the same independent of the initiators. Furthermore, the *cis,trans* to *trans,trans* ratios were similar to those obtained in the homogeneous oxidation of LH in solution at 50 °C.[9)]

In conclusion, the oxidation of methyl linoleate dispersed in an aqueous Triton X-100 was initiated by not only oil-soluble initiator but also water-soluble initiator, and a constant rate of oxygen uptake was observed. Water-soluble inhibitor, L-ascorbic acid, inhibitd the oxidation initiated by water-soluble initiator but it could not inhibited the oxidation initiated by

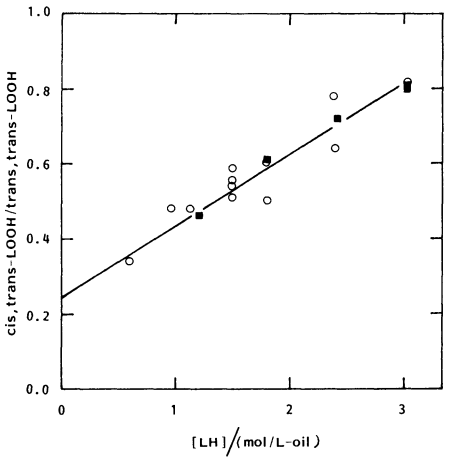

Fig. 6. Plot of *cis,trans*-LOOH/*trans,trans*-LOOH ratio against methyl linoleate (LH) concentration in the AMVN-(○) and AAPH-(■) initiated oxidation of LH in aqueous dispersion at 50 °C.

oil-soluble initiator. Oil-soluble inhibitor, BMP and α-tocopherol, inhibited the oxidation initiated by both inhibitors. The oxidizability for methyl linoleate in an aqueous dispersion was similar to that observed in the homogeneous oxidation in chlorobenzene. Furthermore, conjugated diene hydroperoxides were formed almost quantitatively and *cis,trans/trans,trans* ratios were similar to those obtained in the homogeneous oxidation. Thus, the oxidation of methyl linoleate in an aqueous dispersion proceeds in the oil region by the similar rate and mechanism as in homogeneous solution.

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22) Although it is not clealy established, we believe substantially all of AAPH is distributed in an aqueous phase because AAPH is very soluble in water but insoluble in oil and because L-ascorbic acid can inhibit the AAPH-initiated oxidation whereas it can not inhibit the AMVN-initiated oxidation.
23) Y. Yamamoto, E. Niki, and Y. Kamiya, unpublished data.