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Oxidation of Lipids. IX. Decomposition of Methyl Linoleate and Methyl Linolenate Hydroperoxides in Solution[†]

Yorihiro YAMAMOTO, Noriyuki SAEKI, Schunichi HAGA,
Etsuo NIKI,* and Yoshio KAMIYA
Department of Reaction Chemistry, Faculty of Engineering, University of Tokyo, Hongo, Tokyo 113

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Decompositions of methyl linoleate hydroperoxide (LOOH) induced by di-*t*-butyl diperoxyoxalate and cobalt ions were investigated in benzene at 50 °C under vacuum to elucidate the degradation mechanism. The major products were dimer (LOOL), oxodiene, hydroxydiene and epoxyhydroxyene (and/or epoxyoxoene). It was suggested that these products were formed by the recombination of L· and LOO· radicals, β-scission of LO· radical, hydrogen abstraction of LO· radical, and intramolecular epoxidation of LOO· radical, respectively. The decomposition of methyl linolenate hydroperoxide (L'OOH) was also studied. The major products were dimer, oxotriene, hydroxytriene, and thiobarbituric acid reactive substances, which were suggested to be formed by the intramolecular cyclization of L'OO· radical.

Polyunsaturated fatty acids (PUFA), which are present as phosphoglyceride esters in biological membranes, are quite susceptible to oxidation.1) The oxidation of PUFA gives peroxides as primary products,2) and it is suggested that the formation of so-called "lipid peroxides" is associated with aging and the appearance of geriatric disorders such as heart attack, stroke, diabates, *etc.*3—8) Therefore, it is very important to understand the formation and the decomposition of the lipid peroxide in the living system. The oxidation of PUFA and behavior of hydroperoxides derived from PUFA are also important subjects in food chemistry.5,6)

The decomposition of peroxides is important since it can contribute to chain initiation of lipid peroxidation and xenogenous toxicity. It has been reported that the decomposition of linoleic acid hydroperoxide gave various products such as oxodiene,9—16) epoxyhydroxyene,10,12—14,16—18) epoxyoxoene,10,14,16,17) trihydroxyene,10,14) peroxide-bridged dimer,14) carbon–carbon bridged dimer,19) hydroxydiene,12) oxodihydroxyene,14) oxohydroxyene,10,16) and hydroperoxyoxoene.17) The distribution of products is dependent on experimental variables such as atmosphere, catalysts, absence and presence of hydrogen donors and reductants, and pH.

In this study, we undertook a simple system in order to elucidate the mechanism of homolytic decomposition of linoleate and linolenate hydroperoxides induced by radicals and metal ions in a homogeneous solution.

Experimental

Material. Methyl linoleate (LH) was obtained from Sigma Chemical Co. and used as received. Little peroxide was observed by HPLC analysis.21,22) Methyl linoleate hydroperoxide (LOOH) was synthesized22,23) from LH by the action of soybean lipoxygenase (Sigma Chem. Co.) and purified by passing through a silica-gel column using hexane and ether as eluent. It was found by HPLC analysis that the ratio of (9*Z*,11*E*)-13-hydroperoxy-9,11-octadecadienoic acid methyl ester to total peroxide was higher than 95%. LOOH was stored as a benzene solution below 0 °C. Under these conditions, the decomposition and isomerization22) of LOOH were very slow.

Methyl linolenate(L'H) was obtained from Sigma Chemical Co. and purified by silica-gel column chromatography. Methyl linolenate hydroperoxide (L'OOH) was also prepared using soybean lipoxygenase. It was purified with a silica-gel column and stored as a benzene solution below 0 °C to avoid the decomposition and isomerization of hydroperoxide. More than 95% of hydroperoxide was found to be (9*Z*,11*E*,15*Z*)-13-hydroperoxy-9,11,15-octadecatrienoic acid methyl ester by HPLC analysis.24)

Di-*t*-butyl diperoxyoxalate (DBPO) was prepared by the reaction of oxalyl chloride and *t*-butyl hydroperoxide followed by recrystallization from pentane.25) α-Methylstyrene (α-MS) was purified by the conventional method; washing with alkali and distillation. 2,6-Di-*t*-butyl-4-methylphenol (BMP) was recrystallized from methanol. Bis (acetylacetonato) cobalt (II) (Co(acac)$_2$) and benzene were those of the highest grade available.

Procedure. A 3 ml of benzene solution of LOOH was taken into a 30 ml Pyrex glass ampoule with additives, if necessary, such as DBPO and Co(acac)$_2$. The ampoule was degassed by freeze and thaw method and sealed by a Teflon valve (6VD type, Nupro Co.). Then the ampoule was put into a water bath kept at 50 °C for desired time. The reaction mixture was stirred vigorously.

After the reaction, the ampoule was cooled by liquid nitrogen, opened, and then warmed up to the room temperature. The reaction mixture was analyzed by TLC and HPLC. A portion of the reaction mixture was evacuated to remove the solvent on water aspiration, then hexane was added. UV absorbances of the hexane solution at 235 and 270 nm were measured to determine the conjugated diene and oxodiene; their extinction coefficients were taken from the literature20,26) as 28000 and 20000 $\mathrm{M^{-1}\,cm^{-1}}$, respectively.

Analytical Method. Merck Silica-Gel 60 plate and hexane/ether/acetic acid (60/40/1, by volume) eluent were used for the TLC analysis. Spots were visualized by iodine vapour. TLC/FID analyzer (IATRON model TH-10) was applied for quantitative measurement. HPLC analysis was described in the previous paper.21) GPC spectra were measured on a Simadzu LC model 830 equipped with a UV detector, Shimadzu model UV 202. Shimadzu GPC columns, HSG 15 and 30, were used in series and eluent was tetrahydrofuran. The formation of α-methylstyrene oxide was determined by GLC. 2-Thiobarbituric acid reactive substances (TBARS) were measured by the conventional

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photometric method.[2] Molecular weight of a dimer was measured with a vapour pressure osmometer in benzene at 40 °C. ^{1}H NMR spectroscopy was performed on a Varian 360 spectrometer.

Results and Discussion

Table 1 shows the result of DBPO-induced decomposition of LOOH in 3 ml benzene *in vacuo* at 50 °C for 5 h. LOOH was thermally stable in benzene *in vacuo* at 50 °C (Run 1). However, LOOH was decomposed when incubated with DBPO (Run 2). The half life of DBPO is 23.1 min at 50 °C,[25] therefore more than 99% of DBPO decomposes in 5h at 50 °C. The amount of LOOH decomposed was almost twice as that of DBPO added, which is quite reasonable since DBPO produces 2 moles of *t*-butoxyl radical as discussed later in the text. Table 2 summarizes the result of cobalt-catalyzed decomposition of LOOH in 3 ml benzene *in vacuo* at

TABLE 1. DBPO-INDUCED DECOMPOSITION OF METHYL LINOLEATE HYDROPEROXIDE (LOOH) IN 3 ml BENZENE *in vacuo* AT 50 °C IN 5 h[a]

Run No	1	2[h]	3	4
Reactant				
LOOH	30.0	25.3	23.6	27.5
LH,[b] mmol			1.05	
α-MS,[c] mmol				0.32
DBPO[d]	0	9.4	9.4	17.9
Disappearance of LOOH and formation of products				
Δ LOOH	0	−17.2	−3.6	−24.3
Δ LOH[e]	0	0	0	0
Δ (C=C)$_2$	0	−5.9	9.5	−7.3
Δ Oxodiene	0	0.9	1.0	1.8
Dimer[f]	−	+++	+++++	++
5[f),g]	−	+++	−	+
α-MS oxide				6.6

a) Numbers show μmol in 3 ml. b) Methyl linoleate. c) α-Methylstyrene. d) Di-*t*-butyl diperoxyoxalate. e) Hydroxydiene. f) − and + show that the spot is negative and positive, respectively. The increased numbers of + indicate the relative increase of the formation. g) Assumed to be epoxide (see Text and Fig. 1). h) Ratios of dimer, LOOH, and **5** were 1.0/1.0/1.0 by TLC/FID.

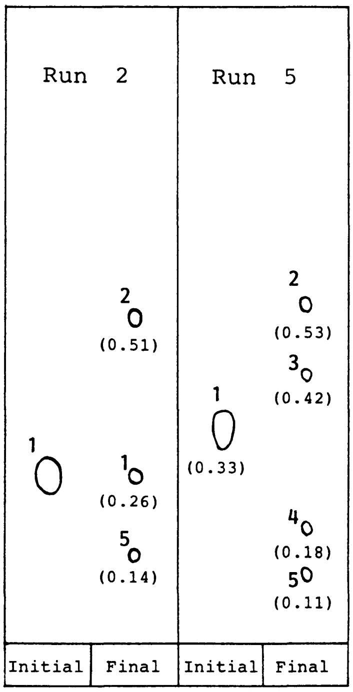


Fig. 1. Thin-layer chromatograms of initial and decomposed LOOH. Numbers in the parentheses are R_f values. Reaction conditions are described in Tables 1 and 2.
1: LOOH, **2**: Dimer (LOOL), **3**: Oxodiene (–C(O)–(CH=CH)$_2$–), **4**: Hydroxydiene (LOH), **5**: Epoxyhydroxyene and/or Epoxyoxoene.
$L=CH_3(CH_2)_4\text{-}CH\text{-}(CH=CH)_2\text{-}(CH_2)_7COOCH_3$.

TABLE 2. COBALT-CATALYZED DECOMPOSITION OF METHYL LINOLEATE HYDROPEROXIDE (LOOH) IN 3 ml BENZENE *in vacuo* AT 50 °C IN 5 h[a]

Run No	5[f]	6	7	8[g]	9
Reactant					
LOOH	24.4	21.8	24.3	24.4	23.3
LH, mmol		0.98			
BMP[b]			15.8		
α-MS, mmol					1.92
Co(acac)$_2$[c]	3.9	4.3	3.5	5.1	3.9
Disappearance of LOOH and formation of products					
Δ LOOH	−24.4	−21.8	−24.3	−24.4	−23.3
Δ LOH	4.1	7.2	7.9	3.7	5.1
Δ (C=C)$_2$	−7.2	0.2	−4.2	−13.2	
Δ Oxodiene	11.5	12.6	10.1	13.0	
Dimer[d]	+++	++++	−	±	++
5[d),e]	+++	−	±	±	+
α-MS oxide					5.8

a) Numbers show μmols in 3 ml. b) 2,6-Di-*t*-butyl-4-methylphenol. c) Bis(acetylacetonato) cobalt(II). d) Same as footnote. f) In Table 1. e) Assumed to be epoxide (see text and Fig. 1). f) Ratios of dimer, oxodiene + LOH, and **5** were 1.0/3.1/1.2 by TLC/FID. g) Under air.

50 °C for 2h. LOOH was decomposed completely in every run, which suggests that Co(acac)$_2$ decomposes LOOH catalytically.

Figure 1 shows the thin layer chromatograms of initial LOOH and decomposition products. As shown, chromatograms were clear. Thin layer chromatography of the reaction mixture was carried out with larger scale than that shown in Fig. 1, and each spot was collected, analyzed, and found as dimer, oxodiene, LOH, and unknown product **5** by the following results.

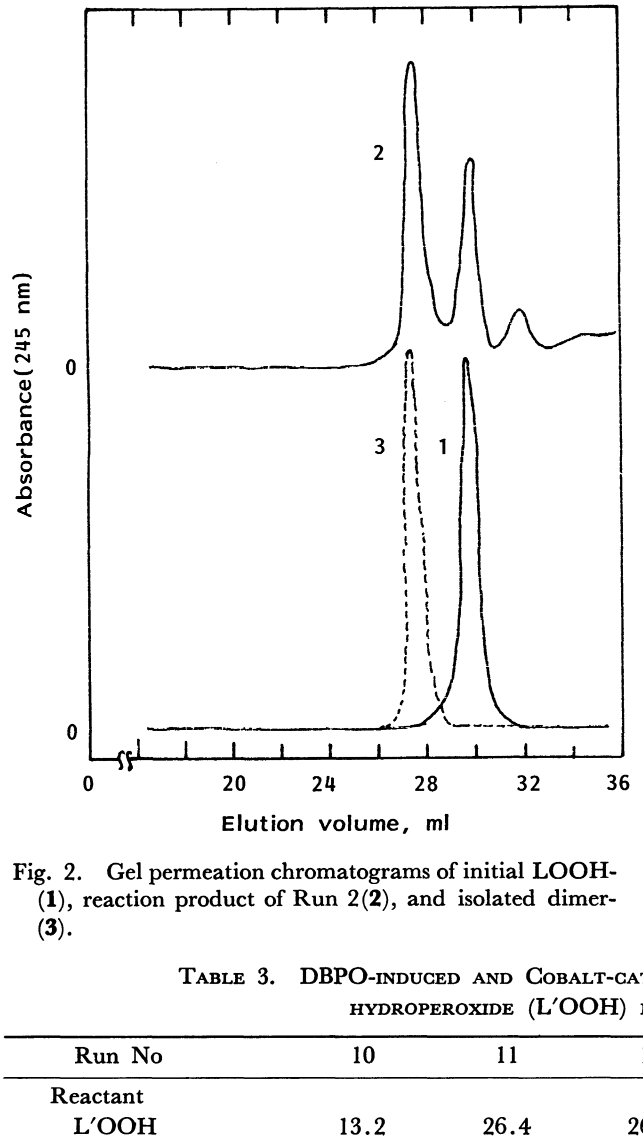


Fig. 2. Gel permeation chromatograms of initial LOOH-(**1**), reaction product of Run 2(**2**), and isolated dimer-(**3**).

2: Figure 2 shows the gel permeation chromatograms of initial LOOH (line 1), reaction mixture of Run 2 (line 2), and isolated **2** (dotted line 3). As shown, a new peak (elution volume=27.5 ml) was observed in the reaction mixture and **2** gave an identical chromatogram. The molecular weight of **2**, as measured with a vapour pressure osmometer, was 619±15. **2** has a strong absorption at 231.5 nm and its extinction coefficient was about 40000 M^{-1} cm^{-1}, suggesting that **2** has two conjugated dienes in a molecule. ^1H NMR spectra $\delta(CCl_4)$; 3.63 (s, 6H,OC$\underline{H}_3$), 4.1—4.5 (m, 2H, O-C-$\underline{H}$), 5.2—6.3 (m, 8H, (C$\underline{H}$=C$\underline{H}$)$_2$) indicate that **2** is a peroxide-bridged dimer LOOL. The calculated molecular weight for LOOL is 619.

3: **3** has a strong characteristic UV absorption at 270 nm and R_f value(0.42) is between those of LOOH(0.33) and LH(0.83). By comparing with the previous results,[14,26] **3** was attributed to oxodiene, -C(O)-(C=C)$_2$-.

4: R_f value of **4** was identical with that of the products obtained by reduction of LOOH with triphenylphosphine. Therefore, **4** must be hydroxyoctadecadienoic acid methyl ester, LOH.

The decrease of LOOH and the formation of LOH shown in Tables 1 and 2 were measured by HPLC. The decrease of conjugated diene and the increase of oxodiene were determined by UV absorbance at 235 and 270 nm, respectively. The yields of dimer and **5** were estimated by the deepness of the color of the spot. The ratios of dimer, oxodiene, LOOH, LOH, and **5** were also measured by TLC/FID in Runs 2 and 5. In other Runs, LH and α-MS disturbed the TLC/FID analysis. α-Methylstyrene oxide was determined by glc.

As shown in Table 1 and Fig. 1, the major products of DBPO-induced decomposition of LOOH were dimer and the unknown compound **5**. The addition of methyl linoleate (LH) decreased **5** (Run 3), while it increased

TABLE 3. DBPO-INDUCED AND COBALT-CATALYZED DECOMPOSITION OF METHYL LINOLENATE HYDROPEROXIDE (L'OOH) IN BENZENE *in vacuo* AT 50 °C[a)]

Run No	10	11	12	13	14	15	16[d)]
Reactant							
L'OOH	13.2	26.4	26.4	13.2	13.2	13.2	13.2
LH, mmol			1.48		1.46		
BMP						37.2	
DBPO	0	23.3	21.9				
Co(acac)$_2$				3.0	3.0	3.0	3.0
Time, h	5	5	5	2	2	2	2
Disappearance of L'OOH and formation of products							
Δ L'OOH	0.7	−26.4	−5.2	−13.2	−13.2	−13.2	−13.2
Δ L'OH	0	0	0	2.2	1.3	4.0	0.6
Δ (C=C)$_2$	0	−4.1	24.7	−1.9	5.2		
Δ Oxotriene	0			4.8	7.1	7.5	Trace
TBARS[b)]	0.2	11.0	7.3	4.5	6.4	2.8	2.6
Dimer[c)]	—	++	+++++	++	+++	—	±

a) Numbers in μmol. b) Thiobarbituric acid reactive substances. c) Same as footnote f) in Table 1. d) Under air.

the dimer. The major products of cobalt-catalyzed decomposition of LOOH were dimer, oxodiene, hydroxydiene (LOH), and **5**. Oxodiene and LOH were not observed in the DBPO-induced decomposition of LOOH. The yield of dimer was increased by the addition of LH (Run 6) and decreased by the addition of BMP (Run 7). Furthermore, little dimer was observed in the decomposition of LOOH under air (Run 8). The yield of **5** decreased by the addition of LH and BMP, and by changing the atmosphere to air (Runs 6—8). Little TBARS was observed in the decomposition of LOOH.

Table 3 summarizes the results of DBPO-induced and cobalt-catalyzed decomposition of methyl linolenate (L'OOH) in benzene *in vacuo* at 50 °C. L'OOH was thermally stable in benzene *in vacuo* at 50 °C (Run 10). The DBPO-induced decomposition of L'OOH gave dimer and TBARS. In addition to them, hydroxytriene (L'OH) and oxotriene were produced in the cobalt catalyzed decomposition of L'OOH. The yield of dimer was increased by the addition of methyl linoleate (LH) (Runs 12 and 14) and decreased by the addition of BMP (Run 15) and by changing the atmosphere to air (Run 16).

The results and discussion described above suggest that the decompositions of LOOH and L'OOH proceed by the scheme shown below. DBPO decomposes to give *t*-butoxyl radical (*t*-BuO·) and carbon dioxide (Eq. 1).

$$\mathrm{DBPO} \longrightarrow 2\,t\text{-BuO}\cdot + 2\mathrm{CO_2}, \tag{1}$$

$$t\text{-BuO}\cdot + \mathrm{LOOH} \longrightarrow t\text{-BuOH} + \mathrm{LOO}\cdot, \tag{2}$$

$$\mathrm{LOOH} + \mathrm{Co^{2+}} \longrightarrow \mathrm{LO}\cdot + \mathrm{OH^-} + \mathrm{Co^{3+}}, \tag{3}$$

$$\mathrm{LOOH} + \mathrm{Co^{3+}} \longrightarrow \mathrm{LOO}\cdot + \mathrm{H^+} + \mathrm{Co^{2+}}, \tag{4}$$

$$\mathrm{LOO}\cdot \rightleftharpoons \mathrm{L}\cdot + \mathrm{O_2}, \tag{5}$$

$$\mathrm{LOO}\cdot + \mathrm{L}\cdot \longrightarrow \mathrm{LOOL}, \tag{6}$$

$$2\mathrm{LOO}\cdot \longrightarrow \mathrm{LOH} + \text{Oxodiene} + \mathrm{O_2}, \tag{7}$$

$$\mathrm{LOO}\cdot \longrightarrow \mathbf{5}\ \text{(epoxyhydroxyene and/or epoxyoxoene)}, \tag{8}$$

$$\mathrm{LO}\cdot \xrightarrow{\mathrm{LOOH,\,LH,\,BMP}} \mathrm{LOH}, \tag{9}$$

$$\mathrm{LO}\cdot \longrightarrow \text{Oxodiene}, \tag{10}$$

$$\mathrm{L'OO}\cdot + \mathrm{L'}\cdot \longrightarrow \mathrm{L'OOL'}, \tag{11}$$

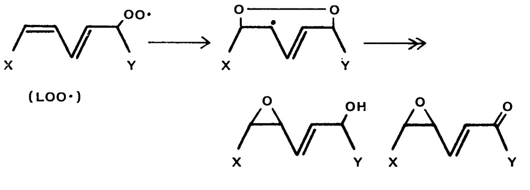
$$\mathrm{L'O}\cdot \xrightarrow{\mathrm{L'OOH,\,LH,\,BMP}} \mathrm{L'OH}, \tag{12}$$

$$\mathrm{L'O}\cdot \longrightarrow \text{Oxotriene}, \tag{13}$$

$$\mathrm{L'OO}\cdot \longrightarrow \mathrm{TBARS}, \tag{14}$$

t-Butoxyl radical abstracts hydrogen from LOOH to give peroxyl radical LOO·(Eq. 2). Equations 3 and 4 show the redox decomposition of LOOH by cobalt ions. Therefore, LOO·radical is a key intermediate in the DBPO-induced decomposition of LOOH. Under low concentration of oxygen, LOO·radical liberates oxygen and gives resonance-stabilized alkyl radical, L·(Eq. 5). The recombination of peroxyl radical and alkyl radical is quite fast and consequently dimer, LOOL, is formed (Eq. 6). The presence of oxygen must suppress the formation of dimer, since the bimolecular interaction of peroxyl radicals gives LOH and oxodiene (Eq. 7) rather than LOOL. Reaction 7 is not important under low concentration of oxygen.[21] In fact, the amounts of LOH and oxodiene were very small in Run 2.

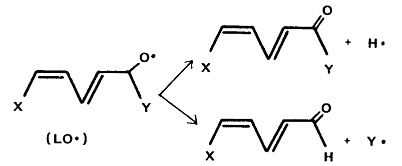
Unknown product **5** is also produced from peroxyl radical (Eq. 8). The formation of α-methylstyrene oxide in the presence of α-methylstyrene (Runs 4 and 9) and the low R_f value of **5** suggest that **5** is an epoxide such as epoxyhydroxyene and/or epoxyoxoene produced by intramolecular epoxidation of LOO·radical as shown below.[27]



(8a)

$$\mathrm{X}=(\mathrm{CH_2})_7\mathrm{COOCH_3},\ \mathrm{Y}=(\mathrm{CH_2})_4\mathrm{CH_3}$$

In addition to peroxyl radical, alkoxyl radical, LO·, plays an important role in the redox decomposition of LOOH and gives LOH and oxodiene by hydrogen abstraction (Eq. 9) and β-scission (Eq. 10), respectively. BMP acts as an efficient hydrogen donor to oxygen radicals and increases LOH.

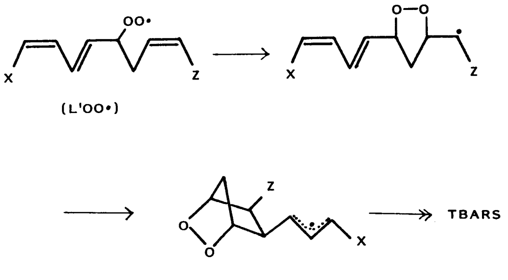


(10a)

(10b)

$$\mathrm{X}=(\mathrm{CH_2})_7\mathrm{COOCH_3},\ \mathrm{Y}=(\mathrm{CH_2})_4\mathrm{CH_3}$$

The decomposition of L'OOH proceeds similarly as LOOH; peroxyl radical, L'OO·gives dimer (Eq. 11) and alkoxyl radical, L'O·gives hydroxytriene, L'OH, (Eq. 12) and oxotriene (Eq. 13). However, TBARS was produced instead of **5**, probably by intramolecular cyclization of peroxyl radical (Eq. 14a).[30—32]



(14a)

$$\mathrm{X}=(\mathrm{CH_2})_7\mathrm{COOCH_3},\ \mathrm{Z}=\mathrm{CH_2CH_3}$$

In conclusion, the homolytic decomposition of linoleate hydroperoxide gave dimer, oxodiene, hydroxydiene, and epoxyhydroxyene (and/or epoxyoxoene) and that of linolenate hydroperoxide gave dimer, oxotriene, hydroxytriene, and TBARS.

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