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New Geometric Isomers of Oxooctadecadienoate in Copper-catalyzed Decomposition Products of Linoleate Hydroperoxide

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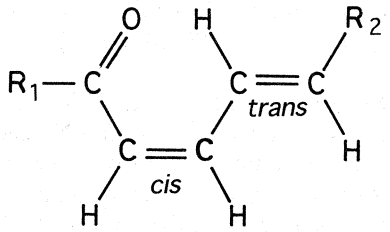
Methyl linoleate hydroperoxide produced by autoxidation was refluxed with 10^{-4} M Cu-naphthenate in benzene. Two new geometrical isomers of oxooctadecadienoate (compounds I and II) were found in addition to the four known isomers. They were isolated by a Sephadex LH-20 column chromatography with chloroform-hexane (2:1) and purified by HPLC on Nucleosil ®100-5 and Zorbax ODS columns. UV, IR, MS, and ^{1}H-NMR spectra were measured. The geometry of conjugated dienes were assigned from the coupling constants of the olefinic protons. Compounds I and II were identified as 13-oxo-*trans*-9, *cis*-11- and 9-oxo-*cis*-10, *trans*-12-octadecadienoate, respectively. Each of them had a *cis* double bond adjacent to the oxo group. The hydroperoxides of the same geometry as compounds I and II were also detected in autoxidation products.

Key words: oxooctadecadienoate; linoleate; hydroperoxide; autoxidation

It is known that the hydroperoxides of lipids and their decomposition products influence the flavor and nutritive value of food, and have various biological effect. Since decomposition of the hydroperoxide mainly occurs by radicals, transition metals have been used as catalysts and the decomposition products have been studied to clarify the mechanism.

Oxooctadecadienoate has been reported as a decomposition product of linoleate hydroperoxide incubated with Cu^{2+},[1] Fe^{2+}, Fe^{3+}-cysteine,[2] and hemoglobin.[3] Four isomers have been reported, which are 13-oxo-*cis*(*Z*)-9, *trans*(*E*)-11-octadecadienoate, 13-oxo-*trans*(*E*)-9, *trans*(*E*)-11-octadecadienoate, 9-oxo-*trans*(*E*)-10, *cis*(*Z*)-12-octadecadienoate, and 9-oxo-*trans*(*E*)-10, *trans*(*E*)-12-octadecadienoate. These oxo compounds are assumed to be produced *via* both alkoxy and peroxy radicals. When the alkoxy radical is an intermediate, the position of the oxygen function is expected to be retained in conversion. Gardner *et al.* and Hamberg have reported that the ratios of the 13-isomer to the 9-one of the oxo compounds were similar to that of the original hydroperoxides when decomposition was catalyzed by Fe^{3+}-cysteine[2] and hemoglobin.[3] On the other hand, when decomposition proceeded by a mechanism involving peroxy radicals, the ratio of the 9-isomer to the 13-one in the oxo compounds was different from that of the original hydroperoxide as reported by Schieberle *et al.*[1] As to the geometry of the conjugated dienes of the oxo compound, the proportion of the *trans trans* isomers was increased in comparison with that of the original hydroperoxide.[1,2]



I : R_1, $-(CH_2)_4CH_3$; R_2, $-(CH_2)_7COOCH_3$

II : R_1, $-(CH_2)_7COOCH_3$; R_2, $-(CH_2)_4CH_3$

Fig. 1. Compounds I and II.

These known oxo compounds have *trans* double bonds adjacent to the carbonyl groups. In this investigation, we detected two new isomers (compounds I and II, Fig. 1) when the hydroperoxide was decomposed by Cu-naphthenate, and separation and identification of these compounds are described. Each of these isomers has a *cis* double bond adjacent to the carbonyl group.

Materials and Methods

Materials. Methyl linoleate was prepared from cottonseed oil and oxidized at room temperature until the concentration of the hydroperoxide reached 500 μmol/ml. The hydroperoxide was purified by silica gel and Sephadex LH-20 column chromatography.[4]

Decomposition of Hydroperoxide. The purified hydroperoxide (5 g) was dissolved in 830 ml of benzene containing 10^{-4} M Cu-naphthenate and the solution was refluxed. The reaction mixture was analyzed for hydroperoxide by HPLC on a Nucleosil ®100-5 column (0.46 × 15 cm) with hexane-diethyl ether (9:1) at a flow rate of 1.5 ml/min, and monitored at 234 nm. When the residual hydroperoxide reached one third of its initial amount, heating was stopped.

Separation of oxo compounds. The reaction mixture was concentrated and chromatographed on a Sephadex

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LH-20 column (1.5×70 cm) with chloroform-hexane (2:1) to separate oxooctadecadienoates. Eluates were fractionated in 2.5-ml portions and each fraction was analyzed by HPLC under the same conditions as described in the section on decomposition, except that the wavelength of the UV detector was 268 nm. Oxooctadecadienoates containing an unknown isomer (compounds I and II) were eluted from 37.5 to 57.5 ml. The separated oxo fractions were then chromatographed on a silica gel column (4.5×20 cm) with 500 ml each of hexane-diethyl ether solvents (95:5, 93:7, 90:10, 85:15, 80:20). The eluates were analyzed by HPLC. The fractions containing compounds I and II were eluted with hexane-diethyl ether (93:7, 90:10, 85:15).

Purification of compounds I and II by HPLC. Compounds I and II were purified first on a Nucleosil ®100-5 column under the same conditions as described in the section on separation of the oxo compounds. They were purified next on a Zorbax ODS column (0.46×15 cm) with methanol-water (9:1) at a flow rate of 1.0 ml/min. The eluates were monitored at 275 nm, and then chromatographed on a Nucleosil ®100-5 column with hexane-diethyl ether (97:3) to isolate each of compounds I and II.

Spectrometry. UV spectra were measured with a Hitachi model 100-50 spectrometer. IR spectra were measured in a KBr tablet with a Hitachi model 285 spectrometer. ^{1}H-NMR spectra were obtained with a Bruker WH-270 spectrometer (270 MHz) in $CDCl_3$ with TMS as an internal standard. Mass spectra were obtained in GC-MS with a JEOL JMS DX-300 mass spectrometer. The GC-column was silar 10C (3 mm×2 m) and column temperature was elevated from 150 to 270°C at a rate of 8°C/min. An EI ionization was used with an ionization current of 70 eV.

Separation of minor isomers of linoleate hydroperoxide. The hydroperoxide of methyl linoleate was injected into HPLC and the minor isomers were isolated. The column was a Nucleosil ®100-5 (0.46×15 cm). The solvent was hexane-diethyl ether (93:7) at a flow rate of 1.5 ml/min. The eluates were monitored at 234 nm. The two isolated isomers were purified by rechromatography under the same conditions as above. They were then reduced to the hydroxy derivatives with ϕ_3P and oxidized to the oxo derivatives with MnO_2[5] in hexane.

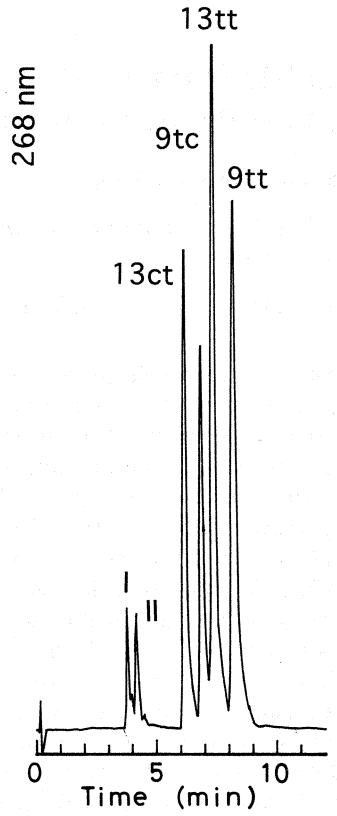

Fig. 2. Separation of Compounds I and II from Known Oxooctadecadienoate by HPLC.

Purified isomeric mixture of oxooctadecadienoates was chromatographed on a Nucleosil ®100-5 column with hexane-diethyl ether (9:1). I, compound I; II, compound II; 13ct, methyl 13-oxo-*cis*-9, *trans*-11-octadecadienoate; 9ct, methyl 9-oxo-*trans*-10, *cis*-12-octadecadienoate; 13tt, methyl 13-oxo-*trans*-9, *trans*-11-octadecadienoate; 9tt, methyl 9-oxo-*trans*-10, *trans*-12-octadecadienoate.

Results and Discussion

Purification of compounds I and II by HPLC

Compounds I and II separated by Sephadex LH-20 and silica gel column chromatography were purified by HPLC. They were chromatographed first on a Nucleosil ®100-5 column with hexane-diethyl ether (9:1). Figure 2 shows a typical chromatogram. Mass spectra of 13ct, 9tc, 13tt, and 9tt in Fig. 2 showed a molecular peak at $m/z=308$, which indicated oxooctadecadienoate.[3,6] After $NaBH_4$ reduction and hydrogenation, mass spectra of TMS derivatives of 13ct and 13tt agreed with that of 13-TMSO octadecanoate and 9tc and 9tt agreed with that of 9-TMSO octadecanoate.[7] Geometry of each isomer was assigned as the hydroxy derivative comparing its retention time with those reported by Chan *et al.*[8] Compound I was eluted at 3.8 min and compound II at 4.2 min and four known oxooctadecadienoates from 6.2 to 8.2 min. Compound I was collected together with compound II and purified on a Zorbax ODS column. The eluting solvent was methanol-water (9:1). Compounds I and II were not separated from each other on this column and eluted together at 10 min. Some peaks of impurities were appeared after compounds I and II were eluted. The separated mixture of compounds I and II was chromatographed again on the Nucleosil ®100-5 column with hexane-diethyl ether (97:3) to isolate each of compounds I and II. The retention times of compounds I and II were 20.6 min and 24.2 min, respectively.

Identification of compounds I and II

The UV spectra of purified compounds I and II showed the absorption maxima at 279 nm in ethanol, which indicated the presence of conjugated dienone chromophores.[9] The mass spectra of compounds I and

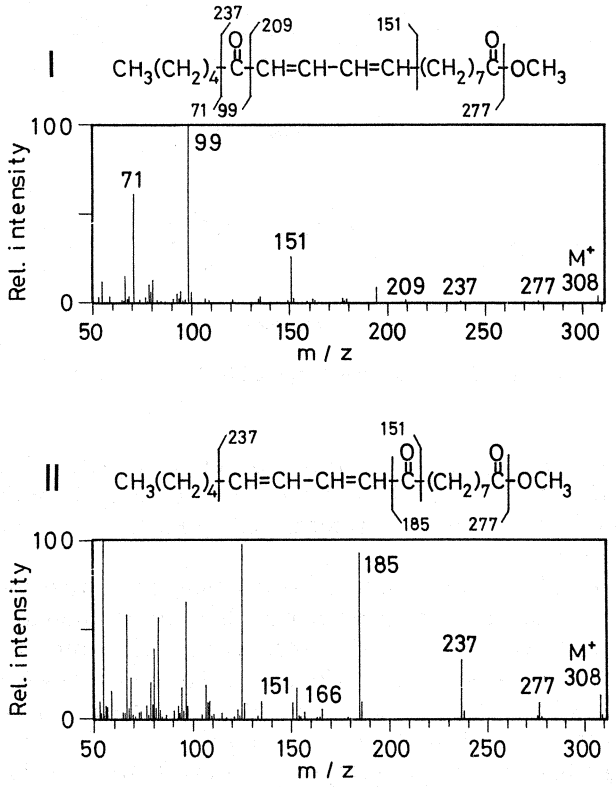

Fig. 3. EI MS of Compounds I and II.

II are shown in Fig. 3. Each spectrum showed peaks at $m/z=308$ and 277, corresponding to the molecular peak and the (M-CH_3O) peak of methyl oxooctadecadienoate, respectively. The fragmentation of compound I resembled that of 13-oxo-9,11-octadecadienoate reported by Hamberg.[3] The major peaks at $m/z=$ 151, 99, and 71 correspond to [M-$(CH_2)_7COOCH_3$], [$CH_3(CH_2)_4CO$], and [$CH_3(CH_2)_4$], respectively. Compound II gave fragment peaks at $m/z=237$ and 185, similarly to 9-oxo-*trans*-10, *trans*-12-octadecadienoate reported by Schieberle *et al.*,[6] and the peaks were assigned to [M-$CH_3(CH_2)_4$] and [$CO(CH_2)_7COOCH_3$]. Compounds I and II were reduced with $NaBH_4$ and hydrogenated with platinum black. The mass spectrum of the TMS derivative of reduced compound I showed fragment peaks at $m/z=173$ (relative intensity, 100%), 315 (41.8%), 73 (55.7%), which indicated that the derivative was 13-TMSO octadecanoate.[7] The mass spectrum of the TMS derivative of reduced compound II showed $m/z=259$ (100%), 229 (96.5%), 73 (81.0%), 355 (3.8%), 371 (3.2%), which indicated that the derivative was 9-TMSO octadecanoate.[7] From these results, it is confirmed that respective compounds I and II are some geometric isomers of methyl 13-oxo-9,11-octadecadienoate and methyl 9-oxo-10,12-octadecadienoate.

The IR spectra of compounds I and II showed absorption bands at 960 and 998 cm^{-1}, which indicated the presence of *cis*, *trans* conjugated dienes.[10]

^{1}H-NMR spectra of compounds I and II in the region of olefinic protons (δ5.91 ~ 7.42 ppm) are shown in Fig.

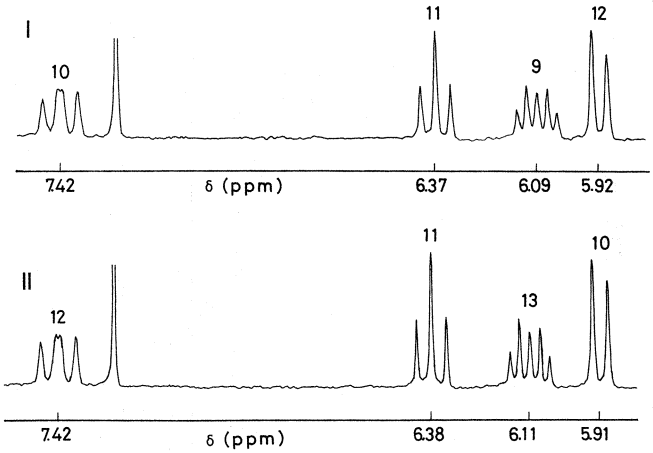

Fig. 4. ^{1}H-NMR Spectra (270 MHz) of Compounds I and II in the Region of Four Olefinic Protons.
9, C-9 proton; 10, C-10 proton; 11, C-11 proton; 12, C-12 proton; 13, C-13 proton.

4. The spectra of compounds I and II were similar. The assignment of these protons were made from consideration of their spin coupling patterns and from decoupring. In compound I, the signal at δ6.37(H-11) coupled with that at δ5.92 (H-12, $J_{11,12}=11.2$ Hz) and the signal at δ6.09 (H-9) coupled with that at δ7.42 (H-10, $J_{9,10}=15.0$ Hz). These coupling constants indicated that C-11 double bond was *cis* and C-9 one was *trans*. In compound II, $J_{10,11}=11.2$ (*cis*) and $J_{12,13}=14.9$ Hz (*trans*) were observed. To confirm the geometry, NOE were analyzed. In compound I, NOE were observed between C-9 and 11, and between C-11 and 12 protons. In compound II, between C-10 and 11 and between C-11 and 13 protons. These results support this geometry assigned from the coupling constants. In every isomer of oxooctadecadienoate hitherto reported, the double bond adjacent to the oxo group is in the *trans* configuration, and the signal of the β-proton to the oxo group appears at a lower magnetic field than the signals of other three olefinic proton do. However, γ-protons appeared at the lower field in compounds I and II. γ-Protons are expected to be affected by oxo groups more than the others when the geometry of adjacent double bonds are in the *cis* configuration. An example in which the signal of γ-proton was shifted to the lower field was reported in the case of 6-oxo-2 (*E*), 4 (*Z*)-heptadienoate,[11] which has an analogous oxo diene structure to compounds I and II. Its chemical shifts of four olefinic protons were 8.271 (H-3), 6.466 (H-4), 6.325 (H-2), and 5.992 (H-5).

From the UV, MS, IR, and ^{1}H-NMR results, compounds I and II were identified as methyl 13-oxo-*trans*-9, *cis*-11-octadecadienoate and methyl 9-oxo-*cis*-10, *trans*-12-octadecadienoate. The amount of each of these isomers was about 2% of total oxo isomers.

Survey of geometric isomers of hydroperoxide

Porter *et al.* have suggested[12] that conjugated hydroperoxides other than the known four isomers (13-hydroperoxy-*cis*-9, *trans*-11-octadecadienoate, 13-hydroperoxy-*trans*-9, *trans*-11-octadecadienoate, 9-

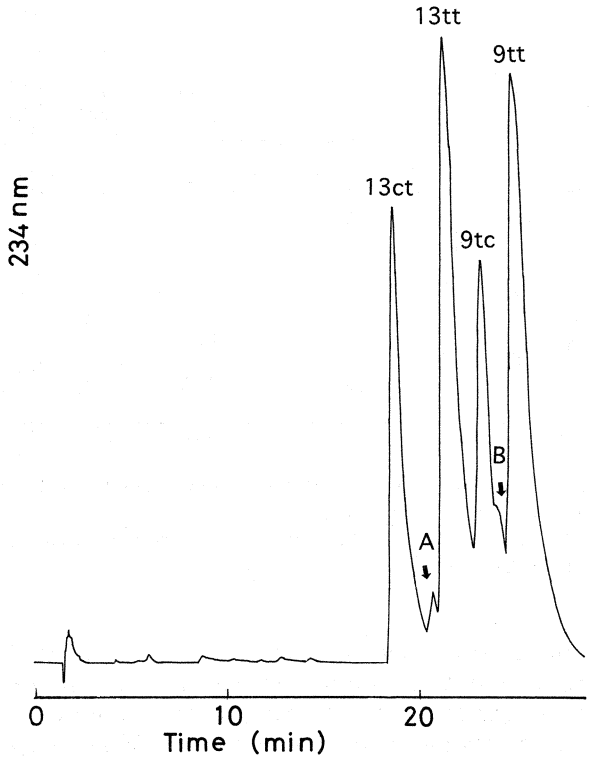

Fig. 5. HPLC Separation of Minor Isomers of Hydroperoxide (A and B).

Hydroperoxide of autoxidation product was injected into HPLC on a Nucleosil ®100-5 column and eluted with hexane-diethyl ether (93:7) at a flow rate of 1.5 ml/min. 13ct, 13-hydroperoxy-*cis*-9, *trans*-11-octadecadienoate; 13tt, 13-hydroperoxy-*trans*-9, *trans*-11-octadecadienoate; 9tc, 9-hydroperoxy-*trans*-10, *cis*-12-octadecadienoate; 9tt, 9-hydroperoxy-*trans*-10, *trans*-12-octadecadienoate.

hydroperoxy-*trans*-10, *cis*-12-octadecadienoate, and 9-hydroperoxy-*trans*-10, *trans*-12-octadecadienoate) were produced when linoleate and three other geometric isomers of 9, 12-octadecadienoate were oxidized in the presence of 1.5–3 M cyclohexadiene. They reported that these minor conjugated hydroperoxides were less than 5 mol% of the total hydroperoxides.

We investigated whether the hydroperoxide produced by autoxidation contains the minor isomers that have the same geometry as those of compounds I and II or not. Methyl linoleate hydroperoxide was analyzed by HPLC (Fig. 5). Four peaks of the hydroperoxide (13ct, 13tt, 9tc, and 9tt in Fig. 5) were isolated by HPLC and reduced with ϕ_3P to hydroxy derivatives. The position of hydroxy group and the geometry of double bonds were assigned as in the same manner as described for known oxooctadecadienoate. Figure 5 shows small peaks at the retention time of 20.8 min (A) and 24.2 min (B). These were isolated and purified by rechromatography and converted to the oxo derivatives. The mass spectra of the oxo derivatives of A and B were the same as those of compounds I and II, respectively. The retention times of the oxo derivatives of A and B on a Nucleosil column by HPLC agreed with those of compounds I and II, respectively. Therefore, A and B were considered to be 13-hydroperoxy-*trans*-9, *cis*-11, and 9-hydroperoxy-*cis*-10, *trans*-12-octadecadienoate. The amount of each of these isomers was about 0.4% of the total hydroperoxides, as measured by HPLC as the oxo derivatives.

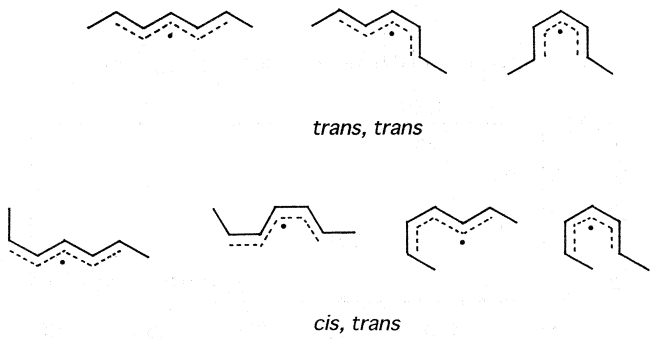

Fig. 6. Dienyl Radicals Proposed by Frankel *et al.*[13]

Frankel *et al.*[13] have reported that hydroperoxides which has the same geometry as those of compounds I and II were produced when three geometrical isomers of 2,5-heptadiene were oxidized. 2-Hydroperoxy-*cis*-3, *trans*-5-heptadiene was produced from the *trans*, *trans* isomer of heptadiene as 6–10% of the total hydroperoxides and from the *cis*, *trans* isomer as 3–5%. From the *cis*, *trans* isomer of heptadiene, a trace of *cis*, *cis* hydroperoxide was also detected. They consider that these minor isomers would be produced not through isomerization of peroxy radical but from pentadienyl radicals of different conformations as shown in Fig. 6.

It has been reported that edible oils such as corn, soybean, and rapeseed oils contain *trans* octadecadienoic acids at less than 0.1% of the total fatty acids.[14] The methyl linoleate used in our experiment may contain *trans* acid. However, the yields of compounds I and II were higher than those expected to be produced from the *trans* acids that would be contained in methyl linoleate. The proportion of compounds I and II in oxooctadienoate were also higher in comparison with that of A and B.

We will examine whether compounds I and II are produced from four major isomers of the hydroperoxides in the course of decomposition catalyzed by Cu-naphthenate.

Acknowledgments

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