Structures of Dimers Produced from Methyl Linoleate during Initial Stage of Autoxidation

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A structural investigation on the main fraction of dimers formed during the induction period of autoxidation in methyl linoleate (ML) was carried out. The dimeric fraction (A2), which was isolated from the autoxidized ML (POV = 18) by various chromatographic techniques and gave a single spot on TLC, was further separated into four major components (components 1 ~ 4) by high performance liquid chromatography (HPLC). The mean molecular weights of these components were found to be 643 ~ 655 and component 4 gave the parent peak 652 on an FD-mass spectrum which corresponded to 2 x ML + 40. The reduced products of each component with stannous chloride were identified in common as methyl 9- or 13-hydroxy octadecadienoate and methyl 9,13-dihydroxy octadecenoate by GC-MS. These results show that all of these dimers contained a peroxide bridge linking between a pair of MLs across C-9 or C-13 on one of the MLs and C-9 or C-13 on the other, with a hydroperoxy group.

It is generally accepted that the primary products of autoxidized lipids are monomeric hydroperoxides and that the secondary products, including such as volatile carbonyls and polymers, are formed by the decomposition or polymerization of accumulated hydroperoxides in the latter half of the autoxidation.1,2) However, in the preceding paper3) we found that a considerable amount of dimers besides MLHPO was formed even during the induction period of ML autoxidation. The dimers could be roughly divided into two main fraction (A1, A2) by TLC, and most of these dimers were linked with a C-O-O-C bond. In this paper, the main components of these dimers were isolated by HPLC and their structures were analyzed by GC-MS and FD-MS.

MATERIALS AND METHODS

Preparation of autoxidized ML. ML was prepared from the methyl esters of safflower oil and purified by silicic acid column chromatography.3) The procedure for autoxidation and the methods for POV and TLC have been described in the previous paper.3)

Isolation of dimers from autoxidized ML. The autoxidized ML (POV = 18, 100 g) was applied to a silicic acid column to remove the intact ML. For the column chromatography, a column of Wakogel C-100 (Wako Pure Chem. Ind., 2.1 kg) was used. The column was eluted in steps with 6% diethyl ether in n-hexane and 100% ether. The ether-eluate fraction was successively fractionated by gel chromatography as described in the preceding paper.3)

The dimeric fraction obtained by gel chromatography was further purified from contaminated MLHPO and separated into two fractions (A1, A2) by a Sep-Pak Cartridge Silica (Waters Assoc. Inc.). Namely, a cartridge which was loaded with the sample was eluted with the following three solvents: 1) ether–hexane (1:9) 10 ml, 2) ether–hexane (2:8) 10 ml, 3) ether 10 ml. The fraction eluted with ether–hexane (2:8) was successively fractionated by HPLC. HPLC was carried out on a 25 x 0.5 cm pyrex glass column packed with Kyowagel MIC SI-10 (Kyowa Seimitsu Co. Ltd.) at a 0.6 ml/min flow rate. The column eluent was monitored with a variable wavelength UV detector (Kyowa Seimitsu KLC-200; Kyowa Seimitsu Co. Ltd.) set at 233 nm. The eluting solvent consisted of 1.5% isopropyl alcohol in n-hexane.

Preparation of derivatives. Chemical reduction of the peroxide groups contained in the dimers by stannous chloride was carried out according to Mizuno.4) The reduced products were silylated by bis trimethyl silyl acetamide (Tokyo Kasei Co. Ltd.).

Analytical procedures. Mean molecular weights (MMW)
were determined by the vapor pressure equilibrium method as described in the preceding paper.\textsuperscript{3)} FD-mass spectrum was determined on a JEOL JMS 01-SG2 apparatus. The mass resolution was 1000 and this spectrum was taken at an emitter heating current of 16 mA. The FD emitter was prepared by high temperature activation at 10 μm dia. The accelerating voltage was 8 kV for the field anode and −5 kV for the slotted cathode plate.

GC-MS was carried with a Shimadzu LKB-9000 apparatus equipped with a glass column (0.3 cm x 200 cm) containing 3% SE-30 on Chromosorb W (60/80 mesh), programmed from 200 to 240°C at 2°C/min. Operational conditions for mass spectrometry were as follows: ion source temp., 270°C; separator temp., 250°C; ionizing electron energy, 25 eV.

RESULTS AND DISCUSSION

The autoxidized ML (POV = 18) was divided into 4 spots (A\textsubscript{1}: R\textsubscript{f}=0.0-0.1, A\textsubscript{2}: R\textsubscript{f}=0.1-0.2, A\textsubscript{3}: R\textsubscript{f}=0.22, A\textsubscript{4}: R\textsubscript{f}=0.5) on TLC as reported in the previous paper.\textsuperscript{3)} Among these spots, it was ascertained\textsuperscript{3)} that both A\textsubscript{1} and A\textsubscript{2} were dimers, whereas A\textsubscript{3} and A\textsubscript{4} were MLHPO and ML, respectively. The dimeric fractions (A\textsubscript{1}+A\textsubscript{2}) were satisfactorily isolated from ML and MLHPO via the successive column chromatography on silicic acid and Bio-Beads gel. The yield of the dimers from the autoxidized ML was approximately 0.025%, which was almost equal to the data reported previously. Because the amount of A\textsubscript{2} between the two fractions of the dimers was about four times of that of A\textsubscript{1}, the structural studies were attempted on A\textsubscript{2} in this paper.

The A\textsubscript{2} fraction could be further isolated from A\textsubscript{1} by Sep-Pak Cartridge Silica. Namely, after adsorption on a Sep-Pak, the small contaminated amount of MLHPO was eluted with ether–hexane (1:9), then A\textsubscript{2} with ether–hexane (2:8), and finally A\textsubscript{1} with ether. The A\textsubscript{2} fraction which was thus obtained gave a single but rather wide spot on TLC.

Figure 1 shows the resolution of A\textsubscript{2} into 4 major fractions (components 1-4) by HPLC. The preparative fractionation of these components on HPLC successfully produced the complete isolation of component 4, but the other components were contaminated with a small amount of neighboring peaks. The measurement of MMW of the components 1-4 (1:650, 2:643, 3:655, 4:644) revealed that all of these components were dimers and were composed of two moles of ML plus 3 to 4 mol of oxygen. As they showed almost the same molecular weights and gave an equal R\textsubscript{f} value on TLC, it was predicted that they should be closely related analogs, presumably isomers.

By reduction with stannous chloride, every component commonly gave three peaks (a-1, a-2 and b) with inherent retention times on gas chromatograms after silylation as shown in Fig. 2. The mass spectra were almost identical between peaks a-1 and -2, and indicated that they were TMS derivatives of methyl 9- and/or 13-hydroxy octadecadienoate by the characteristic peaks (382 [M\textsuperscript{+}], 311, 225). (Fig. 3). Peak b was identified as the TMS derivative of methyl 9,13-dihydroxy octadecenoate by comparing it with the data described by Terao et al.\textsuperscript{5)} and Neff et al.\textsuperscript{6)} Peaks a-1 and -2 were
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expected to be TMS-trans,cis and TMS-trans,trans isomers, respectively.\(^7\) Total peak area of the monohydroxy esters was approximately equal to that of dihydroxy ester on a gas chromatogram. Therefore, one mol of monohydroxy and one mol of dihydroxy ester should be generated by postulating cleavage of a peroxide linkage in a dimer. Consequently, it is presumed that the original dimers contained one peroxide linkage and one hydroperoxy or hydroxyl group per one molecule. However, GC-MS data did not make clear which carbon (9 or 13) of dihydroxy esters contributed to the C–O–O–C bonds.

Figure 4 shows the FD mass spectrum of component 4. In this mass spectrum, com-

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**Fig. 3.** Mass Spectra of a-1, a-2 and b.

**Fig. 4.** FD Mass Spectrum of Component 4.
ponent 4 gave a distinct molecular ion peak (652). The remarkable features which were found to be characteristic in the assignment of this mass spectrum were 635 (M–OH), 619 (M–OOH), 325 (C_{19}H_{33}O_{4}), 309 (C_{19}H_{33}O_{3}) and 293 (C_{19}H_{33}O_{2}). Fragment peaks 325, 309 and 293 were derived from the cleavage across the peroxide linkage. From these data, it was confirmed that component 4 was linked through a peroxide bond and contained one hydroperoxy group per molecule.

The postulated structures of components 1–4 led by the combined results of GC- and FD-MS are given below.

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\begin{align*}
\text{CH}_3-(\text{CH}_2)_4-\text{CH} &= \text{CH} = \text{CH} = \text{CH} = (\text{CH}_2)_7-\text{COOCH}_3 \\
\text{CH}_3-(\text{CH}_2)_4-\text{CH} &= \text{CH} = \text{CH} = \text{CH} - (\text{CH}_2)_7-\text{COOCH}_3 \\
\text{CH}_3-(\text{CH}_2)_4-\text{CH} &= \text{CH} = \text{CH} = \text{CH} = (\text{CH}_2)_7-\text{COOCH}_3 \\
\text{CH}_3-(\text{CH}_2)_4-\text{CH} &= \text{CH} = \text{CH} = \text{CH} = (\text{CH}_2)_7-\text{COOCH}_3 \\
\text{CH}_3OOC-(\text{CH}_2)_7-\text{CH} &= \text{CH} = \text{CH} = \text{CH} = (\text{CH}_2)_4-\text{CH}_3 \\
\text{CH}_3-(\text{CH}_2)_4-\text{CH} &= \text{CH} = \text{CH} = \text{CH} = (\text{CH}_2)_7-\text{COOCH}_3 \\
\text{CH}_3-(\text{CH}_2)_4-\text{CH} &= \text{CH} = \text{CH} = \text{CH} = (\text{CH}_2)_7-\text{COOCH}_3 \\
\text{CH}_3OOC-(\text{CH}_2)_7-\text{CH} &= \text{CH} = \text{CH} = \text{CH} = (\text{CH}_2)_4-\text{CH}_3 (X \text{ is } -\text{OOH} \text{ or } -\text{OH})
\end{align*}
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Presumably the double bond will be located at C: 10–11 or C: 11–12. X was identified as a hydroperoxy group only in component 4. However, judging from the similarity of each component of A_2 on TLC and HPLC, it is assumed that X is a hydroperoxy group in each of the components.

The complexities of the A_2 dimeric fraction on HPLC (Fig. 1) may be attributed to the fact that this fraction was composed of mixtures of positional and geometric isomers.

The mechanism for the formation of these dimers has not been elucidated at all, however, because neither dihydroperoxide nor the dimer without hydroperoxide has been found in the autoxidized ML during the initial stage of autoxidation. It may be reasonable to say that the dimer is formed by the combination of two mol of MLHPO.

Terao et al.\textsuperscript{5) identified further oxygenated MLHPO after reduction with NaBH\textsubscript{4} by GC-MS as methyl 9-hydroxy octadecadienoate, methyl 13-hydroxy octadecadienoate, methyl 9,13-dihydroxy octadecenoate and methyl 9,12,13- or 9,10,13-trihydroxy octadecenoate. Neff et al.\textsuperscript{6) fractionated the NaBH\textsubscript{4} reduced autoxidized ML (POV: 1249) by preparative HPLC and identified them as hydroxy-
trans,cis- and trans,trans-octadecadienoates, dihydroxy-, trihydroxy- and epoxyhydroxy octadecenoates. The NaBH$_4$ reductant severs the C–O–O–C bonds, therefore, it can be assumed that a part of the above hydroxy compounds are derived from dimers linked through C–O–O–C bonds.

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**REFERENCES**