Structures of Ozonolysis Products of Methyl Oleate Obtained in a Carboxylic Acid Medium[1]

Naoki Nishikawa*, Kaoru Yamada, Shigeaki Matsutani, Moriaki Higo, Hitoshi Kigawa and Takeo Inagaki

Lion Corporation, Tokyo, Japan

ABSTRACT: High-performance liquid chromatography–mass spectrometry (LC–MS) and nuclear magnetic resonance spectrometry (NMR) were applied to the analysis of organic peroxide mixtures, which were labile and tended to decompose during analysis. The ozonolysis reaction of methyl oleate gives a peroxide mixture, and, finally, mono- and dibasic acids are obtained by subsequent oxidation. In this study, methyl oleate was ozonized in a nonanoic acid medium, one of the final reaction products. The reaction products were directly analyzed by LC–MS equipped with a frit-fast atom bombardment interface. The molecular ion peak of each peroxide was clearly observed, and its molecular weight was readily determined. On the other hand, each peroxide was fractionated by high-performance liquid chromatography and submitted to structural analysis by NMR. Both results indicated that the reaction products include four peroxidic species: 1,2,4-trioxolane **I**, peroxide oligomer **II**, 1-acyloxyalkyl-1-hydroperoxide **III**, and 1-acyloxyalkyl-1′-hydroxyalkyl peroxide **IV**, as well as an aldehyde **V**. Ozonolysis of methyl oleate in the absence of solvent produces mainly **I**, while that in the presence of a carboxylic acid solvent characteristically produces mainly **III** and **IV** derived from the solvent. *Bis*(1-acyloxyalkyl-1-alkyl) peroxide, which was reported previously as a ozonolysis product of methyl oleate, was concluded to be **IV** in this study.

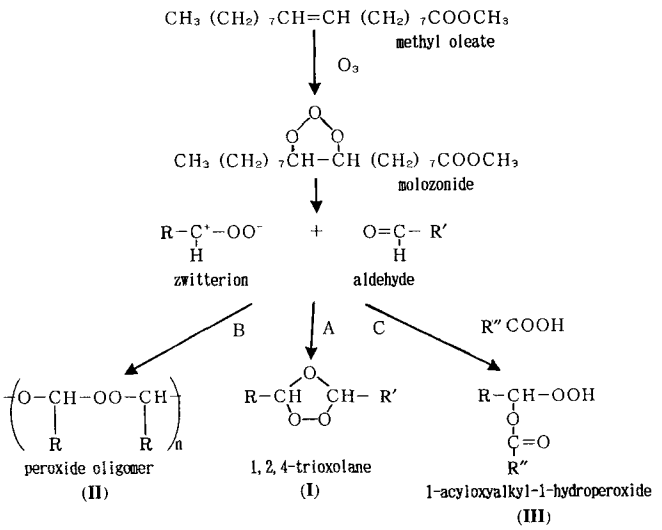
JAOCS 72, 735–740 (1995).

KEY WORDS: 1-Acyloxyalkyl-1-hydroperoxide, 1-acyloxyalkyl-1′- hydroxyalkyl peroxide, LC–MS, methyl oleate, NMR, oleic acid ozonolysis, 1,2,4-trioxolane.

Mass spectrometric analysis of a peroxide mixture is quite difficult because the peroxides have labile structures and tend to decompose during separation or condensation. Mochida *et al.* (1) obtained a molecular ion peak MH^+ of an aliphatic acid hydroperoxide by liquid ionization mass spectrometry (MS). To apply this technique to a peroxide mixture, however, each peroxide needs to be separated by some chromatographic procedure prior to mass analysis. The authors have tried the direct analysis of a peroxide mixture by high-performance liquid chromatography–mass spectrometry (LC–MS), which may be one of the most effective methods for the analysis of such compounds.

Ozonolysis of methyl oleate has been reported in many papers, and the product is a peroxide mixture whose reaction mechanism is of much interest for establishing an industrial process for manufacturing mono- and dibasic aliphatic acids. Much research on the ozonolysis of organic compounds has been carried out with low-molecular weight olefins (2–5). The main ozonolysis products generally include the 1,2,4-trioxolane structure, which is a normal ozonide (5–8). Criegee's interpretation of the ozonolysis reaction mechanism is at present generally regarded as reasonable (2,9). According to the Criegee mechanism, the ozonolysis reaction of methyl oleate in the absence of solvent produces 1,2,4-trioxolane **I** and peroxide oligomer **II**, while that in the presence of a protonic solvent, such as carboxylic acids, produces 1-acyloxyalkyl-1-hydroperoxide **III** (10,11) in addition to the other two compounds (Scheme 1).

Bis(1-acyloxyalkyl-1-alkyl) peroxide was recently identified by ^1H nuclear magnetic resonance (NMR) analysis in the



R = $-(CH_2)_7COOCH_3$ and/or $-(CH_2)_7CH_3$

R′ = $-(CH_2)_7CH_3$ and/or $-(CH_2)_7COOCH_3$

R″ = $-(CH_2)_7CH_3$ (nonanonic acid)

SCHEME 1

[1]This work was presented at the 31th Annual Meeting of the Japan Oil Chemists' Society, 1992, Tokyo, Japan.

*To whom correspondence should be addressed at 13-12 Hirai 7 Chome, Edogawa-Ku, Tokyo 132 Japan.

ozonolysis products of methyl oleate in addition to **I** and **III** (10). In our study, methyl oleate was ozonized in nonanoic acid, one of the final reaction products of ozonolysis and subsequent oxidation. The molecular weight of each reaction product was determined directly by LC–MS, and the detailed NMR structural analysis of each peroxide, fractionated by high-performance liquid chromatography (HPLC), was also carried out.

EXPERIMENTAL PROCEDURES

Materials. Methyl oleate ($C_{17}H_{33}COOCH_3$ purity: 99.9%) and nonanoic acid ($C_8H_{17}COOH$ purity: 99.9%) were purchased from NOF Corp. (Tokyo, Japan) and Sigma Chemical (St. Louis, MO), respectively.

Ozonolysis. One gram (1.0 g) of methyl oleate was weighed out into a sample tube, and 1.0 g of nonanoic acid was added. Ozone was generated by a silent-discharge ozone generator at a rate of 50 mL/min. A mixed gas of ozone and air was bubbled into the sample tube at 25°C for approximately three hours. The reaction mixture was immediately cooled down to −20°C and stored.

LC–MS conditions. LC–MS analysis was carried out under the following conditions: column, Inertsil ODS-2 (4.6 mm i.d. × 150 mm L; GL Science, Tokyo, Japan); mobile phase, methanol/water (95:5 vol/vol, containing 3 mM KCl); flow rate, 1.0 mL/min; matrix, m-nitrobenzyl alcohol (1.5%) in methanol; flow rate, 0.3 mL/min; post-column addition; LC–MS, JMS-DX303 mass spectrometer (JEOL Ltd., Tokyo, Japan) equipped with LC system (Yokogawa Analytical Systems, Tokyo, Japan) and Frit-fast atom bombardment (Frit-FAB) interface (JEOL Ltd.).

NMR. Approximately 20 mg of a sample was dissolved in 1.0 mL $CDCl_3$, and an ^1H NMR spectrum was recorded with a GSX-400 NMR spectrometer (400 MHz; JEOL Ltd.). ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra were also recorded with that instrument (sample 0.2 g). Tetramethylsilane was used as the standard substance.

Gel permeation chromatography (GPC). Peroxide oligomer **II**, mentioned previously, was identified and quantitated by GPC under the following conditions: column, SHODEX G1000HXL (7.8 mm i.d. × 300 mm L; Showa Denko, Tokyo, Japan); mobile phase, tetrahydrofuran; flow rate, 1.0 mL/min; detector, refractive index detector.

RESULTS AND DISCUSSION

LC–MS of ozonolysis products. The ozonolysis products obtained in the nonanoic acid medium were analyzed by LC–MS. The chromatogram is shown in Figure 1. Peak a at 9.8 min corresponds to nonanoic acid. Peak a and peaks 1–5 are the peaks that are not observed in the chromatogram of the ozonolysis products obtained in the absence of the nonanoic acid medium. Peak b at 25.7 min was assigned from the spectrum (not shown) to either the *cis*- or *trans*-isomer of

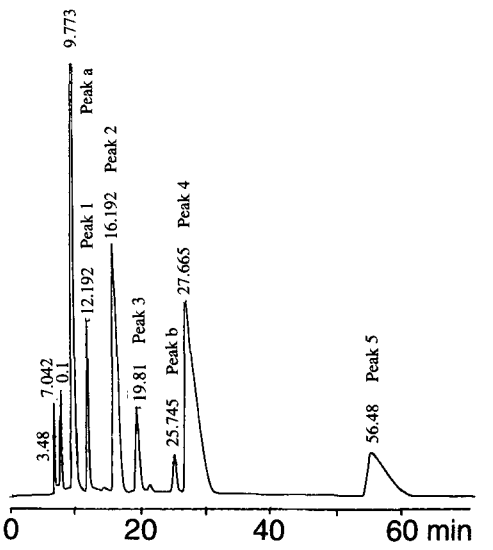


FIG. 1. High-performance liquid chromatography of ozonolysis products of methyl oleate in a nonanoic acid medium.

I (8). The other isomer overlapped on peak 4. FAB–MS spectra of peaks 1–5 are illustrated in Figure 2. Addition of potassium chloride to the mobile phase was effective for easy detection of the molecular ion. Figure 2 reveals that the molecular weights of the components corresponding to peaks 1–5 are 360, 546, 316, 502, and 458, respectively.

Each compound corresponding to the peaks on the chromatogram was fractionated by HPLC. The compounds corresponding to peaks 1–5 were colored by the addition of a 10%

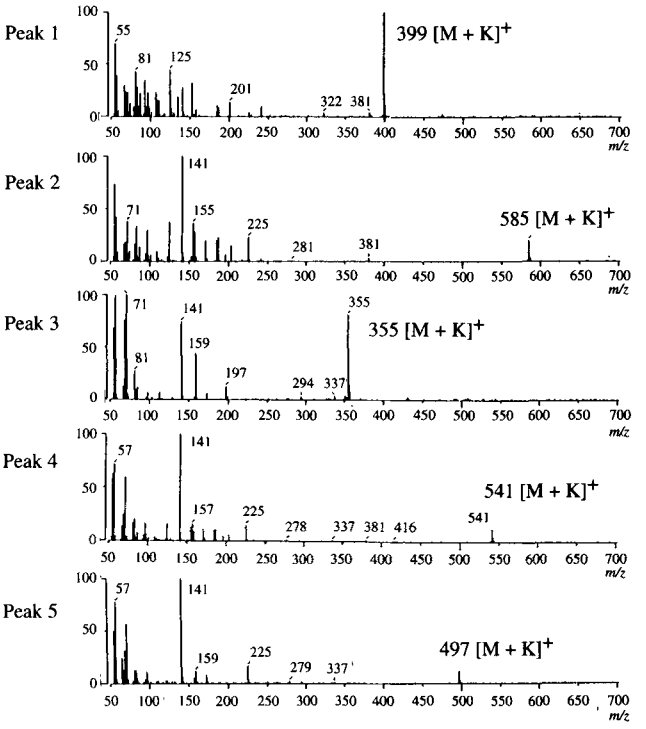


FIG. 2. Fast-atom bombardment–mass spectrometry spectra of ozonolysis products of methyl oleate in a nonanoic acid medium.

KI solution, and that corresponding to peak b was not colored. Therefore, the components of peaks 1–5 are peroxides with higher oxidizing power than an ozonide with the 1,2,4-trioxolane structure.

^1H NMR of ozonolysis products. Figure 3A is a spectrum of the ozonolysis products of methyl oleate in the absence of solvent. According to Criegee's mechanism, both **I** and **II** are produced as shown in Scheme 1 (route A and B). The signal corresponding to the methyl ester group (–$COOCH_3$) is observed as a sharp singlet at 3.7 ppm. Two triplets at 5.13 and 5.18 ppm are assigned to the ring protons of **I**. The resolution of the signal into two triplets may be caused by *cis-trans* isomerism (8). Compound **II** was not confirmed in the spectrum, but was identified and quantitated by GPC. It may be due to the lower quantity and signal broadness caused by the high molecular weight.

Figure 3B illustrates a spectrum of the ozonolysis products of methyl oleate in the presence of a nonanoic acid solvent. The signals of the ring proton of **I** are observed at 5.13 and 5.18 ppm; the latter overlaps the multiplet signal ranging from 5.16 to 5.26 ppm. Although the signal of **II** was not observed in the spectrum, it was identified by GPC. The signals at 5.1–6.5 ppm are, however, distinctly different from those in Figure 3A. The reaction in a carboxylic acid medium may produce **III** as well as **I** and **II**. A recent study (11) reported that the chemical shift of the methine proton of **III** is at 6.19 ppm. Three multiplets at 5.22, 6.30, and 6.40 ppm are, however, not attributed to compounds **I**, **II**, and **III**, that are estimated to be produced in the reaction. Therefore, ozonolysis of methyl oleate in the carboxylic acid medium produces compounds other than **I**, **II**, or **III**. The signal observed at 9.8 ppm corresponds to a formyl proton of an aldehyde of **V**.

*Identification of 1-acyloxyalkyl-1-hydroperoxide **III**: components corresponding to peaks 1 and 3.* The ^1H NMR spectra of the components, fractionated by HPLC and corresponding to peaks 1–5 are illustrated in Figure 4. The compounds corresponding to peaks 1 and 3 are considered to be **III**, based on the signal of the triplet at 6.17 ppm (11) and the molecular weight determined by LC–MS. The singlet at 3.7 ppm (–$COOCH_3$) is observed in the spectrum of peak 1. The signal intensity is nearly equal to that of the terminal methyl at 0.9 ppm. In the spectrum of peak 3, however, this signal is not observed. These signal intensities are listed in Table 1. The LC–MS results re-

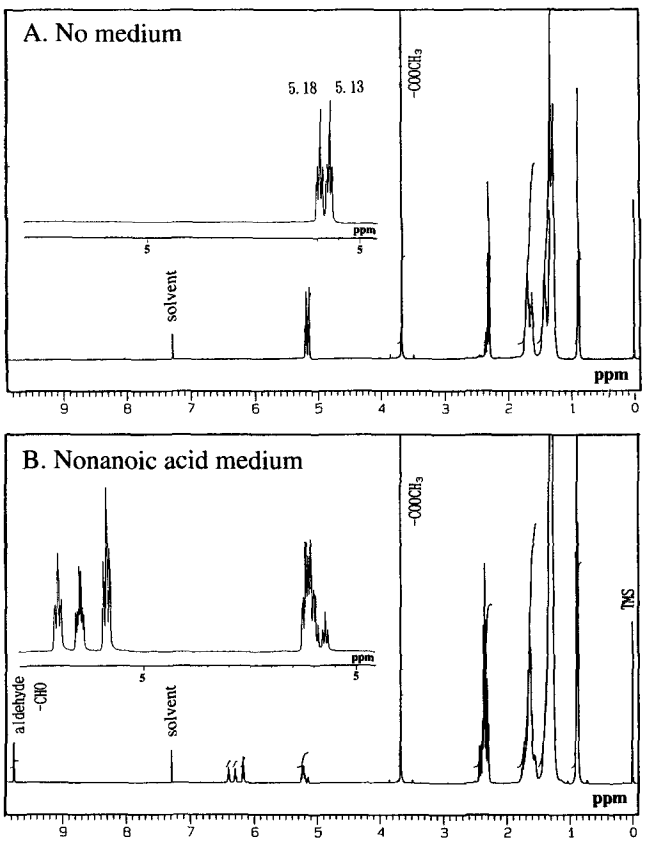


FIG. 3. ^1H Nuclear magnetic resonance spectra of ozonolysis products of methyl oleate. TMS, tetramethylsilane.

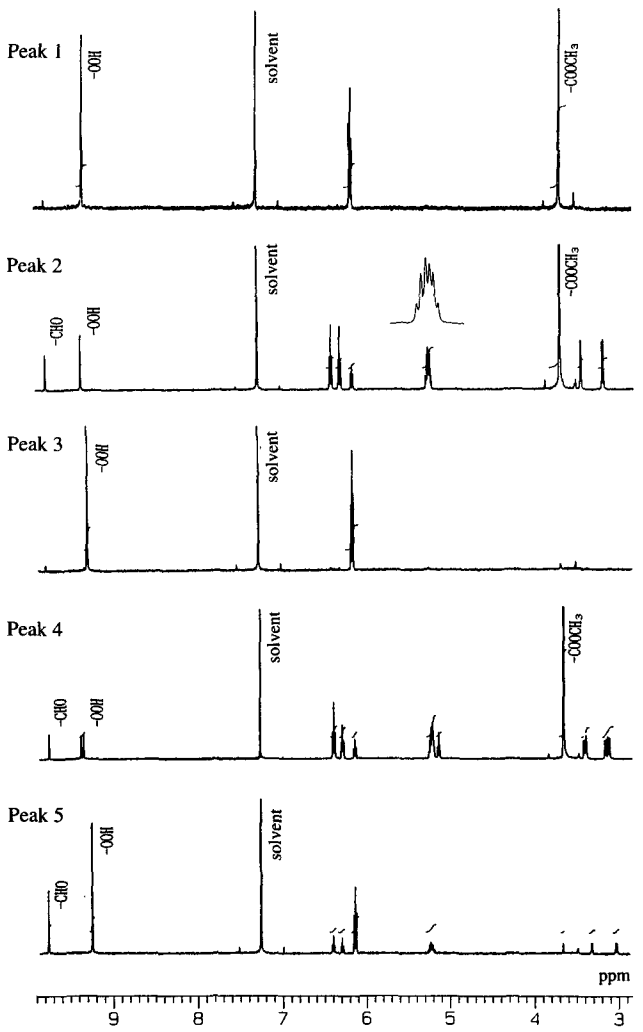


FIG. 4. ^1H Nuclear magnetic resonance spectra of ozonolysis products of methyl oleate fractionated by high-performance liquid chromatography.

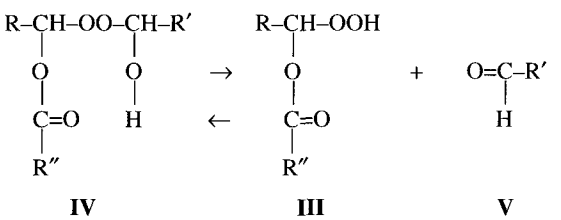
TABLE 1
Signal Intensity of ^{1}H Nuclear Magnetic Resonance of Peroxides

	Intensity				
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Alkyl methyl (CH_3–$(CH_2)_7$–)	3.0	3.0	3.0	3.0	3.0
Ester methyl (CH_3–OOC–$(CH_2)_7$–)	2.8	5.7	0	1.8	0.1

vealed that the molecular weights of these compounds are 360 and 316. It can be concluded, therefore, that the compounds corresponding to peaks 1 and 3 are **III** with different alkyl chains, namely, the addition products of nonanoic acid to the zwitter ion described in Scheme 1. Peaks 1 and 3 are assigned to $CH_3OOCC_7H_{15}$–$CH(OOCC_8H_{17})OOH$ and C_8H_{17}–$CH(OOCC_8H_{17})OOH$, respectively. The signal at 9.3 ppm belongs to the proton of the hydroperoxide group (–OOH).

Structure of 1-acyloxyalkyl-1′–hydroxyalkyl peroxide ***IV****: components corresponding to peaks 2, 4, and 5.* Eight signals with a common chemical shift and different intensities are observed in the range from 3.0 to 9.9 ppm in the spectra of peaks 2, 4, and 5, in addition to a signal of ester methyl proton at 3.6 ppm (Fig. 4). These signals suggest that the three compounds have a similar structure. The structure of the compound corresponding to peak 2 was analyzed in detail. The result of DEPT indicates that four signals, ranging from 5.2 to 6.4 ppm, are attributed to the methine protons. The signals at 3.0–3.4 ppm and at 9.3 ppm disappeared from the spectrum when D_2O was added, which indicates that the protons corresponding to these signals were active protons. Because the singlet signal at 9.3 ppm is the same as that observed in the spectra of peaks 1 and 3, it is assigned to the hydroperoxide group (–OO*H*). It can be inferred from the chemical shift that the signal at 9.8 ppm corresponds to the formyl proton of the aldehyde group. Careful observation of the spectrum reveals that the multiplet at 5.2 ppm comprises two triplets.

Zelikman *et al.* (12) reported, based on his experiment with olefin, that **IV** is produced from **III** and **V** and that the reaction is reversible. For ozonolysis of methyl oleate, it is expected that a similar reaction will occur. Figure 5A is a spectrum of the compound corresponding to peak 2 fractionated by HPLC and measured immediately. The sample was then allowed to stand for 60 h and was analyzed again by NMR (Fig. 5B). The intensities of signals 2 and 6 increased, and those of signals 1, 1′, 3, and 4 decreased. The same



IV **III** **V** [1]

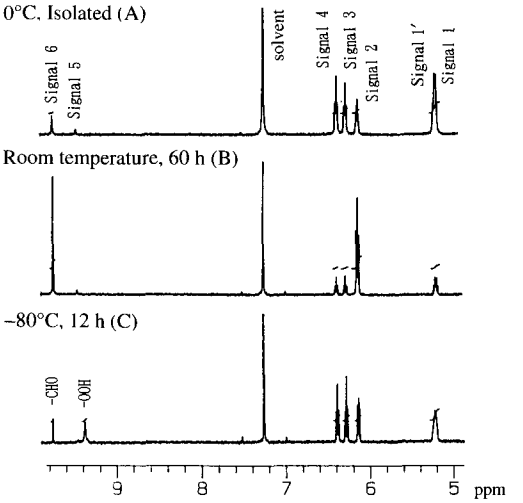


FIG. 5. Variation in ^{1}H nuclear magnetic resonance spectrum of peak 2 with time.

sample was kept at –80°C for 12 h and analyzed once more (Fig. 5C). The intensity of signals 2 and 6 decreased, and those of the other signals increased. These results are summarized in Table 2.

It is concluded, therefore, that signals 2 and 5 are derived from **III**, and signals 1, 1′ 3, and 4 are from **IV**, and that the compound of peak 2 was converted into **III** and **IV** after fractionation by HPLC. The molecular weights obtained by LC–MS and the result of NMR indicates that R and R′ described in Equation 1 are $-(CH_2)_7COOCH_3$. The assignment of signals 1, 1′, 3, and 4 to the structure of **IV** is mentioned later.

Confirmation of diastereoisomer. Because both methine carbons in the structure of **IV** are asymmetric, diastereoisomers exist. The fraction corresponding to peak 2 was further fractionated into two fractions by HPLC, and both were freeze-dried and analyzed by NMR (Fig. 6).

The spectrum of the former fraction reveals that the intensity of signal 3 is higher than that of signal 4, and the intensity of signal 1 is also higher than that of signal 1′. The relationships are opposite to those of the latter fraction. These results indicate that **IV** is a mixture of two components: one is the compound corresponding to signals 1 and 3, and the other corresponds to signals 1′ and 4. Because the molecular weight of both fractions is determined to be 546, they are isomers, as illustrated in Scheme 2. The relationship of a and b vs. c and d is diastereoisometric. Because a vs. b and c vs. d are mirror images, there is no difference between the results of the respective compounds by NMR and HPLC. Their chemical shifts revealed that R–*CH*(OOCR)–OO– can be assigned to signals 3 and 4 and –OO–*CH*(OH)–R′ to signals 1 and 1′. The signals at 3.1 and 3.4 ppm are assigned to –OO–CH(O*H*)R′ in Figure 4. This proton has two chemical shift values due to the existence of the diastereoisomer.

Structure of compounds corresponding to other HPLC peaks. The NMR spectra in Figure 4 reveal that the compo-

TABLE 2
Variation in Signal Intensity of Peak 2 in ^{1}H Nuclear Magnetic Resonance Spectrum with Time[a]

	Intensity						
	Alkyl methyl	Signal 1	Signal 1′	Signal 2	Signal 3	Signal 4	Aldehyde (CHO)
0°C, Isolated (A)	3.00	0.34	0.34	0.23	0.30	0.30	0.06
Room temperature, 60 h (B)	3.00	0.11	0.11	0.71	0.10	0.10	0.49
−80°C, 12 h (C)	3.00	0.34	0.34	0.32	0.31	0.31	0.06

[a]A, B, and C represent results shown in Figure 5.

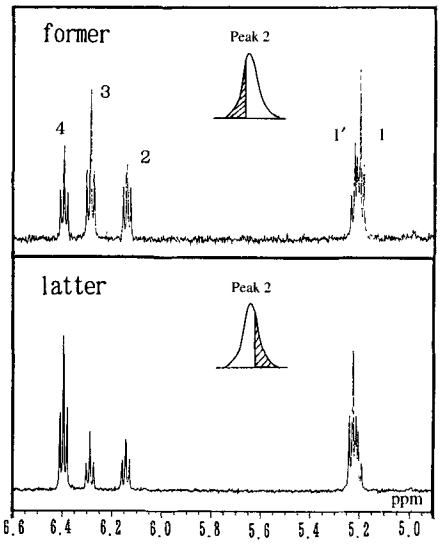
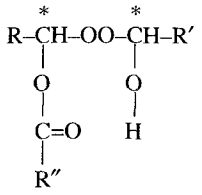


FIG. 6. ^{1}H Nuclear magnetic resonance spectra of peak 2 fractionated by high-performance liquid chromatography.

nents corresponding to peaks 4 and 5 have the same skeletal structure as that corresponding to peak 2. The molecular weights and the signal intensities of NMR indicated that the difference is derived from the end group, methyl or methyl ester, in the same manner as the difference between peaks 1 and 3. The structures of the compounds corresponding to peaks 2, 4, and 5 are as follows (1-acyloxyalkyl-1′-hydroxyalkyl peroxide **IV**):



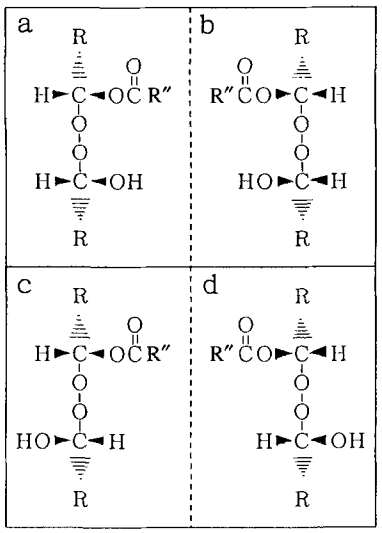
[2]

where peak 2, R,R′ = $-(CH_2)_7COOCH_3$; peak 4, R = $-(CH_2)_7COOCH_3$, R′ = $-(CH_2)_7CH_3$ or R = $-(CH_2)_7CH_3$, R′ = $-(CH_2)_7COOCH_3$; peak 5, R,R′ = $-(CH_2)_7CH_3$; R″ = $-(CH_2)_7CH_3$ (nonanoic acid).

A previous paper (11) reports that signals 3 and 4 are assigned to the two methine protons of *bis*(1-acyloxy-1-alkyl) peroxide: R–*CH*(OOCR)–OO–(OOCR)–*CH*–R. Based on the LC–MS and NMR results in this study, however, it is reasonable that these signals are attributed to the methine proton of R–*CH*(OOCR)–OO–CH(OH)–R′ **V**.

Comparison of peroxides produced in the presence and absence of carboxylic acid medium. The ozonolysis products of methyl oleate with and without the nonanoic acid medium were quantitated by NMR and GPC and are listed in Table 3. With no medium, mainly **I** and some **II** was produced, while with the carboxylic acid medium, mainly **III** is produced in addition to the other two compounds.

The ozonolysis reaction of methyl oleate produces peroxides that are labile and tend to decompose during analysis. LC–MS equipped with a Frit-FAB interface was effectively utilized for the direct analysis of the reaction mixture. The molecular ion peak of each peroxide was readily confirmed. Subsequent NMR analysis of each peroxide fractionated by



SCHEME 2

TABLE 3
Comparison of Peroxides Produced in the Presence and Absence of Carboxylic Acid Medium

Peroxidic species	Carboxylic acid medium (wt%)	No medium (wt%)
1,2,4-Trioxolane (**I**)	5.4	90.1
Peroxide oligomer (**II**)	4.9	9.9
1-Acyloxyalkyl-1-hydroperoxide (**III**)	13.6	ND[a]
1-Acyloxyalkyl-1′-hydroxyalkyl peroxide (**IV**)	76.1	ND

[a]ND, not detected.

HPLC revealed that the reaction products include four peroxidic species: 1,2,4-trioxolane, peroxide oligomer, 1-acyloxyalkyl-1-hydroperoxide, and 1-acyloxyalkyl-1′-hydroxyalkyl peroxide, as well as an aldehyde.

ACKNOWLEDGMENT

LC–MS analysis was performed by Y. Takahashi and K. Matsuura of JEOL Ltd (Tokyo, Japan).

REFERENCES

1. Mochida, Y., T. Kawai, S. Nakamura, Y. Yokoyama and M. Tsuchiya, *Preprint of the 32nd Annual Meeting of the Japan Oil Chemists' Society*, 1993, Toyonaka, Japan.
2. Criegee, R., *Angew. Chem. 14*:745 (1975).
3. Criegee, R., *Record Chem. Progs. 18*:111 (1957).
4. Schroder, G., *Chem. Ber. 95*:733 (1962).
5. Murray, R.W., R.D. Youssefyeh and P.R. Story, *J. Am. Chem. Soc. 89*:2429 (1967).
6. Privett, O.S., and E.C. Nickell, *J. Lipid Res. 4*:208 (1963).
7. Riezelbos, G., J.C. Grimmelikheizsen and D.A. Van Dorp, *Recueil Des Travaux Chimiques Des Pays-Bas 82*:1234 (1963).
8. Nikell, E.C., M. Albi and O.S. Privett, *Chem. and Phys. of Lipids 17*:378 (1976).
9. Hudlicky, M., *186 Oxidations in Organic Chemistry*, American Chemical Society, Washington, D.C., 1990.
10. Pryde, E.H., and J.C. Cowan, *J. Am. Oil Chem. Soc. 39*:496 (1962).
11. Rebrovic, L., *Ibid. 69*:159 (1992).
12. Zelikman, E.S., L.V. Berezova, E.P. Kutueva and Yu.N. Yur'ev, *J. Org. Chem. USSR 7*:641 (1971).

[Received June 17, 1994; accepted March 11, 1995]