Different Rates of Geometric Isomers of Linoleate Hydroperoxide in Acid-catalyzed Decomposition

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Methyl linoleate hydroperoxide was decomposed with 0.1 M HCl in acetone-water (9:1, v/v) at 30°C. The decrease in four isomers of the hydroperoxide was monitored by HPLC without any derivatization. In both isomers having 13- and 9-hydroperoxy groups, those having *trans, trans* dienes decomposed more rapidly than those having *cis, trans* dienes. In all the isomers, the rates of decomposition were first order with respect to concentrations of the hydroperoxides. The yields of 2-nonenal and 12-oxo-10-dodecenoate were also measured by GC-MS. 12-Oxo-10-dodecenoate was produced only from the 13-isomers and 2-nonenal from the 9-isomers. The rapid decomposition of the *trans, trans* isomers didn't seem to be responsible for the formation of these aldehyde products.

Volatile aldehydes produced during acid decomposition of methyl linoleate hydroperoxide have been analyzed, and the mechanism of their formation has been discussed.^{1~4)} If rearrangement of the allylic group from the carbon to the electron deficient oxygen occurs similarly to that of such an aryl group as in cumen hydroperoxide, the mechanism shown in Scheme 1 is conceivable.^{2~4)} Frankel *et al.*⁴⁾

identified four aldehydes (hexanal, 9-oxo-nonanoate, 3-nonenal, and 12-oxo-9-dodecenoate) by GC-MS expected from this mechanism in the decomposition of methyl linoleate hydroperoxide with 5% HCl in methanol.

Though there are many reports which deal with the decomposition products of methyl linoleate hydroperoxide,^{1~4)} there has been little attention to the rate of decomposition, particularly to that of each isomer. In this investigation, the amount of each isomer of methyl linoleate hydroperoxide, 13-hydroperoxy-*cis*-9, *trans*-11-octadecadienoate (13-*cis,trans*), 13-hydroperoxy-*trans*-9, *trans*-11-octadecadienoate (13-*trans,trans*), 9-hydroperoxy-*trans*-10, *cis*-12-octadecadienoate (9-*trans,cis*), or 9-hydroperoxy-*trans*-10, *trans*-12-octadecadienoate (9-*trans,trans*) was measured by HPLC, and its rate of decomposition was calculated. The decomposition products (2-nonenal and 12-oxo-10-dodecenoate) from each isomer were also measured by GC-MS.

SCHEME 1.

MATERIALS AND METHODS

Preparation of methyl linoleate hydroperoxide. Methyl

linoleate (95%) was prepared by fractional freezing (-78°C) of 5% acetone solution of methyl esters obtained from cottonseed oil, then purified by passing through a silicic acid and charcoal column as hexane solution. Oxidation of methyl linoleate and separation of methyl linoleate hydroperoxide by Sephadex LH-20 column chromatography were done similarly to our previous description.⁵⁾ The separated hydroperoxide (30 mg) was put on a Kieselgel GF plate (20×20 , 0.2 cm) and developed twice with hexane-diethyl ether (2:1, v/v). The purified hydroperoxide was dissolved in hexane and stored in liquid nitrogen until use.

HPLC separation of methyl linoleate hydroperoxide into four isomers. Methyl linoleate hydroperoxide (300 μg) was injected into a liquid chromatograph (Shimadzu LC-3A) with a Zorbax SIL column (15×0.46 cm) and eluted with hexane-diethyl ether (95:5, v/v) at the rate of 1.5 ml/min. The effluent was monitored with a spectrophotometric detector (Shimadzu SPD-1) at 234 nm and the separated four peak materials were purified by rechromatography under the same conditions until each separated isomer showed a single peak. After the solvent was removed in a stream of nitrogen, each isomer was dissolved in hexane. Then the concentration of each isomer was measured from its UV absorption and immediately used for the experiment of decomposition with HCl.

UV measure of methyl linoleate hydroperoxide. A part of the stock solution was dried in a stream of nitrogen and dissolved in ethanol. UV absorption was measured on a spectrophotometer (Hitachi Model 100-50) to find the concentration of the hydroperoxide. Molar extinction coefficients, 26000 at 236 nm for 13-*cis*, *trans*, 25900 at 236 nm for 9-*trans*, *cis*, and 28600 at 233 nm for 13-*trans*, *trans* and 9-*trans*, *trans* were used for calculation.⁶⁾

Acid decomposition of methyl linoleate hydroperoxide. Methyl linoleate hydroperoxide (1 mg) in a test tube was treated with 0.1 M HCl in acetone-water (9:1, v/v) (1.0 ml). The test tube was covered with polyethylene film and the temperature was kept at 30°C . The reaction mixture was transferred into hexane (0.5 ml) in a test tube at each sampling time and washed with water (2.0 ml) immediately. The hexane layer (100 μl) was used for HPLC for analysis of the hydroperoxide. For measurement of 2-nonenal and 12-oxo-10-dodecenoate, each isomer of the hydroperoxide purified by HPLC was divided into six portions (25 μg) and they were separately reacted with 0.1 M HCl in acetone-water (9:1, v/v) (100 μl). Half were used for GC-MS analysis and the others for HPLC measurement of the hydroperoxide.

HPLC measurement of methyl linoleate hydroperoxide. The hydroperoxide was measured by HPLC under the same conditions as those used for separation of each isomer except that hexane-diethyl ether (9:1, v/v) was

used as the eluent. The concentration of each isomer in the reaction mixture was calculated based on the peak area of the standard isomer of which the concentration was found from UV absorbance.⁶⁾

GC-MS measurement of 2-nonenal and 12-oxo-10-dodecenoate. The reaction mixture was neutralized with sodium bicarbonate and methyl palmitate was added to it as an internal standard. After extraction with hexane, the hexane layer was injected to GC-MS (JEOL JMS-DX300) by an on-column technique at 50°C . The GC-column was 3% Silar 10C on Diasolid L-1. The temperature was elevated to 250°C at a rate of $8^{\circ}\text{C}/\text{min}$. The chemical ionization (CI) method was used and the reaction gas was isobutane. The ionization potential was 250 eV and the ionization current was 300 μA . The amounts of $M+1$ ions at m/z : 141, 227, and 271 for 2-nonenal, 12-oxo-10-dodecenoate, and methyl palmitate, respectively, were quantified by mass chromatography. The responses of 2-nonenal, 12-oxo-10-dodecenoate, and methyl palmitate were corrected with those of the standard mixture of which the concentration was known.

Analyses of double bond positions of nonenal and 12-oxo-dodecenoate. The decomposition product was injected into a gas chromatograph (Shimadzu GC-6A) with a thermal conductivity detector. The column was a 3 mm $\times$ 2 m glass column packed with 3% Silar 10C on Diasolid L-1. The oven temperature was programmed from 50°C to 27°C at a rate of $8^{\circ}\text{C}/\text{min}$. The carrier gas was helium. The isolated 12-oxododecenoate was analyzed by GC-MS before and after ozonolysis and hydrogenation with platinum catalyst. The isolated nonenal was converted to 2,4-dinitrophenyl hydrazone (DNPH), and the differential absorption spectrum of its chloroform solution was recorded from 300 nm to 450 nm with the reference solution of authentic *trans*-2-nonenal-DNPH.

RESULTS AND DISCUSSION

HPLC analysis of methyl linoleate hydroperoxide

The concentration of the hydroperoxide in the reaction mixture was measured by HPLC; hexane-diethyl ether (9:1, v/v) was used as the eluent. A typical chromatogram is shown in Fig. 1A. For the analysis of the hydroperoxide, mixtures of hexane with alcohol were often used as the solvent,⁶⁾ but two geometric isomers of the 9-hydroperoxides (9-*trans*, *cis* and 9-*trans*, *trans*) could be separated only partly as Chan *et al.*⁶⁾ had reported. As Fig. 1A shows, four isomers of the hydroperoxide were almost completely separated from each other.

FIG. 1. Acid-catalyzed Decomposition of Methyl Linoleate Hydroperoxide as Analyzed by HPLC.

A, before decomposition; B, after 60 min; C, after 120 min. 13c, 13-hydroperoxy-*cis*-9, *trans*-11-octadecadienoate; 13t, 13-hydroperoxy-*trans*-9, *trans*-11-octadecadienoate; 9c, 9-hydroperoxy-*trans*-10, *cis*-12-octadecadienoate; 9t, 9-hydroperoxy-*trans*-10, *trans*-12-octadecadienoate.

Acid decomposition of methyl linoleate hydroperoxide

The hydroperoxide was decomposed with 0.1 M HCl in acetone–water (9:1, v/v). Typical chromatograms are shown in Fig. 1B and 1C and the course of the decrease in concentration of the hydroperoxide is shown in Fig. 2. Logarithms of residual hydroperoxide concentrations versus time are plotted. The rate of decomposition of each isomer was first order with respect to the concentration of the hydroperoxide. The rate constants were calculated from the slopes as follows: 13-*cis,trans* = $8.1 \times 10^{-5} \text{ sec}^{-1}$; 13-*trans,trans* = $2.0 \times 10^{-4} \text{ sec}^{-1}$; 9-*trans,cis* = $8.9 \times 10^{-5} \text{ sec}^{-1}$; 9-*trans,trans* = $2.1 \times 10^{-4} \text{ sec}^{-1}$. It is noticeable that the *trans,trans* isomers decomposed more rapidly than the *cis,trans* isomers. The two positional isomers of similar geometric type had almost equal rates.

Chan *et al.*⁷⁾ reported that methyl linoleate hydroperoxide was rearranged when it was heated at 40 °C for 16 hr in several solvents. It was possible that the rapid disappearance of the *trans,trans* hydroperoxides observed in our experiment was owing to the rearrangement into *cis,trans* isomers. To discover

FIG. 2. Course of Decrease in Each Isomer.

Abbreviations are the same as in Fig. 1.

whether isomerization occurred or not, each isomer was decomposed with 0.1 M HCl. The remaining hydroperoxide in the reaction mixture was extracted with hexane and measured by HPLC. Each isomer decreased with time without appearance of any peaks of other isomers. Therefore the rearrangement of the hydroperoxides rarely occurred under the conditions used in this experiment. Since the reaction time in this experiment was short (90 min) compared with that of Chan *et al.*,⁷⁾ the rearrangement would not occur.

Measurement of 2-nonenal and 12-oxo-10-dodecenoate by GC-MS

In a preliminary experiment, the hydroperoxide was purified by silicic acid column chromatography carefully under oxygen-free conditions. The purified hydroperoxide was decomposed with 0.1 M HCl in acetone–water (9:1, v/v) under oxygen-free conditions. Then the hydroperoxide before and after acid treatment was analyzed by GC-MS. Hexanal was not detected in the reaction mixture catalyzed by the acid solution. A small amount of 9-oxononanoate was detected in the reaction mixture but it was less than before acid treatment. Therefore, hexanal and 9-oxononanoate didn't seem to be produced directly during acid decomposition of the hydroperoxide under anaerobic conditions. They are probably produced by further decomposition of oxidized products of the hydroperoxides. Accordingly, an attempt was made to monitor formation of nonenal and 12-oxododecenoate.

However there is a discrepancy with regard to the positions of the double bond of nonenal and 12-oxododecenoate. According to the mechanism of acid decomposition shown in Scheme 1,²⁻⁴⁾ the production of 3-nonenal and 12-oxo-9-dodecenoate was expected. Frankel *et al.*⁴⁾ detected these aldehydes by GC-MS after decomposition with 5% HCl in methanol, whereas Gardner *et al.*³⁾ reported 12-oxo-10-dodecenoate from the boron trifluoride catalyzed decomposition in diethyl ether. In our experiment, the double bond positions of nonenal and 12-oxododecenoate were investigated. These two aldehydes were isolated by GLC and analyzed by GC-MS after hydrogenation and ozonolysis. After hydrogenation, 12-oxododecenoate gave mass spectra corresponding to 12-oxododecanoate: m/z : 229 ($M+1$), 197 ($M-OCH_3$) (CI) and m/z : 200 ($M-CO$), 197 ($M-OCH_3$) (EI). After ozonolysis, it gave 10-oxodecanoate: m/z : 201 ($M+1$), 169 ($M-OCH_3$) (CI) and m/z : 172 ($M-CO$), 169 ($M-OCH_3$) (EI). The differential absorption spectrum of the separated nonenal-DNPH with authentic 2-nonenal-DNPH didn't show any peaks of alkanal-DNPH in the region from 300 nm to 450 nm. Therefore, these two aldehydes were 12-oxo-10-dodecenoate and 2-nonenal. The migration of the double bond would occur by acid. The isomerization of an α,β -unsaturated carbonyl to a β,γ -unsaturated one generally occurs by acid.³⁾ However, as shown in Scheme 2, it is also possible that not only 3-enal but

SCHEME 2.

also 2-enal was produced by tautomerization of 1, 3-diene-1-ol, which is an intermediate in Scheme 1, and that 2-enal which is stabilized

TABLE I. GC-MS MEASUREMENT OF 2-NONENAL AND 12-OXO-10-DODECENOATE DURING DECOMPOSITION OF EACH ISOMER

Isomer ^a	Time (min)	Hydroperoxide (μg)	Nonenal (μg)	Oxododecenoate (μg)
13c	0	22.0	0.00	0.04
	40	13.2	0.00	0.15
	90	8.2	0.00	0.34
13t	0	21.5	0.00	0.04
	20	13.9	0.00	0.10
	50	6.0	0.00	0.08
9c	0	21.5	0.00	0.01
	40	12.4	0.32	0.01
	90	7.8	0.68	0.00
9t	0	20.5	0.00	0.01
	20	10.9	0.09	0.00
	50	5.7	0.08	0.00

^a Abbreviations are the same as in Fig. 1.

by resonance was mainly formed as the product.

2-Nonenal and 12-oxo-10-dodecenoate were measured by GC-MS and the results are shown in Table I. 2-Nonenal was produced only from the 9-isomers of the hydroperoxide and 12-oxo-10-dodecenoate was from the 13-isomers. This agreed with the results of Gardner *et al.*³⁾ who reported that the decomposition of 13-*cis,trans* with boron trifluoride in diethyl ether had led to the production of 12-oxo-10-dodecenoate and hexanal and 9-*trans,cis* to 9-oxononanoate and nonenal. Although decomposed more rapidly than the *cis,trans* isomers, the *trans,trans* isomers produced less of the aldehydes than the *cis,trans* isomers did. Possibly a mechanism other than the well known rupture (Scheme 1) would participate in the characteristically rapid decomposition of the *trans,trans* isomers.

Effects of temperature

To elucidate the effects of temperature, the hydroperoxide was decomposed with 0.1 M HCl in acetone-water (9:1, v/v) at several temperatures. Logarithms of rate constants (k) were plotted versus the reciprocal of temperature in Fig. 3 and all the isomers gave straight lines. Therefore the relation between tempera-

FIG. 3. Effects of Temperature on Rate of Decomposition of Each Isomer.

Abbreviations are the same as in Fig. 1.

FIG. 4. Effects of Acid Concentration on Rate of Decomposition of Each Isomer.

Abbreviations are the same as in Fig. 1.

tures and k was shown to obey the Arrhenius equation. These four straight lines were almost parallel to each other. The apparent activation energies were as follows: 13-*cis*, *trans* = 23 kcal/mol; 13-*trans*, *trans* = 22 kcal/mol; 9-*trans*, *cis* = 22 kcal/mol; 9-*trans*, *trans* = 22 kcal/mol. It was noted that these values were fairly low notwithstanding that the reactions were postulated to be heterolytic.

Effects of acid concentration

The hydroperoxide was reacted with 0.1~0.3 M HCl in acetone-water(9:1, v/v) at 30°C. The decomposition rate of the hydroperoxide increased with the increase in acid concentration. Fig. 4 shows the relationship between k logarithms of the rate constants and

FIG. 5. Effects of Water Content of Acetone on Rate of Decomposition of Each Isomer.

Abbreviations are the same as in Fig. 1.

TABLE II. EFFECTS OF SOLVENT ON RATE OF DECOMPOSITION OF EACH ISOMER

Solvent	Rate constant ($\times 10^{-4} \text{ sec}^{-1}$)			
	13c	13t	9c	9t
THF ^b	0.48	0.69	0.44	0.88
DMSO ^c	0.37	0.88	0.43	0.92
Acetone	0.81	2.0	0.89	2.1
Ethanol	1.5	6.5	1.6	6.8
Methanol	6.9	19	7.3	19

^a Abbreviations are the same as in Fig. 1.

^b Tetrahydrofuran.

^c Dimethyl sulfoxide.

logarithms of the acid concentration. They deviated a little from straight lines as Gardner *et al.*²⁾ had reported in the decomposition of 13-*cis*, *trans* with HCl in methanol-water. When the relationships in Fig. 4 were assumed to be linear, the slopes of the straight lines were about 1.5 with all isomers, suggesting that the order of reaction with respect to acid concentration is about 1.5. The rate constant would be complicatedly depended on acid concentration as observed for other organic hydroperoxides.⁸⁾

Effects of water content of acetone

When the hydroperoxide was decomposed with 0.1 M HCl at 30°C, the water content of acetone was varied from 5% to 30%. The result is shown in Fig. 5. Increase in the water content tended to slow down the rate of de-

composition,⁸⁾ but little difference was observed in the range from 10% to 30%. The addition of more water than 30% caused separation of the reaction mixture.

Effects of various solvent

The rate constants in the decomposition of the hydroperoxide with 0.1 M HCl at 30 °C in various solvents which contained 10% water are shown in Table II. In all the solvents, the *trans, trans* isomers decomposed more rapidly than the *cis, trans* isomers. The hydroperoxide decomposed more rapidly in protic solvents than in aprotic solvents. In protic solvents, acid easily dissociates and the addition of a proton to the hydroperoxide easily occurs, which would probably affect the decomposition rate of the hydroperoxide.

Investigation of nonvolatile products of the

acid-decomposed hydroperoxide, especially those of the *trans, trans* isomers, is in progress.

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