Analysis of Autoxidized Fats by Gas Chromatography-Mass Spectrometry: II. Methyl Linoleate[1]

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ABSTRACT

The gas chromatography-mass spectrometry (GC-MS) approach developed in the preceding paper was applied for qualitative and quantitative investigations of autoxidation products of methyl linoleate. A GC-MS computer summation method was standardized with synthetic 9- and 13-hydroxyoctadecanoate. Equal amounts of 9- and 13-hydroperoxides were found in all samples of linoleate autoxidized at different temperatures and peroxide levels. The results are consistent with the classical free radical mechanism of autoxidation involving a pentadiene intermediate having equivalent sites for oxygen attack at carbon-9 and carbon-13. Minor oxygenated products of autoxidation indicated by GC-MS include keto dienes, epoxyhydroxy monoenes, di- and tri-hydroxy monoenes. These hydroxy compounds are presumed to be present in the form of hydroperoxides. The quantitative GC-MS method was found suitable for the analysis of autoxidized mixtures of oleate and linoleate. By this method, it is possible to determine the origin of the hydroperoxides formed in mixtures of these fatty esters.

INTRODUCTION

In the preceding paper (1), gas chromatography-mass spectrometry (GC-MS) was used for qualitative and quantitative investigations of autoxidized methyl oleate. In this paper, we report an extension of these studies to methyl linoleate.

MS and GC-MS have been used in a number of studies to help identify products from the reaction of lipoxygenase with linoleic acid and some of their enzymatic and nonenzymatic decomposition products (2-7). Apparently, only one report has appeared recently where GC-MS was used with autoxidized linoleate to characterize hydroperoxides and their thermal decomposition products (8). Partial separation of the 9- and 13-hydroxyoctadecanoate derivatives by GC permitted an estimate of their relative concentrations but no authentic compounds were used as references. We have shown in the preceding paper (1) that the application of GC-MS for quantitative analyses requires careful standardization with known synthetic compounds. In the present paper, a quantitative GC-MS computer summation method was so standardized with known mixtures of synthetic 9- and 13-hydroxyoctadecanoates. Although work has been published on the relative rates of oxidation of oleate, linoleate, and linolenate (9,10), no suitable method has been available for the analysis of the individual hydroperoxides formed in mixtures of these fatty esters. We used a direct GC-MS method to determine the isomeric hydroperoxide composition of autoxidized mixtures of oleate and linoleate. GC-MS provided also qualitative information on some of the secondary oxygenated products in autoxidized linoleate that may be precursors of off-flavors in fats.

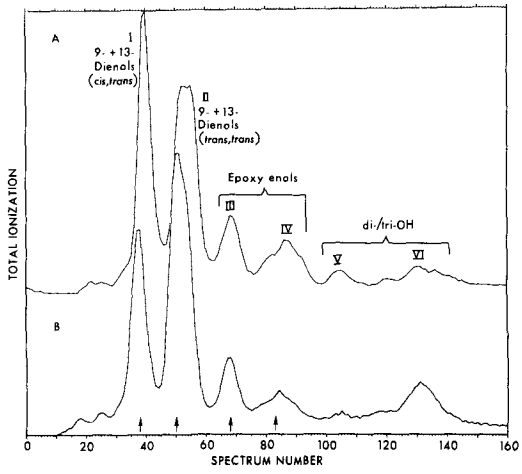

FIG. 1. GC-MS of silyl ethers of reduced-autoxidized methyl linoleate (PV 2128): computer traces of MS total ionization vs. spectrum numbers corresponding to full MS scan recorded every 12 sec. A. $NaBH_4$-reduced; B. KI-reduced.

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TABLE I

Mass Spectral Data

Peak	Spectrum no.	Characteristic fragments m/e (rel. abundance)	Identification[a] (C-18 OTMS methyl esters)	References
Figure 1A[b] $NaBH_4$ reduced-oxidized linoleate				
I	40	225(100), 311(76.4)	9-OH-diene + 13-OH-diene	16
II	54	225(100), 311(90.0)	9-OH + 13-OH-diene	16
III	69	199(100) 285(88.4), 383(7.3) M - 15	11-OH-9,10-epoxy-ene + 11-OH-12,13-epoxy-ene	4-7
IV	87	173(100), 241(59.2) 259(68.6), 327(28.5)	9-OH-12,13-epoxy-ene + 13-OH-9,10-epoxy-ene	3,7
V	106 120	173(18.0), 259(16.8), 355(3.8) 173(56.7), 259(100), 355(17.1)	9,13-diOH-ene 9,13-diOH-ene	8
VI	131 136	173(89.8), 259(100), 301(9.8) 173(100), 259(91.8), 301(20.3)	9,12,13-triOH-ene + 9,10,13-triOH-ene	3,5,6,8
Figure 1B[b] KI reduced-oxidized linoleate				
III	68	151(100), 237(54.1), 308(74.0)	9-keto diene + 13-keto diene	5,7
Figure 3[c] Hydrogenated-oxidized linoleate				
I	15	155(87.0), 185(43.7), 281(27) M - 31 99(100), 241(30.6)	9-keto-ane 13-keto-ane	14
II	23	229(87.5), 259(94.5) 173(100), 315(59.1)	9-OH-ane 13-OH-ane	8
III	40	173(57.4), 301(25.2)[d] 259(70.2), 215(100)	11-OH-9,10-epoxy + 12,13-diOH-ane + 9,10-diOH-ane	3,4,7
IV	53	215(34.8), 259(64.0), 273(100) 173(100), 301(41.5)	9,10-/10,13-diOH-ane 12,13-diOH-ane	8
V	81	173(46.1), 213(59.8) 259(53.7), 299(62.5)	9,12,13-/9,10-13-triOH-ane	3,5,8

[a]See fragmentation schemes in Figure 2 and in preceding paper (1).

[b]m/e normalized from 100 to 400.

[c]m/e normalized from 0 to 400.

[d] 

EXPERIMENTAL PROCEDURES

Methyl linoleate was prepared from methyl esters of safflower oil and purified by counter double current distribution (11), silicic acid chromatography, and vacuum distillation. It analyzed 99.6% by GC (probable impurity, 0.4% conjugated diene).

The procedure for autoxidation, methods for peroxide values and GC, procedures for KI and $NaBH_4$ reduction, catalytic hydrogenation, silylation, and GC-MS were described in the previous paper (1). Authentic methyl 9- and 13-hydroxyoctadecanoate were prepared by $NaBH_4$ reduction of the corresponding keto derivatives synthesized by literature methods (12).

RESULTS

The GC-MS computer chromatogram of silyl ethers of oxidized-reduced samples of linoleate showed two major peaks due to the allylic 9- and 13-dienols from corresponding hydroperoxides (Fig. 1). Peak I was assigned to *cis,trans*-dienol and peak II to *trans,trans*-dienol on the following bases. With increasing level of oxidation, the relative area of peak II increased, and infrared (IR) analyses showed more intense bands at 990 (*trans,trans* + *cis,trans*) than at 955 cm^{-1} (*cis,trans*) (13). Also, the relative porportion of peak II increased when KI was the reducing agent (Fig. 1B). Apparently, somerization of *cis,trans*- to *trans,trans*-dienols

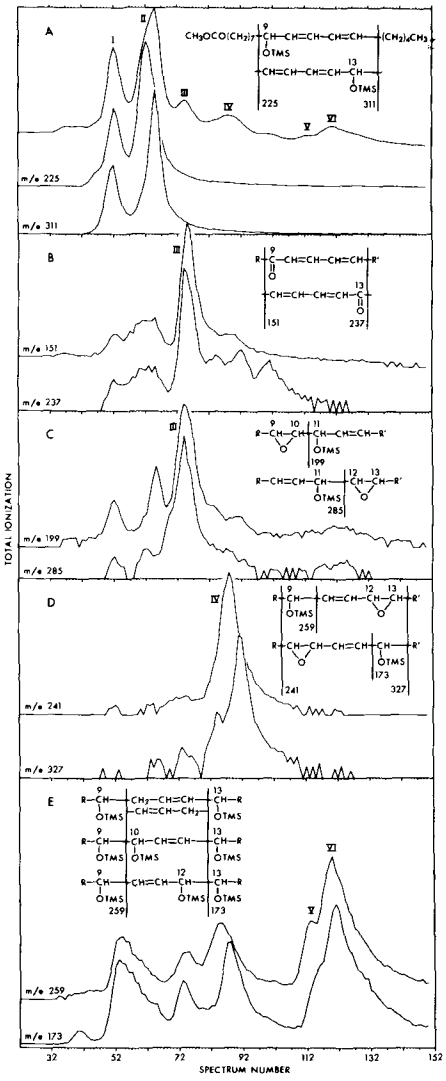

FIG. 2. Mass Chromatography of KI reduced-autoxidized methyl linoleate (PV 1403) as TMS ethers, for the identification of: A. I, II = methyl 9- and 13-hydroxyoctadecadienoate; B. III = methyl 9- and 13-keto octadecadienoate; C. III = methyl 11-hydroxy-9,10-epoxy-12-octadecenoate + methyl 11-hydroxy-12,13-epoxy -9- octadecenoate; D. IV = methyl 13-hydroxy-9,10-epoxy-11-octadecenoate + methyl 9-hydroxy-12,13-epoxy-10-oxtadecenoate; E. V, VI = methyl 9,13-dihydroxyoctadecenoate + methyl 9,12,13-/9,10,13-trihydroxyoctadecenoate.

was catalyzed by the I_2 produced from oxidized KI. The MS evidence indicates that epoxyenols are eluted from peaks III and IV, dihydroxy esters from peak V, and trihydroxy esters from peak VI (Table I). In the KI-reduced sample (Fig. 1B), components eluted from peak III show also MS evidence of small

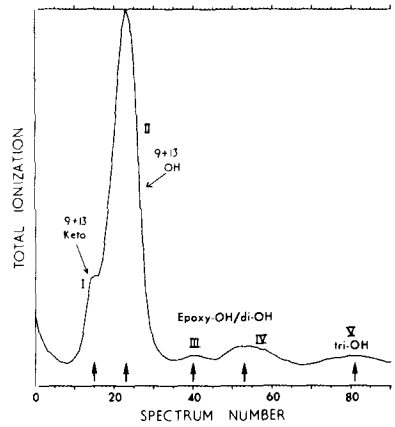

FIG. 3. GC-MS of silyl ethers of hydrogenated-autoxidized methyl linoleate (PV 2128).

TABLE II

GC-MS Analysis[a] of Synthetic Hydroxyoctadecanoate

Known mixtures	Relative percent 9-OH	13-OH
Mixture 1	48.7	51.3
Found	49.6	50.4
Mixture 2	44.9	55.1
Found	46.1	53.9
Mixture 3	77.5	22.5
Found	77.0	23.0
Mixture 4	50.7	49.3
Found	51.4	48.6
Mixture 5	81.0	19.0
Found	81.8	18.2
Mean relative %		
5 Mixtures	60.56	39.44
Found	61.18	38.82
St. deviation	0.92	

[a]Based on computer summation of masses 229 + 259 for 9-OH isomer and masses 173 + 315 for 13-OH isomer (1).

amounts of keto dienes. These components are reduced by $NaBH_4$ into the corresponding dienols. The identifications given in Table I are based on published fragmentation schemes (3-8,14-17) and confirmed below by mass chromatography and GC-MS analyses after catalytic hydrogenation of the autoxidized samples.

Mass chromatography (1) showed that the *cis,trans*-9-, and 13-dienols are not separated in peak I, and that the corresponding *trans,trans*-isomers are partially separated in peak II (Fig. 2A). Characteristic masses for keto dienes (m/e 151 and 237) and 11-hydroxy-9,10-/12,13-epoxy-enes (m/e 199 and 285) fall in peak III

(Fig. 2B, 2C), for 13-/9-hydroxy-9,10-/12,13-epoxy-enes (m/e 173, 241, 259, and 327) in peak IV (Fig. 2D, 2E), for 9,13-dihydroxyenes and 9,12,13-/9,10,13-trihydroxyenes (m/e 173 and 259) in peaks V and VI (Fig. 2E).

After double bond hydrogenation of the autoxidation products, the gas chromatogram shows evidence of 9- and 13-ketooctadecanoate (peak I), 9- and 13-hydroxyoctadecanoate (peak II), epoxyhydroxyoctadecanoate, dihydroxyoctadecanoate (peaks III-IV), and trihydroxyoctadecanoate (peak V) (Fig. 3, Table I). The diminished amount of epoxy esters indicates some reduction of the epoxy group during catalytic hydrogenation. There was no evidence of 11-hydroxyoctadecanoate (m/e 201 and 287) coming from peak II. These identifications were confirmed by mass chromatography. The evidence for dihydroxy esters with one hydroxy group on carbon-9 or -12 and the other on carbon-10 or -13 means that the corresponding monounsaturated dihydroxy esters were present before hydrogenation. Mass chromatography showed partial separation of the TMS ethers of 9- and 13-hydroxyoctadecanoate by GC. The 9-TMS ether isomers was eluted in the first half of peak II and the 13-TMS ether isomer in the second half. Because of this separation, quantitative determination of the 9- and 13-hydroxy esters requires careful summation of total ions for all mass spectra taken within peak II.

The quantitative GC-MS analysis was standardized with synthetic mixtures of 9- and 13-hydroxyoctadecanoate. The same computer summation method was used as that developed in the preceding paper for oleate (1). The results in Table II show that this computer summation method is quantitatively reliable, with a standard deviation of 0.92. Samples of methyl linoleate oxidized to different peroxide values and at different temperatures show a uniformly equal distribution of the 9- and 13-hydroxy esters (Table III). These results support the general belief (18) that carbons-9 and -13 of methyl linoleate are equivalent sites to oxygen attack.

The quantitative GC-MS method was also applied to the analysis of autoxidized mixtures of oleate and linoleate. Five mixtures were autoxidized at different levels, and the hydroxyoctadecanoate TMS derivatives were anal-

TABLE III

GC-MS Analysis[a] of Isomeric Hydroxyoctadecanoate in Autoxidized Methyl Linoleate

Peroxide value	Temp., °C	Relative percent 9-OH	13-OH
152	40	50.2	49.8
261	40	51.7	48.3
686	40	49.7	50.3
918	40	49.6	50.4
93	60	47.3	52.7
505	60	51.5	48.5
1403	60	49.0	51.0
1249	80	52.5	47.5

[a]Based on computer summation of masses 229 + 259 for 9-OH isomer and masses 173 + 315 for 13-OH isomer (1).

TABLE IV

GC-MS Analysis of Autoxidized Mixtures of Oleate:Linoleate (80 C)

Mixtures oleate:linoleate (Ol)	(Lo)	Peroxide value	Relative percent 8-OH	9-OH	10-OH	11-OH	13-OH	Origin[a] Ol	Lo
9:1		84	13.2	36.7	11.3	14.4	24.4	51.2	48.8
		152	13.3	33.4	11.7	16.2	25.4	49.2	50.8
		528	14.3	35.8	10.8	13.8	25.3	49.4	50.6
		1109	25.8	23.4	21.7	25.8	3.3	93.4	6.6
2:1		414	5.2	43.3	4.3	8.3	38.9	22.2	77.8
		995	5.5	42.9	5.8	11.1	34.7	30.6	69.4
		1047	9.5	40.3	7.0	9.1	34.1	31.8	68.2
1:1		106	3.1	46.9	1.8	3.2	45.0	10.0	90.0
		407	4.1	47.6	3.0	3.5	41.8	16.4	83.6
		1070	6.9	42.5	5.5	6.3	38.8	22.4	77.6
1:2		515	2.6	46.5	2.1	3.6	45.3	9.5	90.5
		1037	4.5	44.8	3.0	3.4	44.4	11.2	88.8
1:9		593	1.5	48.8	0.4	0.7	48.6	2.8	97.2
		936	0.3	49.2	1.2	1.2	48.1	3.8	96.2

[a]Assuming that amount of 9-OH = 13-OH in oxidized linoleate. Lo - hydroperoxides = 13-OH x 2; Ol - hydroperoxides = 8-OH + (9-OH - 13-OH) + 10-OH + 11-OH.

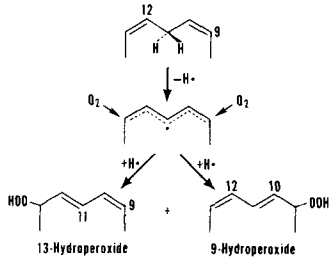

FIG. 4. Mechanism of linoleate autoxidation.

yzed quantitatively by GC-MS. With these mixtures, the 9-hydroxy ester comes from both oleate and linoleate hydroperoxides, whereas the 13-hydroxy ester comes only from linoleate hydroperoxides. Assuming that equal amounts of 9- and 13-hydroperoxides are formed from linoleate (as shown in Table III), it is possible to estimate the origin of the hydroperoxides in these autoxidized mixtures (Table IV). With the 1:1 mixture of these esters oxidized to peroxide values of 106, 407, and 1070, the total hydroperoxides originating from linoleate varied from 78 to 90%. With the 9:1 oleate-linoleate mixture oxidized to peroxide values of 84, 152, and 528, as much as 50% of the hydroperoxides formed come from linoleate. At the more advanced level of oxidation (PV 1109), as expected, oleate hydroperoxides become dominant. With 2:1 and 1:2 oleate-linoleate mixtures, linoleate hydroperoxices are again dominant and vary from 68 to 90% of the total. The quantitative GC-MS method thus appears suitable to determine the kind of hydroperoxide formed in autoxidized mixtures of oleate and linoleate.

DISCUSSION

Much work has been reported on the autoxidation of linoleic acid and related compounds, and it is generally agreed that equal amounts of 9- and 13-hydroperoxides are formed as initial products (2,8,18). In the analyses of different samples of linoleate autoxidized at different levels and temperatures, we also found equal amounts of the 9- and 13-hydroxy isomers. These results agree with the accepted mechanism for linoleate autoxidation (18) involving hydrogen abstraction at carbon-11 (Fig. 4). The interaction of the unpaired electron on carbon-11 and the π-electrons of the adjacent double bonds produce an allylic radical in which the electrons are delocalized over five carbon atoms. This pentadiene radical has two equivalent sites for O_2 attack: carbons-9 and -13, and the products expected by this scheme are equal amounts of the 9- and 13-isomers. Although it has been spectulated before that an 11-hydroperoxide may be formed from linoleate by autoxidation (19-22), our present studies show no evidence for it. However, there is now evidence in the literature that the 9- and 13-hydroperoxides of linoleate undergo facile interconversion (23).

Minor products indicated by GC-MS in reduced- or hydrogenated- oxidized linoleate include ketodienes, epoxyenols, di- and trihydroxyesters. Both heterolytic and homolytic mechanisms have been invoked for the nonenzymatic thermal or metal-catalyzed decomposition of linoleate hydroperoxides, usually in aqueous or alcoholic solutions (24). In the neat linoleate system used in this study, the homolytic removal of •OH from the diene hydroperoxides would likely lead to oxy radicals. These intermediates can then form either keto dienes by further abstraction of H• (7) or epoxyhydroxy esters by cyclization with an α double bond and addition of •OH on either end of an allyl 3-carbon system (24). The dihydroxy or trihydroxy esters may be formed either by 1,2- or 1,4-addition of •OH to the conjugated diene system of the 9- and 13-dienol intermediates (24), or by formation of dihydroperoxides (18) or even trihydroperoxides. The role of these oxygenated products as possible precursors of off-flavors in oxidized fats remains to be established.

It is known that the presence of linoleate greatly accelerates the autoxidation of oleate (9,25). In a recent kinetic study of oleate-linoleate mixtures, the rates of oxidation were dependent on oleate concentration (26). Different kinetics observed at different peroxide values were explained by the different propagation and termination rates of oleate and linoleate. In the present study, although we started with equal mixtures of oleate and linoleate, about 80% of the hydroperoxides formed at three peroxide levels originated from linoleate. Even when the mixture contained only 10% linoleate, 50% of the hydroperoxides formed below 9% oxidation came from the linoleate. These results reflect the greater ease of hydrogen abstraction from linoelate compared with that from oleate. Since rates of propagation and termination during autoxidation are greatly influenced by such factors as temperature, catalysts, antioxidants, and peroxide level, it is extremely difficult to predict the contribution of different fatty acids when present in mixtures as in natural fats. The GC-MS method was shown to be suitable for the determination of individual hydroperoxides formed in autoxidized mixtures of oleate and linoleate. This

approach will form the basis of a direct method to analyze the origin of hydroperoxides in autoxidized fats that include mixtures of these fatty acids.

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