Gas–liquid chromatography–mass spectrometry of hydroxylated octadecanols derived from hydroxylated stearic acids

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ABSTRACT A gas–liquid chromatographic–mass spectrometric method of determining the position of oxygen atoms on polyfunctional fatty acids has been explored. The method consists of reduction of keto, hydroperoxy, epoxy, and carboxylic acid groups with $LiAlH_4$ to the corresponding alcohols; trimethylsilylation with bis(trimethylsilyl)acetamide; and analysis by means of the combined gas–liquid chromatograph–mass spectrometer.

The following compounds were analyzed: 9-mono-, 9,10-di-, 9,10,12-tri-, and 9,10,12,13-tetrahydroxystearic acids and the corresponding derivatives of octadecan-1-ol. The reduction products of 9,10-epoxystearic acid and a mixture of linoleic acid 9- and 13-hydroperoxides were also analyzed.

The position of the oxygen function in the original molecule can be deduced rapidly and accurately.

SUPPLEMENTARY KEY WORDS hydroxy fatty acids · hydroxyoctadecanes · $LiAlH_4$ reduction · trimethylsilyl derivatives · autoxidation

THE DETERMINATION of the position of unstable oxygen functions such as keto, hydroperoxy, hydroxy, or epoxy groups on fatty acids by chemical methods is a tedious and difficult process (1). A simple and accurate method involves the stabilization of these groups by $LiAlH_4$ reduction followed by combined GLC-mass spectrometry of the trimethylsilyl (TMS) ethers of the resulting alcohols.

This method was developed for determination of the exact position of the oxygen atoms on several gas-chromatographically unstable polyfunctional fatty acids that are products of the metabolism of linoleic acid 13-hydroperoxide (13-hydroperoxy-9 *cis*, 11 *trans*-octadecadienoic acid). This paper describes GLC–mass spectrometry of several known hydroxy fatty acids and of their $LiAlH_4$-reduced counterparts, which resemble the expected reduction products of the unknown metabolites mentioned above.

The hydroxy fatty acids examined in this work were 12-hydroxy-, 9,10-dihydroxy-, 9,10,12-trihydroxy-, and 9,10,12,13-tetrahydroxystearic acids. The corresponding reduction products are: 1,12-dihydroxy-, 1,9,10-trihydroxy-, 1,9,10,12-tetrahydroxy-, and 1,9,10,12,13-pentahydroxyoctadecanes. 9,10-Epoxystearic acid and linoleic hydroperoxide were also reduced and analyzed. TMS ethers and esters of these compounds were used for the GLC–mass spectrometry. Bis(trimethylsilyl)-acetamide (BSA) was used for the synthesis of the TMS ethers, esters, and ether-esters because it reacts rapidly and completely with alcohols and acids (2).

METHODS

12-Hydroxystearic acid was synthesized by catalytic reduction (PtO_2 and H_2) of ricinoleic acid (12-hydroxy-9 *cis*-octadecenoic acid). The other fatty acids were synthesized by a controlled oxidation (dilute permanganate at 0°C) of oleic, ricinoleic, and linoleic acids to yield the dihydroxy-, trihydroxy-, and tetrahydroxystearic acids, respectively (3). 9,10-Epoxystearic acid was prepared according to the method of Swern, Findley, and Scanlan (4). Linoleic hydroperoxide was prepared by the oxidation of linoleic acid catalyzed by soybean lipoxidase (5).

Compounds were trimethylsilylated with BSA (Su-

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Abbreviations: TMS, trimethylsilyl; OTMS, trimethylsilyloxy; BSA, bis(trimethylsilyl)acetamide; M ion, molecular ion; GLC, gas–liquid chromatography; MS, mass spectrometry; amu, atomic mass number.

pelco, Inc., Bellefonte, Pa.) by a method based on that of Klebe, Finkbeiner, and White (2). An excess of the reagent was added to the solid hydroxy fatty acids or to a dry ether solution of the octadecanols at 25°C. The progress of the reaction was determined by direct injection of the reaction mixture into the GLC apparatus or by observing the dissolution of the solid fatty acid into the reagent. For the more crystalline acids (i.e., the more hydroxy substituted) the derivatives were formed only when the reaction mixture was heated to 50°C for 5 min.

The hydroxy fatty acids were reduced by means of a modification of Gaylord's procedure (6). A saturated ethereal solution of $LiAlH_4$ was added dropwise to a solution of the TMS derivatives (ether-esters) of hydroxy acids in diethyl ether until there was no further evolution of hydrogen (after about 1 min at 25°C). Dilute HCl was

TABLE 1 RETENTION TIMES OF TMS ESTERS AND ETHERS

Group(s) Bearing TMS Function	Retention Time
	min
Stearate derivatives	
1-COOH	1.4
1-COOH, 12-OH	3.0
1-COOH, 9,10-OH	5.0
1-COOH, 9,10,12-OH	8.0
1-COOH, 9,10,12,13-OH	12.0
Octadecanol derivatives	
1-OH	1.4
1,12-OH	2.4
1,9,10-OH	3.8
1,9,10,12-OH	6.2
1,9,10,12,13-OH	10.0

The 50 cm column of OV-1, 3% on Celite, was at 220°C; flow rate of helium, 60 ml/min.

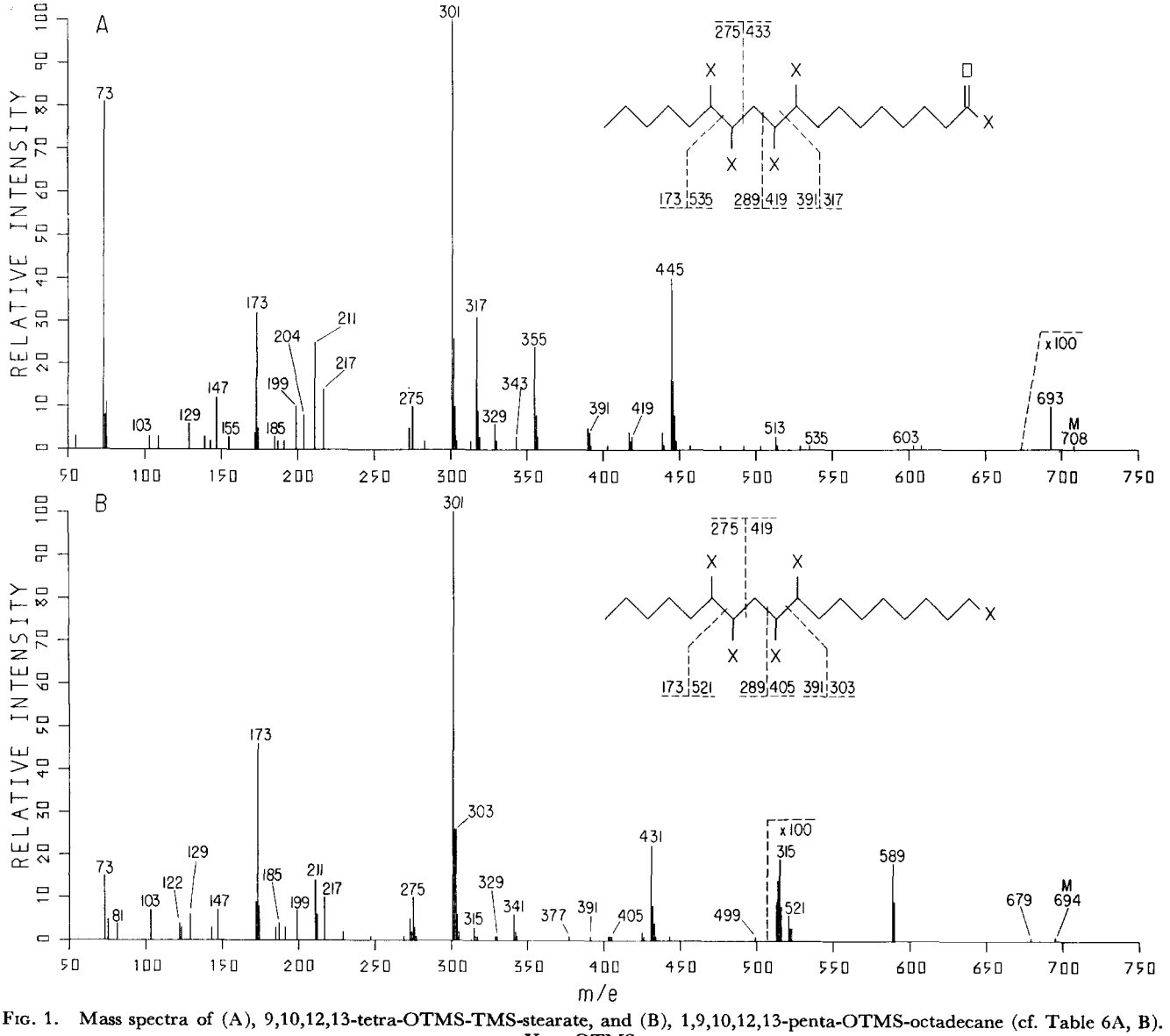

FIG. 1. Mass spectra of (A), 9,10,12,13-tetra-OTMS-TMS-stearate, and (B), 1,9,10,12,13-penta-OTMS-octadecane (cf. Table 6A, B). X = OTMS.

added and the mixture was extracted with ether. The ether solution was evaporated to dryness and the new hydroxyl group was silylated by the addition of more BSA. GLC showed 100% conversion to the reduced form.

The compounds were dissolved in ether and separated by GLC from the unreacted BSA on a 50 cm column of OV-1 (a nonpolar silicone phase), 3% on Celite (Supelco) at 225°C, with a Varian Aerograph model 202b.

The TMS fatty acids and octadecanols were analyzed without prior purification on an LKB-9000 gas chromatograph–mass spectrometer equipped with a 2 m column of SE-30 (methylpolysiloxy gum), 1% on Celite, operated isothermally between 200 and 225°C. The mass spectrometer was operated at an ionization potential of 20 ev and with an ion source temperature of 290°C. Magnetic scans were taken at the peak maxima as the compounds were eluted from the gas chromatograph.

RESULTS

Mixtures of the TMS fatty acids or of the TMS octadecanols can be resolved on a 50 cm column of OV-1; in addition, the TMS octadecanols are resolved from the corresponding TMS fatty acids (except for the unsubstituted octadecanol and stearate) (Table 1).

Mass spectra of the various TMS derivatives are described in Tables 3–6, with reference to the structure and cleavages indicated in Table 2. Various major peaks are designated by a letter (Tables 3–6) and the m/e value. The notation of a letter –90 or –180 (–2 × 90) indicates the loss of one or two trimethylsilanol groups [90 atomic mass units (amu)]. In all cases the most probable fragmentation is adjacent to the trimethylsilyloxy (OTMS) groups, except that the more substituted fragments also show a high probability of loss of trimethylsilanol. For example in Table 6 the base (ma-

TABLE 2 STRUCTURES AND MAJOR CLEAVAGES OF THE OTMS ESTERS (R_1) AND THEIR REDUCTION PRODUCTS (R_2)

Structure and Cleavage		For Observed m/e Values Refer to:
	R_1—C(=O)—OTMS	Table 3A
	↓ $LiAlH_4$, BSA	
	R_2—CH_2-OTMS	Table 3B
A $CH_3(CH_2)_5$—CH(OTMS)—$(CH_2)_{10}$—$R_{1,2}$ [345]; 359 *b*; *a* 187		
	R_1	Table 4A
	R_2	Table 4B
B $CH_3(CH_2)_7$—CH(OTMS)—CH(OTMS)—$(CH_2)_7$—$R_{1,2}$ [405]; 419 *b*; *d* 317; *a* 215; 317 *c* [303]		
	R_1	Table 5A
	R_2	Table 5B
C $CH_3(CH_2)_5$—CH(OTMS)—CH_2—CH(OTMS)—CH(OTMS)—$(CH_2)_7$—$R_{1,2}$ *a* 187; [405]; 419 *b*; *c* 303; 317 *d* [303]		
	R_1	Table 6A
	R_2	Table 6B
D $CH_3(CH_2)_4$—CH(OTMS)—CH(OTMS)—CH_2—CH(OTMS)—CH(OTMS)—$(CH_2)_7$—$R_{1,2}$ [419]; *c* 275; 433 *d*; [303]; *g* 391; 317 *h*; *a* 173; 535b [521]; *e* 289; 419 *f* [405]		

The numbers adjacent to the dashed lines are the m/e values calculated for the indicated fragmentation. The m/e values of fragments toward the carboxyl end are decreased by 14 atomic mass units (amu) on reduction of the esters to the ethers and are shown in brackets.

TABLE 3 MS ANALYSIS OF 12-OTMS-TMS-STEARATE

m/e	Relative Intensity A Ester	B Reduced Ester
73	15	42
97	7	22
103	5	13
132	6	
147	2	13
149		19
165		8 *b*–180
187	100 *a*	100 *a*
204	8	
217	15	
255		1 *b*–90
269	1 *b*–90	
325		1 M–180
330	15	
339	7 M–180	
340	2	1 M–90
345		16 *b*
359	92 *b*	
399		0.2 M–31
413	11 M–31	
415	1	0.3 M–15
429	12 M–15	
430	4	0.1 M
444	1 M	

TABLE 4 MS ANALYSIS OF 9,10-DI-OTMS-TMS-STEARATE

m/e	Relative Intensity A Ester	B Reduced Ester
73	65	15
103	7	10
147	13	11
155	3	
204	12	
215	77 *a*	100 *a*
217	21	5
227	6 *c*, *d*–90	1 *d*–90
303		73 *c*
315		1 *b*–90
317	100 *c*, *d*	1 *d*
329	1 *b*–90	
337	1 M–15–180	
390	14	
405		1 *b*
413		1 M–15–90
427	1 M–180	1
428		1 M–90
503		5 M–15
517	6 M–15	
518	3	0.1 M
532	1 M	

TABLE 5 MS ANALYSIS OF 9,10,12-TRI-OTMS-TMS-STEARATE

m/e	Relative Intensity A Ester	B Reduced Ester
73	12	4
103	1	3
147	2	2
155	1	
187	100 *a*	100 *a*
204	6	
213	1 *c*–90	1
217	11	4
227	2 *d*–90	
303	1 *c*	10 *c*, *d*
315		2 *b*–90
317	59 *d*	
329	1 *b*–90	0.4
390	21	
419	1 *b*	
425	1 M–15–180	
501		0.9 M–15–90
515	5 M–15–90	
591		0.1 M–15
605	1 M–15	
606		0.1 M
620	<0.1 M	

TABLE 6 MS ANALYSIS OF 9,10,12-13-TETRA-OTMS-TMS-STEARATE

m/e	Relative Intensity A Ester	B Reduced Ester
73	81	15
103	3	7
147	12	7
155	3	
173	32 *a*	46 *a*
185	3 *c*–90	3 *c*–90
199	10 *e*–90	7 *e*–90
204	8	
211	25 *g*–180	14 *g*–180
217	14	10
275	10 *c*	10 *c*
301	100 *g*–90	100 *g*–90
303	10	26 *h*
315		3 *f*–90
317	31 *h*	1
329	6 *f*–90	1 *d*–90
343	3 *d*–90	1
355	24 *b*–180	
390	5	
391	4 *g*	1 *g*
405		1 *f*
419	3 *f*	
431		22 *b*–90
445	40 *b*–90	
499		1 M–15–180
513	3 M–15–180	0.1
521		0.1 *b*
535	1 *b*	
589		0.2 M–15–90
603	1 M–15–90	
679		<0.1 M–15
693	0.1 M–15	
694		<0.1 M
708	<0.1 M	

jor) peak for both alcohol and acid is the fragment *g*–90.

The mass spectra of the most complex compounds studied are given in Fig. 1 as an aid to visualizing the type of breakdown patterns encountered throughout this study.

The molecular ion (M ion) appears in all of the spectra, but for the more highly substituted esters and all of the reduced compounds its intensity is low compared to that of the base ion because of the instability introduced by extensive substitution. A recent paper by McCloskey, Stillwell, and Lawson (7) indicates that M–15 (Tables 3–6) and M–31 (Table 3 only) correspond to the loss of CH_3 and $CH_3 + CH_4$ from the TMS groups. In addition they report the structures corresponding to lines at m/e 73, 75, 103, 147, and 149, which also appear in our spectra. The line corresponding to the McLafferty carboxylic ester rearrangement, m/e 132, is an abundant ion (as expected from the intensity of m/e 74 in methyl esters) in the stearic, oleic, and linoleic acid spectra (not shown). However, in 12-OTMS-TMS-stearate (Table 3A) the m/e 132 is only about 6% and in the other carboxylic ester spectra it is nonexistent (see Discussion). The probable structures of the above and other common peaks are given in Table 7.

Several peaks appear in the TMS ester spectra that are difficult to explain without postulating highly rearranged structures. These peaks are m/e 204, 217, 330 (Table 3A only) and 390 (Tables 4A, 5A, and 6A). No

outstanding rearrangement peaks are consistently present in the TMS octadecanols.

Mass spectral analysis of the reduced and derivatized 9,10-epoxystearic acid and linoleic acid 9- and 13-hydroperoxides are given in Tables 8 and 9, respectively. Since the spectra have fragmentation patterns similar to those of the other spectra only major peaks are given.

DISCUSSION

Aside from the previously mentioned rearrangements, practically all of the major peaks are explicable in terms of simple cleavage with loss of one or more trimethylsilanol groups. This makes determination of the position of hydroxy groups on the stearates and the octadecanols a straightforward process.

It is easy to deduce the number of OTMS groups in a compound or unknown structure from the mass of the compound because of the large mass of the OTMS substituent (Δamu = 89). Interpretation is aided by the fact that the M ion is always observed in conjunction with an intense M–15 line and the number of silicon atoms in a fragment corresponding to a given line can be determined from the P + 1 and P + 2 values arising from the high percentages of isotopes of silicon (^{29}Si, 5.07% and ^{30}Si, 3.31%).

The fragment *a*, resulting from cleavage toward the methyl end of the chain, m/e 173 (Table 2D), 187 (Table 2A,C), or 215 (Table 2B) gives rise to an intense line and allows the number of carbons from this end up to and including the first OTMS group to be determined. In Tables 3 and 4 the second most favored cleavage is toward the —CO-OTMS or —CH_2-OTMS group and allows the determination of the number of carbons from this terminal OTMS group up to and including the next position of oxidation. In Tables 5 and 6, however, the major expected fragments minus 90 must be considered. For example, in the 1,9,10,12,13-penta-OTMS-octadecane (Table 6B) the base peak is fragment *g*–90, m/e 301. Other major peaks (with the exception of fragment *a*, m/e 173 discussed above) are m/e 589, M–15–90, which represents loss of a methyl group and trimethylsilanol; m/e 499, which is loss of a methyl group and two trimethylsilanols; m/e 431, which is fragment *b*–90; and m/e 211 which is *g*–(2 × 90). These peaks appear in addition to the unmodified fragments *c* and *h*, which are present at a relatively high intensity. The more substituted fragments (*b*, *c*, *d*, *e*, *f*, and *g*, Table 2D) show a distinct tendency to lose one or more trimethylsilanol molecules.

The spectra of the TMS octadecanols are similar to those of the TMS acids. The major cleavage peaks are identical except that cleavage toward the reduced acid end in the octadecanols is decreased by 14 amu (i.e., minus oxygen plus two hydrogens). The TMS derivatives of reduced compounds generally show M and M–15 peaks of decreased intensity relative to the TMS acids. This is expected because under electron bombardment the carbonyl in the acid group would have a greater tendency to form an ion than would the entirely saturated oxygens in the reduced compounds.

The absence of the McLafferty carboxylic ester re-

TABLE 7 Common Mass Peaks Appearing in Tables 3–6

m/e	Structure	Comments
73	$Si(CH_3)_3^+$	Appears in all spectra
75	$H\overset{+}{O}{=}Si(CH_3)_2$	Appears in all spectra (see Refs. 7 and 9)
103	$CH_2{=}\overset{+}{O}Si(CH_3)_3$	Appears in all spectra (see refs. 7 and 9)
132	$[CH_2{=}C(OH){-}OSi(CH_3)_3]^+$	Appears in Table 3A only. McLafferty carboxylic rearrangement.
147	$(CH_3)_2Si{=}\overset{+}{O}{-}Si(CH_3)_3$	Appears in all spectra (see Refs. 7 and 9)
155	$CH(CH_2)_7C{\equiv}\overset{+}{O}$	Tables 4A, 5A, and 6A (see Ref. 9)
204	Rearrangement	Appears in all acid esters(Tables3A, 4A, 5A, and 6A)
217	$CH_2{=}CH{-}C({=}\overset{+}{O}{-}Si(CH_3)_3){-}O{-}Si(CH_3)_3$	Appears in all acid esters(Tables3A, 4A, 5A, and 6A) Probable structure (see Ref. 9).
390	$\cdot CH(OSi(CH_3)_3)(CH_2)_7C({=}\overset{+}{O}{-}Si(CH_3)_3){-}OSi(CH_3)_3$	Appears in most acid esters (Tables 4A, 5A, and 6A). Probable structure (see Ref. 9).
M–(15+16)	Loss of CH_3 + CH_4	Appears in Table 3A, B (see Ref. 7)
M–15	Loss of CH_3	Apears in all spectra

TABLE 8 Reduced and Derivatized 9,10-Epoxystearic Acids

m/e	Relative Intensity
215	100
229	69
303	56
317	50
415	2 M–15
430	1 M

TABLE 9 Reduced and Derivatized Linoleic Hydroperoxides

m/e	Relative Intensity
73	37
103	12
173	100
225	25
303	3
355	45
426	20 M

arrangement peak may perhaps be related to the m/e 217 peak through a highly rearranged structure (Table 7) which is similar to a rearranged structure reported by McCloskey et al. at m/e 177 (7). The suggestion of this rearranged structure, m/e 217, is supported by the fact that the presence of two or more OTMS groups (Tables 4A, 5A, and 6A) on one molecule coincide with the complete disappearance of m/e 132. In addition, m/e 217 does not appear in the reduced compounds where no carboxylic ester rearrangement is possible. The isotopic distribution of m/e 217 also indicates the presence of two silicon atoms.

The method described is applicable to determining the position of epoxy groups on fatty acids in addition to keto and hydroxy groups. For example, $LiAlH_4$ reduction of 9,10-epoxystearic acid (Table 8) yielded a mixture of the 1,9- and 1,10-dihydroxyoctadecanols. The epoxy ring opened toward either the 9 or 10 carbon, randomly; the cleavage toward the methyl end gave both m/e 215 and 229, and the cleavage toward the 1-OTMS group gave both m/e 303 and 317. The mixture of 9- and 10-dihydroxyoctadecanols was not resolved under the GLC conditions used and the mass spectrum indicated ring opening about 50% in each direction. The position of the epoxy group might also be determined by hydrolysis to dihydroxystearic acid followed by TMS formation and MS, in which case the data would be those of Table 4A.

The oxidation of linoleic acid with soybean lipoxidase is reported to yield a mixture of hydroperoxides, 70% in the 13 position and 30% in the 9 position (5). A mixture of linoleic 9- and 13-hydroperoxides was subjected to analysis. Reduction with $LiAlH_4$ yielded a mixture of 1,13-dihydroxy-9,11- and 1,9-dihydroxy-10,12-octadecadiene. GLC of the TMS derivatives showed no separation at 220°C on the OV-1 column. MS (Table 9) indicated that the major fragmentation occurred adjacent to the OTMS group: m/e 355 and 173 representing cleavage at the 13-OTMS, and m/e 225 and 303 representing cleavage at the 9-OTMS. The intensity of the 13-OTMS set is greater than the 9-OTMS set, which supports the reported positions and distributions.

Double bonds can also be located by this method if they are first oxidized to the dihydroxy compound; the standards in this work were in fact prepared in this way. A method results that is similar to that reported by Niehaus and Ryhage for the corresponding methyl ethers and esters (8).

Chemical and spectral investigations of one of the products of the metabolism of linoleic acid 13-hydroperoxide indicate that an epoxy group and a keto group conjugated with a double bond are present. Reduction, formation of the TMS derivative, and MS of the compound established the presence of the expected trihydroxyoctadecane. Further investigations with the use of $LiAl^2H_4$ and $^{18}O_2$ are in progress.

Several relevant papers have appeared since the preparation of this manuscript. The proposed rearrangements accounting for m/e 155, 217, and 390 (Table 7) are supported by the recent paper of Capella and Zorzut (9). Using ^{18}O and ^2H they have shown the conversion of methyl 9,10-diOTMS-octadecanoate to m/e 332, with further decomposition to m/e 155. A similar decomposition of the TMS ester in our studies yielded m/e 390 with decomposition to m/e 155. The m/e 217 compares with m/e 159 for the methyl ester.

Smaller fragments and rearrangements—for example m/e 73, 75, 103, and 147—are not discussed in this paper since Diekman, Thomson, and Djerassi (10) have recently described the mechanism of their formation from OTMS hydrocarbons.

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