Gas Chromatography-Mass Spectrometry of Methyl Esters of Unsaturated Oxygenated Fatty Acids[1]

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ABSTRACT

Silylation of hydroxyl groups in methyl esters of unsaturated hydroxy acids provides compounds that give mass spectra which can be readily interpreted, whereas spectra of underivatized esters are extremely difficult to evaluate. The relationship of the double bond(s) to the trimethylsiloxy (TMS) group results in specific mass spectral patterns. In esters that have the TMS group separated from the double bond by one methylene group, the ions caused by α-cleavage at the TMS group on the side closest to the olefinic group are much more abundant than those produced from α-cleavage on the other side of the TMS group. In esters that have the TMS group and the double bond separated by two methylene groups, α-cleavage ions are approximately equal. When the TMS group and the double bond are allylic, no fragmentation results between them. Cleavage does occur on either side of this system, and those ions resulting from cleavage alpha to the TMS group are in greatest abundance. Silylation of esters that have a conjugated diene or ene-yne system adjacent to a hydroxyl group also gives derivatives amenable to gas chromatography-mass spectrometry. In these esters, large peaks are observed that arise from α-cleavage at the TMS group and at the other end of the olefinic system. No fragmentation between the TMS group and the sites of unsaturation occurs. Unsaturated epoxy methyl esters produce spectra difficult to interpret. When the epoxide is converted to methoxy-hydroxy derivatives by BF_3-methanol, the spectrum locates the position of the epoxide group. Silylation of the hydroxyl group produces a compound that gives a less complicated spectrum which also locates the original epoxy group. Mass spectrometry of a series of unsaturated keto-esters, without derivatization, provides spectra that are easily interpretable.

INTRODUCTION

Although mass spectral information about methyl esters of saturated oxygenated fatty acids is extensive (1), only a

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TABLE I

Methyl Esters Analyzed

Trivial name	Systematic name	Source	Reference
Ricinoleate	12-Hydroxy-*cis*-9-octadecenoate[a,b]	*Linum mucronatum*	(11)
Densipolate	12-Hydroxy-*cis*-9,*cis*-15-octadecadienoate[a]	*Lesquerella auriculata*	(12)
Auricolate	14-Hydroxy-*cis*-11,*cis*-17-eicosadienoate[a]	*Lesquerella auriculata*	(12)
	9-Hydroxy-*cis*-12-octadecenoate[a,b]	*Nerium oleander*	(13)
	13-Hydroxy-*trans*-11-octadecenoate[a,b]	Reduction of methyl coriolate	(14)
	9-Hydroxy-*trans*-10-octadecenoate[a]	Reduction of methyl α-dimorphecolate	(14)
	13-Hydroxy-*cis*-9-octadecenoate[a]	Reduction of methyl coriolate	(14)
	2-Hydroxy-*cis*-9,*cis*-12,*cis*-15-octadecatrienoate[b]	*Thymus vulgaris*	(15)
Coriolate	13-Hydroxy-*cis*-9,*trans*-11-octadecadienoate[a,b]	*Coriaria nepalensis*	(16)
Dimorphecolate	9-Hydroxy-*trans*-10,*trans*-12-octadecadienoate[a,b]	*Dimorphotheca sinuata*	(17)
	9,10,18-Trihydroxyoctadec-*cis*-12-enoate[a,b]	*Chamaepeuce afra*	(18)
Phloionolate	9,10,18-Trihydroxyoctadecanoate[a,b]	*Chamaepeuce afra*	(18)
	7-Hydroxy-*trans*-10,16-heptadeacdien-8-ynoate[a]	*Acanthosyris spinescens*	(9)
	7-Hydroxy-*trans*-10-heptadecen-8-ynoate[a]	*Acanthosyris spinescens*	(9)
	8-Hydroxy-*trans*-11,17-octadecadien-9-ynoate[a]	*Acanthosyris spinescens*	(9)
	9,10-Epoxy-*trans*-3,*cis*-12-octadecadienoate[a]	*Stenachaenium macrocephalum*	(19)
	9,10-Epoxyoctadecanoate[a]	Reduction product	(19)
	9,10-Epoxy-*trans*-3-octadecenoate[a]	Reduction product	(19)
Coronorate	9,10-Epoxy-*cis*-12-octadecenoate[a,b]	*Stenachaenium macrocephalum*	(19)
Vernolate	12,13-Epoxy-*cis*-9-octadecenoate[a,b]	*Vernonia anthelmintica*	(20)
	15-Oxo-*cis*-18-tetracosenoate[b]	*Cuspidaria pterocarpa*	(21)
	17-Oxo-*cis*-20-hexacosenoate[b]	*Cuspidaria pterocarpa*	(21)
	19-Oxo-*cis*-22-octacosenoate[b]	*Cuspidaria pterocarpa*	(21)

[a]Analyzed as a pure compound.
[b]Separated from a mixture during gas chromatography-mass spectrometry.

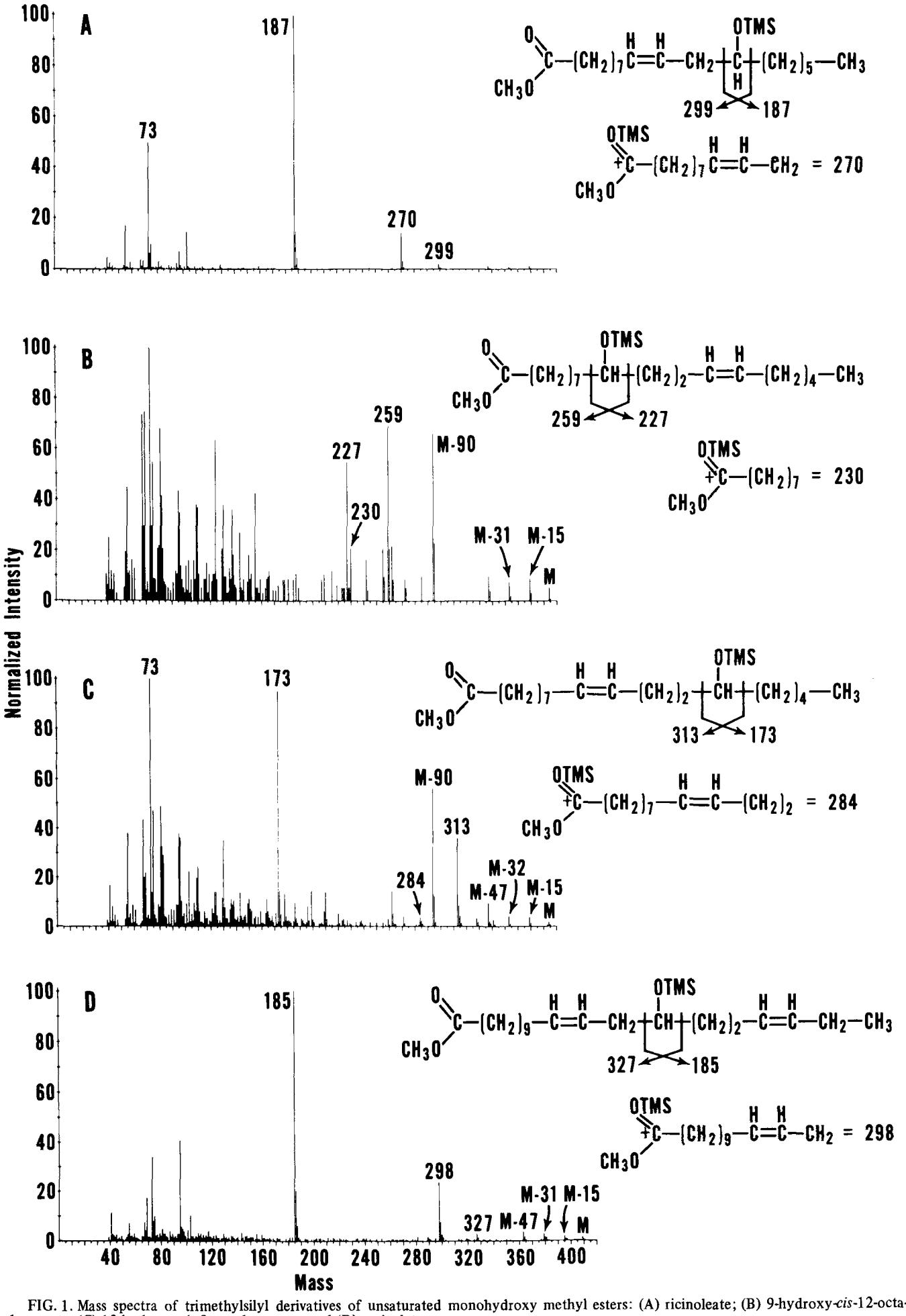


FIG. 1. Mass spectra of trimethylsilyl derivatives of unsaturated monohydroxy methyl esters: (A) ricinoleate; (B) 9-hydroxy-*cis*-12-octadecenoate; (C) 13-hydroxy-*cis*-9-octadecenoate; and (D) auricolate.

few workers have reported specific analysis of unsaturated oxygenated esters (2-6). Reluctance to analyze these unsaturated esters is understandable, because unsaturation favors rearrangements in the mass spectrometer that produce spectra difficult to interpret. To avoid these difficulties and to obtain meaningful spectra, we silylated hydroxyl groups to give compounds which, even though unsaturated, were amenable to gas chromatography-mass spectrometry (GC-MS). For epoxy esters, we first converted the epoxide to a methoxy-hydroxy group and then silylated.

Advantages from analysis of the silylated unsaturated oxygenated esters over analysis of their saturated analogs are: (a) unsaturation, in addition to the oxygen function, is located; (b) side reactions that occur during catalytic hydrogenation (7-9) and complicate GC analysis are avoided; (c) rearrangements during MS are minimized; and (d) molecular ions (M) are usually present in significant amounts.

A number of oxygenated fatty acids encountered in seed oils (10) have been subjected, as derivatized methyl esters, to GC-MS; these spectra serve as the basis for this paper.

MATERIALS AND METHODS

Some esters investigated were pure materials. Others were part of the original mixed esters derived directly from a seed oil (Table I). All esters were introduced into the mass spectrometer through a Packard Model 7401 gas chromatograph. The gas chromatograph was used with a 2 ft x 1/4 in. or 6 ft x 1/4 in. glass column packed with 3% OV-1 on Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pa.) or a 2 ft x 1/8 in. stainless steel column packed with 3% Dexsil 300 GC on Anakrom Q (Analabs, Inc., North Haven, Conn.). The column effluent was split between a flame ionization detector and the mass spectrometer. Components in the effluent arriving at the mass spectrometer were detected by a total ionization monitor. Mass spectra were taken at the apexes of peaks as sensed by the total ionization monitor.

The Du Pont (CEC) 21-492-1 mass spectrometer was equipped with a jet-type helium separator and a mass marker. The mass marker was calibrated with perfluorodecalin (22) (Aldrich Chemical Co., Inc., Milwaukee, Wis.). Transfer lines, separator and source temperatures were kept between 200 and 250 C. The filament current was 50 μA; the voltage was 70 eV; and the source pressure was 4-6 x 10^{-6} Torr. The analyzer pressure was held at 4 x 10^{-7} Torr.

Hydroxyl groups were silylated either with a mixture of hexamethyldisilazane and trimethylchlorosilane (2:1) (Pierce Chemical Co., Rockford, Ill.) in pyridine or with bis(trimethylsilyl)-trifluoroacetamide (Regis Chemical Co., Chicago, Ill.) in acetonitrile.

RESULTS AND DISCUSSION

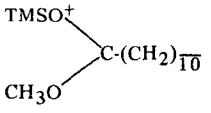
In general, all silylated hydroxy methyl esters exhibit M-15 (methyl), M-31 (methoxyl) and M-47 (methyl plus methanol) ions. In all these esters, the ions at m/e 73 $(CH_3)_3Si^+$ and m/e 75 $(CH_3)_2SiOH^+$ are major peaks (3).

Nonconjugated Systems

In 1969, Capella et al. (4) reported the mass spectrum of methyl ricinoleate. Since the intensities of the significant peaks were low, they concluded that the spectrum might be subject to misinterpretation. The spectrum of the trimethylsilyl (TMS) derivative of methyl ricinoleate (Fig. 1A) allows positive identification. The base peak is m/e 187 from α-cleavage at the unsaturated side of the silyl ether group. Cleavage of the molecule on the other side of the oxygenated site produces a much less intense, though significant, peak at m/e 299. The intensities found for the α-cleavage peaks are in contrast to those found from MS of the silylated saturated analog of methyl ricinoleate (23) where both α-cleavage peaks are greater than 50% (normalized intensity). The effect of the double bond in the molecule may be considered in two different ways: (a) Either ions formed that contain the olefinic site undergo further cleavage and are therefore diminished or (b) placement of the double bond, one methylene group from the oxygenated carbon atom, enhances cleavage on the side of the oxygenated carbon atom closest to the double bond. When other unsaturated hydroxy esters are examined, it appears that both effects may be operative. In one compound with two methylene groups between the oxygenated atom and the double bond, the TMS derivative of methyl 9-hydroxy-*cis*-12-octadecenoate (Fig. 1B), both α-cleavage peaks (m/e 227 and 259) are large and approximately the same size even though one fragment is unsaturated (m/e 227). From the TMS derivative of an ester with similar but reversed structure, methyl 13-hydroxy-*cis*-9-octadecenoate (Fig. 1C), both α-cleavage fragments (m/e 173 and 313) give large peaks, but the one from the unsaturated fragment (m/e 313) is only about one-third as large as the one from the saturated fragment (m/e 173). The olefinic fragment here possibly underwent additional cleavage(s).

Methyl auricolate (Table I, Fig. 1D) contains structures present in both ricinoleic and 9-hydroxy-*cis*-12-octadecenoic acids, i.e., the oxygenated atom is separated from one double bond by one methylene group and from the other by two. From the TMS derivatives of these esters, the peak representing the α-cleavage on the side of the nearest double bond is much larger than that from cleavage on the other side. Since both fragments contain a double bond, the fragmentation pattern presumably is due to a directing effect rather than to unequal destruction of the fragments.

There are a few additional points to be made about mass spectral analyses of these unsaturated monohydroxy esters. To some degree in all these esters, the "migration ions" produced by movement of the TMS group to the ester function are present, e.g.,



m/e 272 from methyl 12-hydroxystearate (23). In esters with one methylene group separating the olefinic bond and the oxygenated carbon atom, this ion (m/e 270 from the TMS derivative of methyl ricinoleate and methyl densipolate [Table I], m/e 298 from methyl auricolate) helps confirm the location of the oxygenated atom revealed by the corresponding low intensity α-cleavage ion. The migration ion is easily recognized, since it has an even number of mass units. This type of ion appears clearly in the analysis of the TMS derivatives of esters with two methylene units separating the functional groups (in methyl 9-hydroxy-*cis*-12-octadecenoate m/e 230 and in methyl 13-hydroxy-*cis*-9-octadecenoate [Fig. 1C] m/e 284).

In addition, analysis of the esters with two methylene units separating the functional groups also showed intense peaks (m/e 294 or M-90) resulting from the loss of $(CH_3)_3SiOH$ (3,24) comparable to the M-90 peaks from TMS derivatives of sterols (25). In contrast, M-90 peaks are very small from esters with just one methylene group separating the functional groups.

We examined TMS derivatives of two trihydroxy esters derived from *Chamaepeuce* seed oil. Both esters gave spectra (Fig. 2A and B) expected from their structures reported by Mikolajczak and Smith (18). One ester, methyl phloionolate, gave the spectrum (Fig. 2A) reported by Eglinton et al. (3), in which the major peaks are m/e 259,

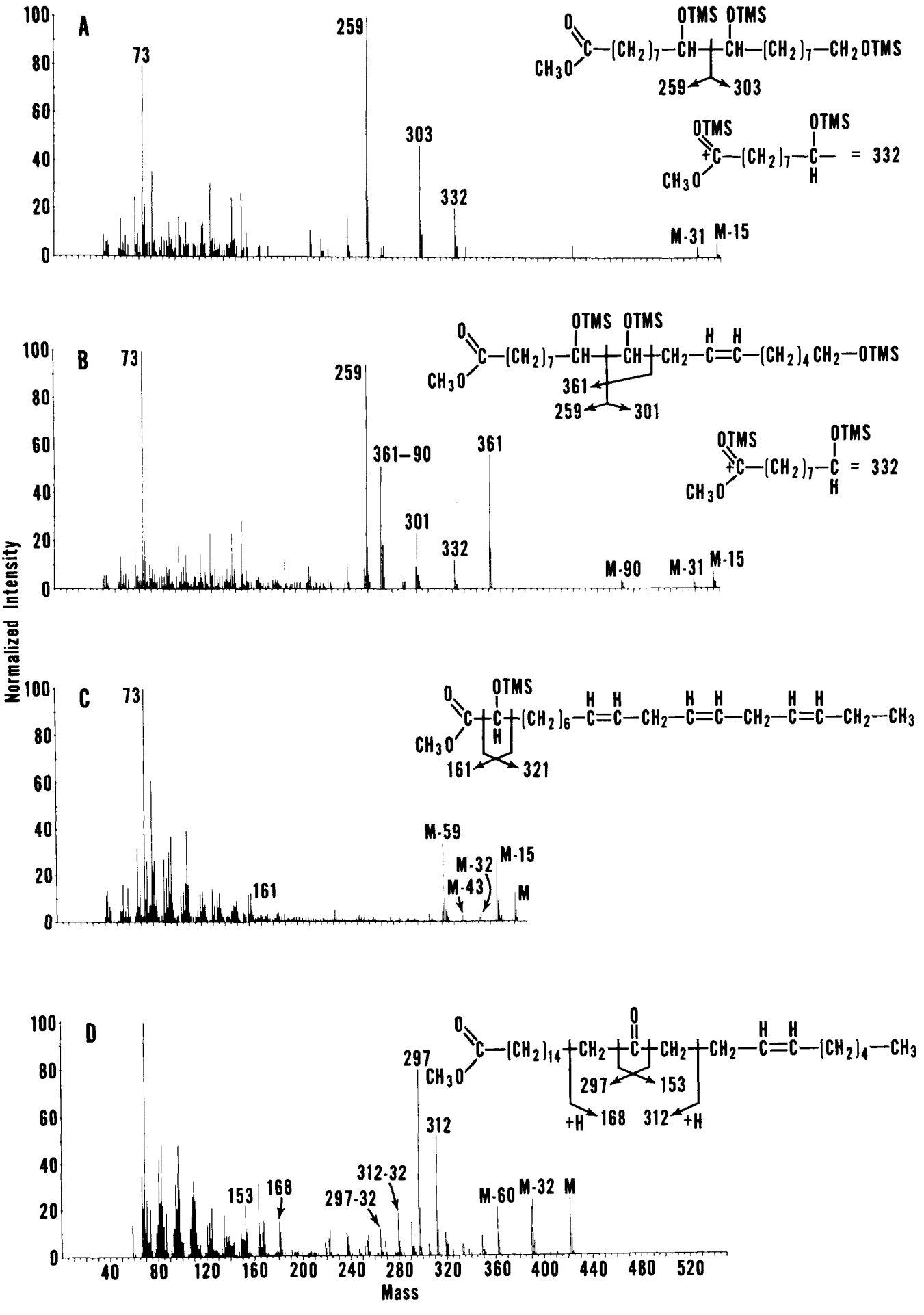


FIG. 2. Mass spectra of the trimethylsilyl derivatives of: (A) methyl 9,10,18-trihydroxy-stearate; (B) methyl 9,10,18-trihydroxy-*cis*-12-octadecenoate; (C) 2-hydroxy-all-*cis*-9,12,15-octadecatrienoate; and (D) the mass spectrum of methyl 17-oxo-hexacos-*cis*-20-enoate.

303 and 332. The spectrum of the other trihydroxy ester (Fig. 2B) was consistent with that of an unsaturated analog. The ions resulting from cleavage between the carbon atoms containing the TMS groups (m/e 259 and 301) are again quite conspicuous, and two additional peaks (m/e 271 and 361) become prominent. Their formation is presumably caused by the presence of a double bond located one methylene group away from the oxygenated carbon atom. This m/e 361 ion corresponds to the intense α-cleavage ions formed in analogous monohydroxy esters mentioned above; the double bond here tends to increase production of ions that locate the oxygenated carbon atom and suggest the location of the double bond. In the unsaturated ester spectrum we again find an M-90 ion and a large m/e 271 (361-90) ion; neither is present in significant amounts in the spectrum from the saturated trihydroxy ester. The m/e 332 ion is found because of TMS group migration (3,26), similar to that found with the derivatives of the monohydroxy esters (23), and is present in about the same degree in both trihydroxy esters.

One nonconjugated monohydroxy trienoic acid that occurs naturally, 2-hydroxy-all-*cis*-9,12,15-octadecatrienoic acid (15), after methylation and silylation showed the ions expected for an α-hydroxy ester (3) (m/e 321 [M-59], 161, M-15, M-32 and M-43) but in addition exhibited a large molecular ion (m/e 380) (Fig. 2C). Interestingly, the molecular ion is not present at all in the spectrum of a saturated C_{16} analog (3).

Conjugated Diene and Ene-Yne Systems

First consideration in the analysis of these compounds is their behavior in GC. Both methyl dimorphecolate (Table I) and its acetate dehydrate to conjugated trienes during GC analysis (27). Even the methyl ether of methyl dimorphecolate is not stable enough to undergo GC analysis (28). However silylation of the hydroxyl group of dimorphecolate esters produces GC-stable derivatives (29-31). We found that TMS derivatives of methyl dimorphecolate and methyl coriolate (Table I) maintain their stability through the GC and into the source of the mass spectrometer.

The spectrum resulting from GC-MS of the TMS derivative of methyl dimorphecolate (Fig. 3A) has three major peaks that define the structure of the ester: m/e 225, M-$(CH_2)_7COOCH_3$ (base peak), m/e 311, M-C_5H_{11} and m/e 382 (the molecular ion). Two of these ions clearly define the location of the conjugated dienol system, while the large m/e 225 peak shows the location of the TMS group itself. The m/e 311 peak locates the double bonds in the 10 and 12 positions. Failure of the molecule to cleave between the TMS group and the 10,11 double bond is expected because of the allylic relation between the double bond and the hydroxyl group (4). This same protective action is evident in the mass spectrum of the TMS derivative of methyl 9-hydroxy-*trans*-10-octadecenoate (Fig. 3B), which includes a base peak at m/e 227 and a peak at m/e 285 that define the hydroxyl at the 9 position and the double bond at the 10,11 position. Absence of a m/e 259 peak demonstrates that no cleavage occurs between the TMS group and the allylic double bond.

The TMS derivative of a compound related to methyl dimorphecolate, methyl coriolate, shows upon MS (Fig. 3C) the same set of major peaks (m/e 225, 311 and 382). However the spectrum of this ester reveals a larger 311 peak than the one at 225 and confirms that the TMS group is at the 13 position. Methyl coriolate and methyl dimorphecolate both exhibited M-90 but no migration ions. The spectrum from the TMS derivative of methyl 13-hydroxy-*trans*-11-octadecenoate (Fig. 3D) again shows the basic cleavage products, m/e 313 (α to the TMS group) and m/e 199 (α to the olefinic group). No significant cleavage was observed between the TMS group and the allylic double bond (no m/e 173).

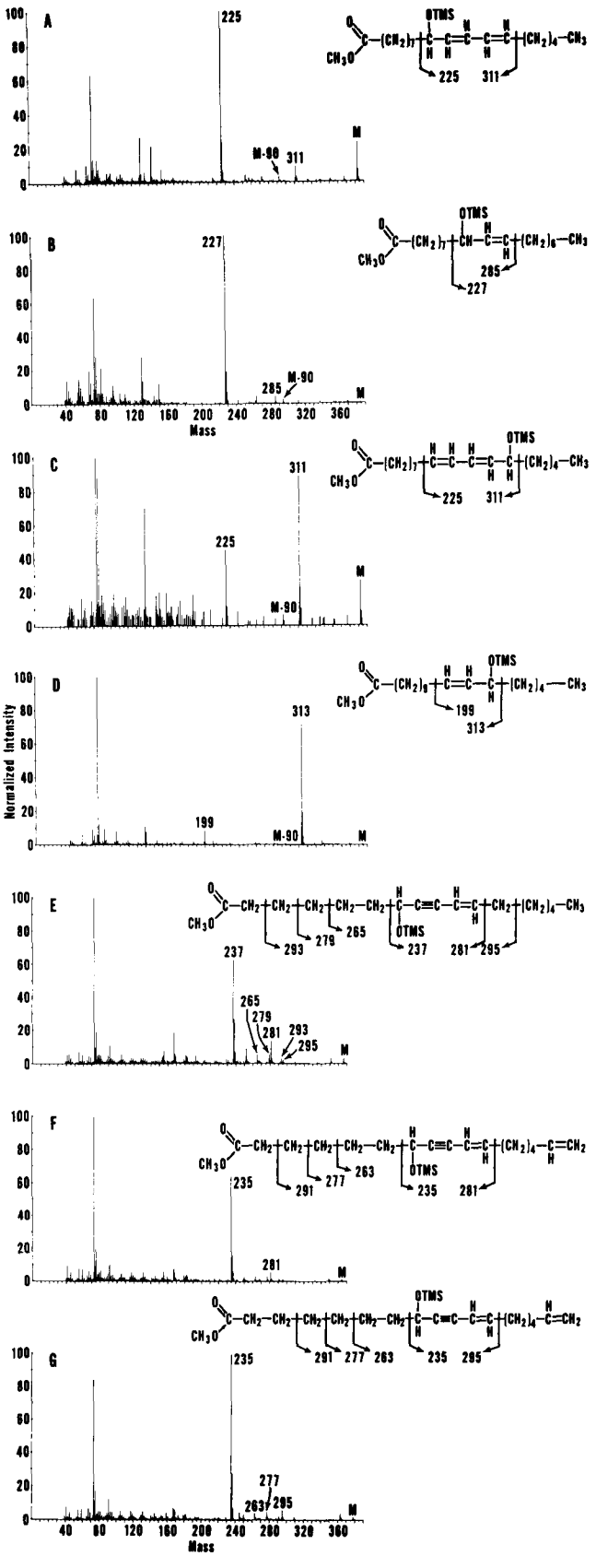


FIG. 3. Mass spectra of trimethylsilyl derivatives of: (A) methyl dimorphecolate; (B) methyl 9-hydroxy-*trans*-10-octadecenoate; (C) methyl coriolate; (D) methyl 13-hydroxy-*trans*-11-octadecenoate; (E) methyl 7-hydroxy-*trans*-10-heptadecen-8-ynoate; (F) methyl 7-hydroxy-*trans*-10,16-heptadecadien-8-ynoate; and (G) methyl 8-hydroxy-*trans*-11,17-octadecadien-9-ynoate.

A series of hydroxy acids with conjugated ene-yne systems was isolated from *Acanthosyris spinescens* and characterized by Powell et al. (9). The TMS derivatives of these esters have excellent GC properties, and no degra-

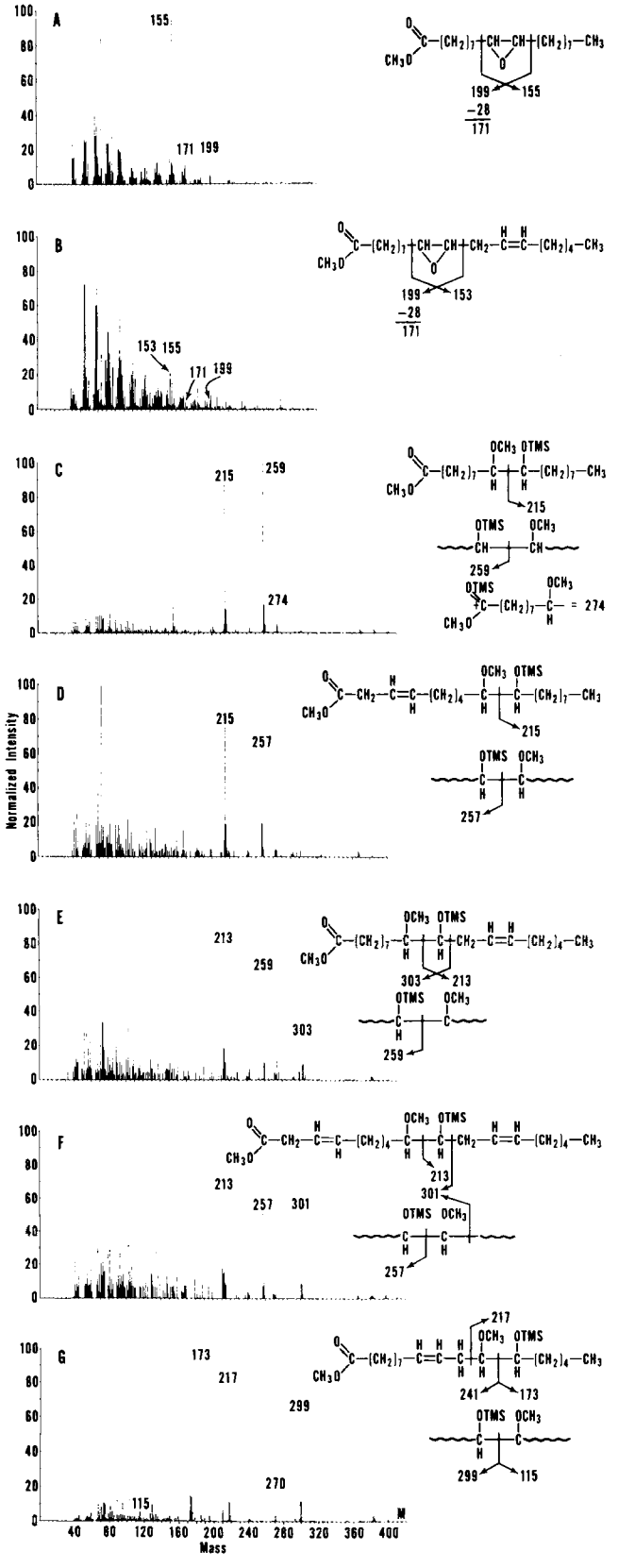


FIG. 4. Mass spectra of (A) methyl 9,10-epoxystearate; and (B) methyl 9,10-epoxy-*cis*-12-octadecenoate, and the trimethylsilyl derivatives of methoxy-hydroxy derivatives of: (C) methyl 9,10-epoxystearate; (D) methyl 9,10-epoxy-*trans*-3-octadecenoate; (E) methyl 9,10-epoxy-*cis*-12-octadecenoate; (F) methyl 9,10-epoxy-*trans*-3,*cis*-12-octadecadienoate; and (G) methyl vernolate.

dation is revealed by either the total ionization monitor or flame ionization detector.

The spectrum from the TMS derivative of methyl 7-hydroxy-*trans*-10-heptadecen-8-ynoate (I) (Fig. 3E) was similar to those for the hydroxy-conjugated diene esters. Major peaks arise from α-cleavage at the TMS group (m/e 237) and α-cleavage at the double bond (m/e 281) and mark the location of the functional groups. Because this method of analysis does not distinguish between an ene-yne or an yne-ene system, we know only the location of the entire hydroxy-ene-yne system and the specific position of the hydroxyl group. Both double and triple bonds must be located by other procedures.

The stability of the trimethylsilyloxy-ene-yne system permits ions formed by cleavage at the methylene groups surrounding it to be observed. Ions at m/e 265, 279 and 293 are most likely formed by rupture of the bonds between the 2 and 3, 3 and 4, and 4 and 5 methylene groups, whereas ions at m/e 295 and 309 come from cleavage between methylene units 11 and 12, and 12 and 13 on the other side of the hydroxy-ene-yne system. No ions from cleavage between the TMS carbon atom and the triple bond were observed, and no migration ion was present.

Analysis of another ester with the same structure as I, except for a terminal methylene group, helped confirm the assignments of ion structures. This ester, 7-hydroxy-*trans*-10,16-heptadecadien-8-ynoate (II), has the same general spectrum (Fig. 3F) as I except that all ions which contain the intact terminal part of the molecule are two mass units less than those from I. These include the large m/e 235 peak (237 in the spectrum of I), which locates the TMS group, and the cleavages at the methylene groups (m/e 263, 277 and 293). Ions not containing the terminal end of the ester are the same in both spectra (m/e 281 and 295).

We have analyzed another ester, methyl 8-hydroxy-*trans*-11,17-octadecadien-9-ynoate (III), which has the same hydroxy-ene-yne system in the terminal end of the molecule as II, but has one more methylene unit than I and II in the carboxyl end. Therefore the spectrum of III (Fig. 3G) has peaks m/e 235, 263, 277 and 291 in common with II and peaks m/e 295, 309 and 323 14 mass units longer than the corresponding peaks in both I and II.

Keto Fatty Esters

In 1966, Smith reported the presence of an interesting homologous series of unsaturated keto-acids in the seed oil of *Cuspidaria pterocarpa* (21). The major keto-ester derived from the oil was methyl 17-oxo-*cis*-20-hexacosenoate. The spectrum for this ester (Fig. 2D) resembled that for a saturated keto-ester (32). α-Cleavage was observed on both sides of the keto group with ions m/e 153 and 297 present. However the m/e 297 ion was much stronger than expected from the behavior of an analogous saturated compound (32). Whether this effect was caused by the double bond is not known, since reference compounds were lacking. The β-cleavage ion on the olefin side, m/e 312, is more abundant than the corresponding ion on the other side of the keto group (m/e 168). Ryhage and Stenhagen (32) showed large β-cleavage ions from both sides of the keto group in saturated keto-esters. All three keto-esters in *Cuspidaria* had analogous spectra. In addition to the structure-revealing ions, all three had large M-32, M-60 and molecular ions.

Epoxy Fatty Esters

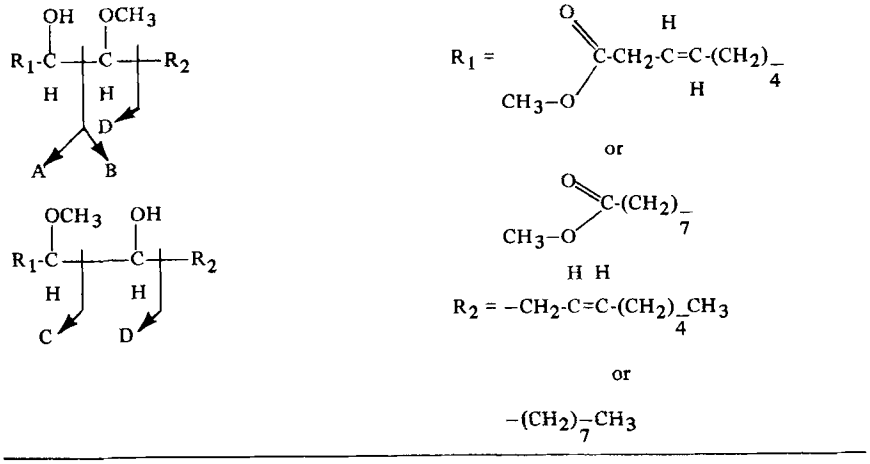
Saturated epoxy esters give mass spectra, the interpretation of which is so straightforward that epoxidation and MS form an established procedure to locate double bonds (3,33). For example, the spectrum of methyl 9,10-epoxystearate (32) has a base peak of m/e 155 arising from cleavage alpha to the epoxide ring. α-Cleavage on the other side of this functional group produces a much smaller but significant peak at m/e 199 (Fig. 4A). In contrast, addition of a double bond into the molecule changes the spectrum so radically that assigning the location of the epoxide ring is almost impossible (Fig. 4B).

When methyl esters of epoxy fatty acids are reacted with

TABLE II

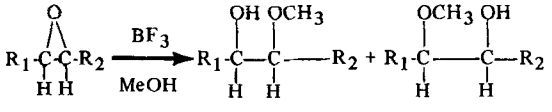
Mass Spectra of Methoxy-Hydroxy Derivatives of 9,10-Epoxy C_{18} Methyl Esters

	Unsaturation							
	Saturated		*trans*-3		*cis*-12		*trans*-3,*cis*-12	
Peak type	m/e	%	m/e	%	m/e	%	m/e	%
A	187	100	185	100	187	25	185	52
A-32[a]	155	66	153	34	155	23	153	43
A-31[b]	156	11	154	2	156	2	154	12
A-(32+18[c])	137	12	135	45	137	16	135	67
A-(31+18)	138	18	136	69	138	12	136	77
B	157	62	157	40	155	23	155	32
B-32	125	6	125	17	123	13	123	30
D	231	0.4	229	1	231	28	229	52
D-32	199	4	197	1	199	100	197	32
D-18	213	2	211	1	213	37	211	27
D-(32+32)	167	3	165	5	167	12	165	85
C	201	78	199	116	201	36	199	17
C-32	169	15	167	53	169	16	167	77
C-31	170	29	168	24	170	18	168	27



[a]Loss of methanol.
[b]Loss of methoxyl.
[c]Loss of water.

BF_3/methanol, essentially quantitative ring opening occurs as follows (28):



Although two products are formed from each epoxide, GC analysis is little affected, since with nonpolar columns both products emerge together. In Table II we include key data from the spectra of the methoxy-hydroxy derivatives of methyl 9,10-epoxystearate and methyl 9,10-epoxy-*cis*-12-octadecenoate. The major ions that locate the methoxy-hydroxy group are the result of either cleavage between or adjacent to the oxygen functions and loss of methanol, methoxyl or water from these ions. The double bond one methylene unit removed from the methoxy-hydroxy group results in ions (D series, Table II) from cleavages similar to those from the first group of unsaturated monohydroxy esters described in the section on "Nonconjugated Systems." These ions (m/e 231 and 199) arise from cleavage adjacent to the oxygenated system nearest the double bond and are the most abundant in the spectrum. Their presence here, and their near absence in the spectrum of the analogous saturated compound, reveal the strong influence of the olefinic group. Analysis was made of two more esters with the methoxy-hydroxy group in the 9,10 position—the *trans*-3 and the *trans*-3,*cis*-12 analogs. The *trans*-3 bond was useful in confirming the identification of the cleavage fragments, since those ions containing it were two mass units less than those ions without this bond. Prominent features from spectra of the methoxy-hydroxy derivatives of the *trans*-3 esters are also given in Table II.

Though GC-MS of the methoxy-hydroxy derivatives of the epoxy-containing esters defines the location of the original epoxy ring, the many major ions from loss of methanol, methoxyl and water give a somewhat complex picture. Silylation of these derivatives produces compounds which, upon MS, give simpler spectra but the same amount of information. In Figures 4C-G, spectra of TMS derivatives of five methoxy-hydroxy esters are shown. In esters without a double bond one methylene unit from the oxygenated site (Fig. 4C and D), two peaks define the location of the oxygenated group. Both peaks arise from cleavage between the carbon atoms containing the methoxyl and the siloxyl groups. The significant ions contain the siloxyl groups and not the methoxyl groups. In esters that have a methylene group separating a double bond from the methoxy-trimethylsilyloxy groups (Fig. 4E-G), we also have the ions described above plus an ion from α-cleavage on the side of the methoxy-trimethylsilyloxy substituent closest to the double bond. MS of esters containing either one or more methoxyl groups (34,35) or siloxyl groups (3,26) result in strong α-cleavages; however, in esters that have both groups, ions containing siloxyl groups completely overshadow those with methoxyl groups.

ACKNOWLEDGMENTS

Methyl esters of reduction products of hydroxy-conjugated

dienoic acids and the acids from *Acanthosyris sponescens* were provided by R.G. Powell. Methyl esters of the trihydroxy acids were supplied by K.L. Mikolajczak.

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