Mass Spectrometry of Triglycerides: I. Structural Effects[1]

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ABSTRACT

Mass spectra of several triglycerides of specific structure or with specific deuterium labeling have been measured with a low resolution mass spectrometer. With saturated triglycerides the abundances of ions characteristic of the component acids, $[M-RCO_2]^+$, increase with increasing chain length, and $[M-RCO_2CH_2]^+$ decrease with increasing chain length. Unsaturation in the acyl moiety causes the abundant formation of $[RCO-1]^+$. Structures have been suggested for a number of the main peaks obtained from saturated triglycerides, and high resolution spectra of one triglyceride agree with the postulated structures. The peaks, $[RCO+74]^+$, $[RCO+115]^+$ and $[RCO+128+14n]^+$, represent structures which contain the glyceryl portion of the triglyceride, since in case of the replacement of its hydrogens with deuteriums, these peaks are shifted accordingly. Evidence which indicates the possibility of determining the location of unsaturation by the interruption of homology of the $[RCO+128+14n]^+$ series, brought about by the addition of deuterium to the unsaturated linkages, is introduced. Further evidence is also presented, which indicates that the $[M-RCO_2CH_2]^+$ ions arise from the positions 1 and 3 and, in agreement with earlier studies from other laboratories, it is thus possible to identify the acyl groups attached to the 1 and 2 positions of the glyceryl moiety.

INTRODUCTION

Ryhage and Stenhagen (1) and Barber et al. (2) have shown that the spectra of triglycerides are characterized by major peaks, which are readily interpreted. One of these major peaks results from the loss of an acyloxy group from the parent molecular ion, and in the case of mixed triglycerides, peaks corresponding to the loss of each acyloxy group are obtained. An acyl ion results from each fatty acid residue present in the mixed triglyceride. Peaks with mass numbers 74 and 128 higher than each acyl ion are also prominent, but structures have not been previously postulated for them.

The present study revealed an additional type of ion $[RCO+115]^+$ and a homologous series of ions $[RCO+128+14n]^+$ of which $[RCO+128]^+$ is the simplest member. This series is of special interest because it presents the possibility to determine the location of unsaturation in triglycerides, for its homology is interrupted by unsaturation or substituents in the acyl chains.

The studies of Dinh-Nguyen et al. (3,4), dealing with isotopically substituted normal long chain methyl esters demonstrated that different mechanisms exist for the formation of the methoxycarbonyl ions, $[CH_3-CO-O-(CH_2)_n]^+$ where $n > 1$. One perhaps unexpected source of this type of ion involves the extrusion of part of the hydrocarbon chain and therefore militates against the possibility of locating the position in the chain at which the homology is interrupted. Hydrogen-deuterium exchange reactions also complicate the problem. Dinh-Nguyen has delineated the behavior of the methyl ester of octadecanoic acid by utilizing isotopically-substituted preparations (deuterium and ^{13}C) and has discussed the applicability of this method for locating double bonds in long chain methyl esters. Our results with triglycerides appear to parallel the behavior of the long chain methyl esters as established by these studies.

EXPERIMENTAL PROCEDURES

The mass spectra were measured using two Hitachi RMU 6D instruments operated at 70 eV. Samples of triglycerides of short chain acids were introduced through the liquid sample insertion system and less volatile triglycerides were directly inserted. The results with the two instruments were consistent. High resolution mass spectra were measured on AEI MS-9 double focussing mass spectrometer.

Glyceryl triacetate, tripropionate, tributanoate, tripentanoate, trihexanoate and trioctanoate were obtained from Eastman Organic Chemicals. Glyceryl tridecanoate, tridodecanoate, tritetradecanoate, trihexadecanoate, tri-

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octadecanoate, trihexadec-9-enoate, trioctadec-6-enoate, trioctadec-9-enoate and trioctadeca-9,12-dienoate were obtained from The Hormel Institute, Lipids Preparation Laboratory. These triglycerides were found to be better than 95% pure by thin layer chromatography (TLC). The saturated diacid triglycerides used in this study were obtained from E.S. Lutton of the Procter and Gamble Co., and from R.E. Jensen of the University of Connecticut. The preparations of perdeuterio-glyceryl trioctadecanoate and 2-deuterio-glyceryl trioctadecanoate are described in a subsequent paper (5).

Methyl-6,7-dideuterio-octadecanoate was prepared according to the method of Rohwedder et al. (6). One milliliter of hydrazine-d_4 solution prepared from 5.6 g hydrazine hydrate-d_6 and 1.6 g of deuterium oxide was added every 12 hr to a solution of 313 mg methyl octadec-6-enoate in 20 ml dry dioxane. Dry oxygen was bubbled continuously through the mixture, which was maintained at 60 C. After 64 hr, the solvent was removed, water was added to the residue and the ester was extracted with petroleum ether. Gas liquid chromatography (GLC) revealed that less than 1% of the starting material remained. The ester was crystallized from petroleum ether [235 mg, 74%, mp 37.5 C Lit. (7) 37.78 C methyl octadecanoate].

The mass spectrum showed a strong parent ion at m/e 300 and major ions at m/e 269 $[M-OCH_3]^+$, 257 $[M-CH_2CH_2CH_3]^+$ and 256 $[M-CHDCH_2CH_3]^+$. The latter was stronger than that at m/e 257.

6,7-Dideuterio-Octadecanoic Acid

The methyl ester (235 mg) was hydrolyzed by refluxing 2 hr in a solution of potassium hydroxide (2 ml, 90%) and methanol (20 ml). The reaction mixture was acidified with dilute hydrochloric acid and then extracted with ether. Removal of the solvent left 171 mg of a solid with a yield of 76%. The impure acid was crystallized, once from petroleum ether and twice from acetone, to yield 121 mg of a product which melted at 69.5 C [Lit. (8) 69.42 C for octadecanoic acid]. Isotopic purity: 86% d_2, 13.7% d_1. The mass spectrum showed a parent ion at m/e 286, and ions at m/e 242 and 243 corresponding to $[M-CHDCH_2CH_3]^+$ and $[M-CH_2CH_2CH_3]^+$, respectively, the former being the more abundant.

Glyceryl Tri-6,7-Dideuterio-Octadecanoate

A mixture consisting of 89 mg 6,7-dideuterio-octadecanoic acid, 6 mg glycerol and 9 mg *p*-toluenesulfonic acid was heated at 80 C at 1 mm for 8 hr. The triglyceride was purified by preparative TLC and recrystallizations from petroleum ether yielding 16 mg of a crystalline product. The triglyceride which was chromatographically indistinguishable from authentic glyceryl trioctadecanoate melted at 72 C [glyceryl trioctadecanoate (9): 72 C]. Isotopic purity of RCO^+ ion: 87% d_2, 13% d_1.

Glyceryl tri-9,10-dideuterio-hexadecanoate and -tri-9,10-dideuterio-octadecanoate were prepared as described above using methyl hexadec-9-enoate and methyl octadec-9-enoate, respectively, as starting materials.

2-Deuterio-Glyceryl Tributanoate

Thirty-seven milligrams of 2-deuterio-glycerol (5), 20 ml butanoic acid and 2 mg *p*-toluenesulfonic acid were heated at 115 C for 39 hr. After removing the excess acid by distillation, the residue was fractionated by TLC. Homogeneous 2-deuterio-glyceryl tributanoate was obtained (77 mg, 64%).

1,3-Dihexanoyloxyacetone was prepared by stirring 1 g dihydroxyacetone and 13.3 g hexanoyl chloride in 25 ml anhydrous benzene plus 2 ml dry pyridine at room temperature for 60 hr. Water was added and the mixture extracted with chloroform. The acid was removed by distillation under vacuum. The remaining yellow oil was crystallized from petroleum ether at 0 C. The crystals, 802 mg (32%), melted at 52 C. The IR spectrum exhibited at intense absorption at 1740 cm^{-1} (CO-stretch) and no absorption in the 3500 cm^{-1} region (OH-stretch). The product had an appropriate NMR spectrum displaying a singlet ((4 H) at 4.78 ppm, a triplet (4 H) at 2.45 ppm, a multiplet (12 H) in the region 1.9–1.1 ppm and a triplet (6 H) at 0.91 ppm. Major peaks in the high mass region of the mass spectrum were m/e 171 $[M-C_5H_{11}CO_2]^+$, 157 $[M-C_5H_{11}CO_2CH_2]^+$, 141 and 99 $[C_5H_{11}CO]^+$. The compound could not be detected on TLC charred with sulfuric acid indicating facile hydrolysis to volatile products. It reacted, however, with 2,4-dinitrophenylhydrazine to give a yellow spot.

2-Deuterio-glyceryl 1-hexanoate was prepared from 100 mg 1,3-dihexanoyloxyacetone by stirring with 30 mg of sodium borodeuteride in 2 ml of ethanol-d_1 for 1 hr at room temperature. Water was added and the mixture extracted with ether. Removal of the solvent left 80 mg which was preparatively chromatographed on a thin layer plate, yielding 17 mg of chromatographically pure material. Its mobility corresponded to a monoglyceride. Important peaks in its mass spectrum were m/e 160 $[M-CH_2OH]^+$ and m/e 99 $[C_5H_{11}CO]^+$.

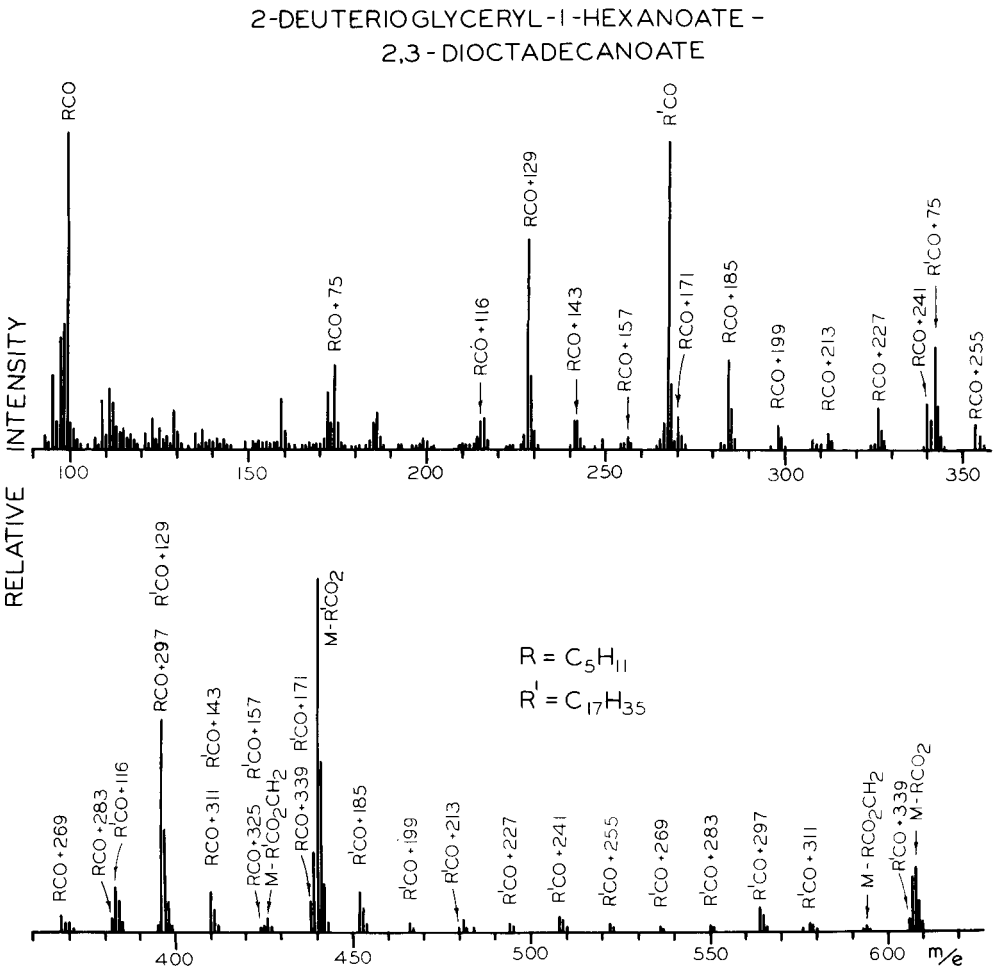


FIG. 1

2-Deuterio-glyceryl 1-hexanoate 2,3-dioctadecanoate was prepared from 17 mg 2-deuterio-glyceryl 1-hexanoate and 116 mg octadecanoyl chloride held under vacuum (ca. 1 mm) at 40 C for 6 hr. The triglyceride was purified by preparative thin layer chromatography twice, and crystallization yielded 13 mg of needles (20%) melting at 45 C. Estimated isotopic purity 83% d_1, based on RCO+74 (75), uncorrected for perhydro analog.

RESULTS AND DISCUSSION

Effect of Chain Length of Fatty Acid Upon the Mass Spectra of Triglycerides

Monoacid triglycerides of the very short chain fatty acids have no molecular ion in their mass spectra. The molecular ion generally increases with increasing chain length of the fatty acid. Even with long chain fatty acids the molecular ion is of low abundance, amounting to 0.013% of the total ionization and 0.22% of the base peak in the case of glyceryl trioctadecanoate.

The ions $[M-RCO_2CH_2]^+$ have been suggested (1) as a means of identifying the fatty acids in the 1 and 3 positions in a triglyceride. In the series of saturated triglycerides studied, the ratio of $[M-RCO_2]^+/[M-RCO_2CH_2]^+$ increased from virtually zero for glyceryl triacetate to 31 for glyceryl trioctadecanoate. Thus the chain length of the fatty acid has a profound effect upon the relative yields of these ions, and the elucidation of structure of mixed triglycerides is thus rendered more difficult.

Mixed triglycerides have mass spectra in which each acyloxy group present is manifested by an ion $[M-RCO_2]^+$ (Fig. 1). The size of this group rather than its location appears to

TABLE I

Per cent Yield of Ions $[M\text{-}RCO_2]^+$ Characteristic of Each Acid in Mixed Triglycerides

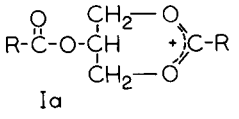
Acid	Atoms %	Atoms %	Atoms %
6:18:18	12.8	43.6	43.6
12:18:18	19.8	40.1	40.1
18:12:18	39.6	20.8	39.6
18:14:18	36.1	27.8	36.1
18:16:18	35.0	30.0	35.0
18:18:18[b]	33.3	33.3	33.3
16:12:16	37.8	24.3	37.8
16:14:16	35.4	29.1	35.4
16:16:16[b]	33.3	33.3	33.3
16:18:16	30.8	38.3	30.8

[a]Saturated acids are designated by their numbers of carbon.

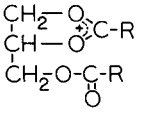
[b]Assuming equivalence of the three positions.

determine the relative amounts of these ions. This statement is based on studies of a number of mixed triglycerides containing two different acyloxy groups. In the case of glyceryl 2-hexadecanoate-1,3-dioctadecanoate (18:16:18) the observed height of the $[M-C_{17}H_{35}CO_2]^+$, peak is 220 and that of the $[M-C_{15}H_{31}CO_2]^+$ peak is 94. Thus, if there is constant sensitivity in this region of the spectrum, the yields of these ions are 35.0% each for the two 18:0 residues and 30.0% for the 16:0. Table I is the result of similar measurements with a series of mixed triglycerides. A comparison between 18:16:18 and 16:18:16 reveals that in both cases the $[M-C_{17}H_{35}CO_2]^+$ fragment is approximately 1.2 times more abundant than the $[M-C_{15}H_{31}CO_2]^+$ fragment. The table also reveals that in two series in which two acyl groups remain constant and one changes, the yield of $[M-RCO_2]^+$ increases with chain length of the acid. This phenomenon has been observed for glyceryl 1-acyl-2,3-diacetates and glyceryl 2-acyl-1,3-diacetates (10) and in diesters of short chain diols (11).

The molecular ion presumably is formed by the loss of an unshared electron from an oxygen, and the $[M-RCO_2]^+$ may be produced from the molecular ion by the loss of one acyloxy group from the 1 or 2 position giving rise to ions Ia or Ib, or both.



Ia



Ib

The larger the acyloxy group lost, the more intense is the residual ion, I.

TABLE II

Relative Intensities of $[M\text{-}RCO_2CH_2]^+$ Ions in Spectra of Saturated Triglycerides and Their 2-Deuterio-Glyceryl Counterparts

Ion	m/e	Relative intensities
Glyceryl trioctadecanoate		
	592	38
$[M - RCO_2CH_2]^+$	593	100
	594	40
Glyceryl tributanoate		
	199	12.7
	200	3.6
$[M - RCO_2CH_2]^+$	201	100
	202	14.3
	203	2.6
2-Deuterio-glyceryl trioctadecanoate		
$[M - RCO_2CHD]^+$	593	48
$[M - RCO_2CH_2]^+$	594	100
	595	37
2-Deuterio-glyceryl tributanoate		
	200	12.0
$[M - RCO_2CHD]^+$	201	3.0
$[M - RCO_2CH_2]^+$	202	100
	203	11.8
	204	1.8

Position of Fatty Acid in Triglyceride Molecule

Earlier studies (1,2) indicated that the acids esterified at the 1 and 2 positions in a mixed triglyceride could be distinguished on the basis of the relative populations of the two ions, $[M-R^1CO_2CH_2]^+$ and $[M-R^2CO_2CH_2]^+$ derived from positions 1 and 3 or from the 2 position, respectively. This method has been applied to a natural triglyceride of unusual structure (12). The ionic population of $[M-R^2CO_2CH_2]^+$ from the 2 position, is very small or negligible. In the case of the higher members of the series of saturated triglycerides, the ions $[M-R^1CO_2CH_2]^+$ are of low intensity, but are readily recognizable. The $[M-CH_3CO_2CH_2]^+$ ion from glyceryl triacetate is very prominent, but as the molecular weight of the triglyceride increases, the corresponding ions become much less abundant.

A comparison of the spectra of glyceryl trioctadecanoate and 2-deuterio-glyceryl trioctadecanoate revealed a maximum contribution of 10% due to $[M-C_{17}H_{35}CO_2CHD]^+$ ions (Table II). A much more significant example is given by comparison of spectra of glyceryl tributanoate and 2-deuterio-glyceryl tributanoate. In this case, unlike the preceding one, the $[M-RCO_2CH_2]^+$ peak is prominent. Table II

TABLE III

Relative Intensities of Members of the Series, $[RCO+128+14n]^+$ (% of base peak)

RCO	Glyceryl tridodecanoate	Glyceryl tritetradecanoate	Glyceryl trihexadecanoate
+128	29.0	89.0	100.0
+142	2.7	5.9	11.2
+156	1.9	1.6	7.6
+170	4.0	8.9	19.5
+184	6.9	23.7	46.0
+198	1.6	5.4	7.6
+212	5.1	3.0	11.9
+226	2.3	4.0	13.2
+240	0.5	12.1	10.5
+254	1.3	3.8	6.2
+268	---	0.6	27.0
+282	---	2.6	10.3
+296	---	---	2.9
+310	---	---	8.4

also shows that the abundance of $[M-C_3H_7CO_2CHD]^+$ ions formed from 2-deuterio-glyceryl tributanoate is within experimental error of the measurement.

The synthesis of mixed triglycerides without any rearrangement is difficult (13), and as a consequence samples regarded as pure may be contaminated with small amounts of rearranged products. Accordingly, a mixed triglyceride, ABA, may contain some AAB which could give rise to a small amount of ion $[M-RCO_2CH_2]$ corresponding to the acid B whereas the pure triglyceride ABA should not do so. It appears that the $[M-RCO_2CH_2]^+$ ions originate from the 1 and 3 positions and that little, if any, arises from the 2 position. Use of these ions to determine structure of pure triglycerides is possible if the effects of chain length of acyl groups are considered.

Effect of Unsaturation

Unsaturation in the acyl chains would be expected to interrupt the homology in the $[RCO+128+14n]^+$ series. The mass spectra of glyceryl trihexadec-9-enoate, trioctadec-6-enoate, trioctadec-9-enoate and trioctadeca-9,12-dienoate revealed that this was the case. However, the mobility of the double bonds caused formation of many ions in relatively equal abundances which obscure the interruption of homology needed for assignment of double bond position. This is analogous to the phenomenon observed with unsaturated methyl esters (14,15). In the case of unsaturated triglycerides, the RCO^+ ions are accompanied by prominent $[RCO-1]^+$ ions. This loss of one mass unit occurred in all cases studied. With glyceryl trioctadeca-9,12-dienoate, prominent ions representing RCO^+, $[RCO-1]^+$, $[RCO-2]^+$ and $[RCO-3]^+$ occurred. With glyceryl trioctadec-6-enoate, trihexadec-9-enoate, trioctadec-9-enoate and with glyceryl-1-hexadecanoate-2-octadecanoate-3-octadec-9-enoate (16:0,18:0,18:1), only the RCO^+ and $[RCO-1]^+$ ions were prominent in this part of the spectrum. The ratio of $[RCO-1]^+/RCO^+$ varied between 1.5 and 0.7. In the single case, glyceryl trihexadec-9-enoate, in which low electron voltage (15 eV) was employed, the intensity of the $[RCO-1]^+$ ion showed a considerable relative increase when compared with the value obtained at 70 eV. The loss of hydrogen might give rise to a ketene, which is an attractive possibility for the structure of this $[RCO-1]^+$ ion.

Locating Double Bond Positions via Deuteration

The $[RCO+128]^+$ peaks are very prominent in the spectra of triglycerides. In the case of glyceryl trihexadecanoate it proved to be more

TABLE IV

Composition of Ions of the Series $[RCO+128+14n]^+$ in the Mass Spectrum of Glyceryl Tritetradecanoate

$C_{13}H_{27}CO$	Ion	Calculated	Found
+128	$C_{20}H_{35}O_4$	339.2535	339.2519
+142	$C_{21}H_{37}O_4$	353.2692	353.2672
+156	$C_{22}H_{39}O_4$	367.2848	367.2808
+170	$C_{23}H_{41}O_4$	381.3005	381.2973
+184	$C_{24}H_{43}O_4$	395.3161	395.3155
+198	$C_{25}H_{45}O_4$	409.3318	409.3316
+212	$C_{26}H_{47}O_4$	423.3474	423.3470
+226	$C_{27}H_{49}O_4$	437.3631	437.3606
+240	$C_{28}H_{51}O_6$	451.3787	451.3764
+254	$C_{29}H_{53}O_4$	465.3944	465.3948
+282	$C_{31}H_{57}O_4$	493.4257	493.4254

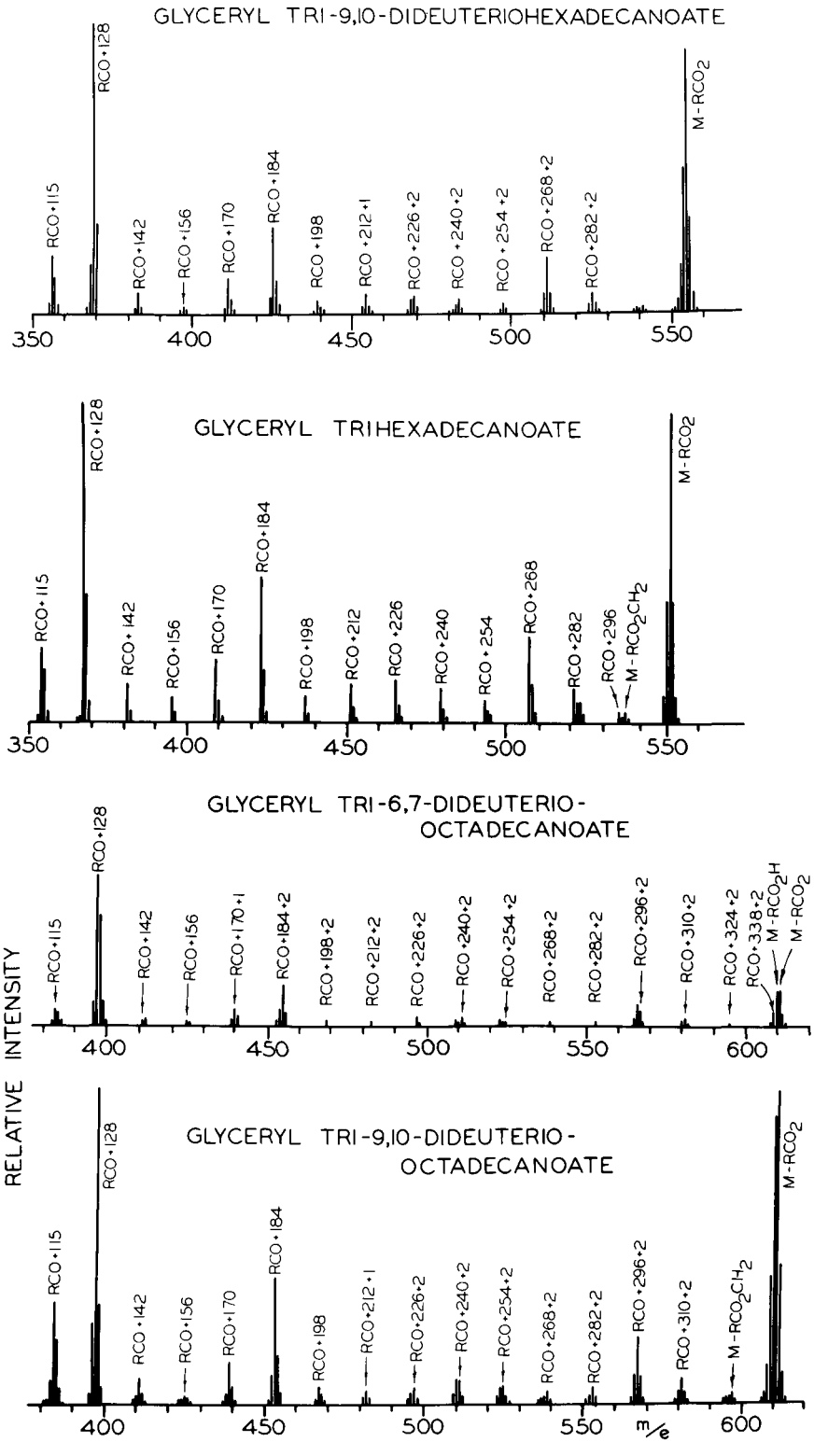
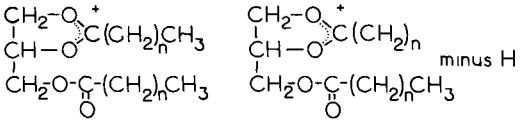

GLYCERYL TRI-9,10-DIDEUTERIOHEXADECANOATE
RCO+128
M-RCO2
RCO+115
RCO+142
RCO+156
RCO+170
RCO+184
RCO+198
RCO+212+1
RCO+226+2
RCO+240+2
RCO+254+2
RCO+268+2
RCO+282+2
350
400
450
500
550
GLYCERYL TRIHEXADECANOATE
RCO+128
M-RCO2
RCO+115
RCO+142
RCO+156
RCO+170
RCO+184
RCO+198
RCO+212
RCO+226
RCO+240
RCO+254
RCO+268
RCO+282
RCO+296
M-RCO2CH2
350
400
450
500
550
GLYCERYL TRI-6,7-DIDEUTERIO-
OCTADECANOATE
RELATIVE INTENSITY
RCO+128
RCO+115
RCO+142
RCO+156
RCO+170+1
RCO+184+2
RCO+198+2
RCO+212+2
RCO+226+2
RCO+240+2
RCO+254+2
RCO+268+2
RCO+282+2
RCO+296+2
RCO+310+2
RCO+324+2
RCO+338+2
M-RCO2H
M-RCO2
400
450
500
550
600
GLYCERYL TRI-9,10-DIDEUTERIO-
OCTADECANOATE
RCO+128
M-RCO2
RCO+115
RCO+142
RCO+156
RCO+170
RCO+184
RCO+198
RCO+212+1
RCO+226+2
RCO+240+2
RCO+254+2
RCO+268+2
RCO+282+2
RCO+296+2
RCO+310+2
M-RCO2CH2
400
450
500
550
m/e
600

FIG. 2

intense than the RCO^+ or $[M-RCO_2]^+$ peaks under the conditions used. It is the first member of a series, the successive members of which differ by 14 mass units and which therefore can be used for locating double bonds which have been saturated by deuterium. The intensities of the members of the series vary widely, but their recognition presents little difficulty, because for each the neighboring peaks are less intense. Table III gives the uncorrected values for three typical simple triglycerides.

The composition of the ions of this series was established by high resolution studies in the case of glyceryl tritetradecanoate and these data are shown in Table IV. It is apparent that this series of fragment ions results from the molecular ion, $C_{45}H_{86}O_6$, by loss of one acyloxy ion, $C_{14}H_{27}O_2$, plus an alkane, or by the loss of $C_{14}H_{28}O_2$ plus an alkyl radical. Several structures may be assigned, but structures II and III have been adopted as a working hypothesis. These ions may also include species containing six-membered rings involving all three carbon atoms of glycerol, analogous to ion Ia. Similar cyclic structures have been suggested for ions formed from ethylene ketals (16-18), deuterated glyceryl 1,3-dioctadecanoates (19) and from 1-acyl-2-alk-1'-enyloxy ethanols (20).



II III

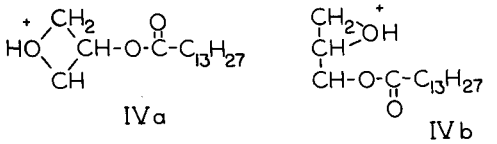
Glyceryl trihexadec-9-enoate was deuterated with tetradeuterio-hydrazine and the spectrum of the resulting glyceryl tri-9,10-dideuteriohexadecanoate was compared with that of glyceryl trihexadecanoate. The pertinent portions of the spectra are shown in Figure 2. Figure 2 also presents similar results obtained in the cases of glyceryl tri-6,7-dideuterio-octadecanoate, and glyceryl tri-9,10-dideuterio-octadecanoate. Inspection of the series of ions, $[RCO+128+14n]^+$, in the spectra of the three deuterated triglycerides, revealed discontinuities related to the original double bond position. The first members of the series are normal in all cases, but when the first carbon atom which bore the double bond appears in the series, the ion bearing one deuterium atom is one mass too large. All subsequent members of the series are two mass units too large because they include both carbon atoms bearing deuterium. In these three cases, expulsion and exchange reactions do not constitute a series limitation (5). The method likewise seems applicable to locating double bonds in all positions in a fatty acid residue, for the series plus ion e (5) subtend all the carbon atoms of a fatty acid residue. The applicability of the method may be limited because of the relatively low intensities of the ion in which n=2 and the last member of the series and the possibility of exchange and extrusion reactions. Mixed triglycerides, such as 16:18:16, give two series; one $[C_{15}H_{31}CO+128+14n]^+$ and another, $[C_{17}H_{35}CO+128+14n]^+$, some members of which overlap.

Structures of Other Prominent Ions in Mass Spectra of Triglycerides

A McLafferty rearrangement occurs, for in the case of glyceryl tritetradecanoate a peak appears at mass 554 (calculated for $C_{33}H_{62}O_6$, 554.45465; found 554.45501), which corresponds to the loss of $C_{12}H_{24}$, leading to the replacement of the acyl group with an acetyl group. A series of such ions, in which CH_3CO is the acyl group is indeed found. The $[CH_3CO+115]^+$ peak, m/e = 158, is present as well as the series $[CH_3CO+128+14n]^+$, m/e 171, 185, 199, etc. The presence of an acetyl group, resulting from a McLafferty rearrangement manifested itself in the spectra of all of the saturated triglycerides studied. A second series of ions produced from the McLafferty product by loss of a fatty acid molecule likewise occurs. Their structures would be analogous to ion III in which the remaining acyl group is CH_3CO. When the triglycerides contained glycerol labeled in the 2 position with deuterium, all these ions shifted by one mass unit, indicating that they all include the glyceryl moiety.

$[RCO+74]^+$

This peak is a prominent one, as indicated by earlier studies (1,2). High resolution measurement of this ion in the spectrum of glyceryl tritetradecanoate indicated a composition of $C_{17}H_{33}O_3$ (calculated 285.2429; found 285.2420). The structures IVa and IVb are viewed as probable for this ion.



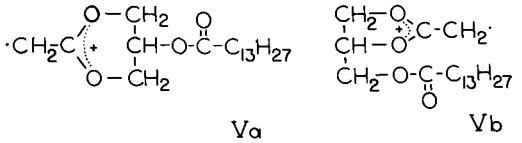
IVa IVb

Its formation may involve the loss of a ketene, $C_{12}H_{25}CH=C=O$, from the $[M-C_{13}H_{27}CO_2]^+$ ion. In spectra of mixed triglycerides there is a prominent $[RCO+74]^+$

peak corresponding to each RCO in the triglyceride structure. In the cases of 2-deuteriogiyceryl trioctadecanoate and perdeuteriogiyceryl trioctadecanoate the peak appears at $[RCO+75]^+$ and $[RCO+79]^+$, respectively, indicating that the entire glycerol moiety is involved, consistent with the structures postulated.

$[RCO+115]^+$

The spectra, published by Ryhage and Stenhagen (1), and by Barber et al. (2), as well as those obtained in the present study show prominent $[RCO+115]^+$ peaks. In the case of glyceryl tritetradecanoate, the composition of $C_{19}H_{34}O_4$ was established by high resolution measurements (calculated 326.2457; found 326.2433). This corresponds to a loss of $C_{13}H_{27}CO_2$ plus $C_{12}H_{25}$, or to $C_{13}H_{27}CO_2H$ plus $C_{12}H_{24}$ from glyceryl tritetradecanoate, $C_{45}H_{86}O_6$.



Va

Vb

Formulas Va and Vb represent possible structures of this ion. In the cases of 2-deuteriogiyceryl trioctadecanoate and perdeuteriogiyceryl trioctadecanoate this peak is shifted to $[RCO+116]^+$ and $[RCO+120]^+$, respectively, indicating retention of the entire glycerol moiety.

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REFERENCES

1. Ryhage, R., and E. Stenhagen, J. Lipid Res. 1:382 (1960).
2. Barber, M., T.O. Merren and W. Kelly, Tetrahedron Letters 1063 (1963).
3. Dinh-Nguyen, Ng, R. Ryhage, S. Stallberg-Stenhagen and E. Stenhagen, Ark. Kemi 18:393 (1961).
4. Dinh-Nguyen, Ng, Ibid. 28:289 (1968).
5. Aasen, A., W.M. Lauer and R.T. Holman, Lipids 5:869-877 (1970).
6. Rohwedder, W.K., C.R. Scholfield, H. Rakoff, J. Nowakowska and H.J. Dutton, Anal. Chem. 39:820 (1967).
7. King, A.M., and W.E. Garner, J. Chem. Soc. 1936:1372.
8. Meyer, J.D., and E.E. Reid, J. Amer. Chem. Soc. 55:1574 (1933).
9. Clarkson, C.E., and T. Malkin, J. Chem. Soc. 1948:985.
10. Johnson, C.B., and R.T. Holman, Lipids 1:371 (1966).
11. Baumann, W.J., J. Seufert, H.W. Hayes and R.T. Holman, J. Lipid Res. 10:703 (1969).
12. Sprecher, H.W., R. Maier, M. Barber and R.T. Holman, Biochemistry 4:1856 (1965).
13. Malkin, T., and T.H. Bevan in "Progress of the Chemistry of Fats and Other Lipids," Vol. 4, Edited by R.T. Holman, Pergamon Press, Oxford and New York, 1957, p. 63-77.
14. Hallgren, B., R. Ryhage and E. Stenhagen, Acta Chem. Scand. 13:845 (1959).
15. Christie, W.W., and R.T. Holman, Chem. Phys. Lipids 1:407 (1967).
16. Mutzenbecher, G.V., Z. Pelah, D.H. Williams, H. Budzikiewicz and C. Djerassi, Steroids 2:475 (1963).
17. Pelah, Z., D.H. Williams, H. Budzikiewicz and C. Djerassi, J. Amer. Chem. Soc. 86:3722 (1964).
18. Audier, H., A. Diara, M.J. deDurazo, M. Fetizon, P. Foy and W. Vetter, Bull. Soc. Chim. Fr. 1963:2827.
19. Morrison, A., M.D. Barratt and R. Aneja, Chem. Phys. Lipids 4:47 (1970).
20. Kramer, J.K.G., and H.K. Mangold, Chem. Phys. Lipids 4:332 (1970).

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