Mass Spectrometry of Triglycerides: II. Specifically Deuterated Triglycerides and Elucidation of Fragmentation Mechanisms[1]

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ABSTRACT

Deuterium labeled monoacid triglycerides were synthesized and their mass spectra were measured. The spectra provided further support for proposed (1) structures of principal ions, knowledge about the formation of $[M-18]^+$, the interexchange of hydrogen atoms between 2 and the 5, 6 or 7 positions and the expulsion of part of the alkyl chain.

INTRODUCTION

Mass spectrometry has been used to only a limited extent in characterizing glyceryl esters. Brief notes by Ryhage and Stenhagen (2) and Barber et al. (3) recognized the major ions, $[M-18]^+$, $[M-RCOOH]^+$, $[M-RCO_2]^+$, $[M-RCO_2CH_2]^+$, RCO^+, and stated that the acyl groups attached to 1, 3 and 2 positions in a mixed triglyceride could be distinguished by comparing the abundances of [M-acyloxymethylene]$^+$ ions. Johnson and Holman (4) studied the di-trimethylsilyl ethers of 1 and 2 monoglycerides and found the populations of the ion $[M-CH_2OSi(CH_3)_3]^+$ to differ greatly in the two cases. Morrison et al. (5) recently discussed the mass spectra of some deuterated glyceryl 1,3-dioctadecanoates.

Practical applications of mass spectrometry of glycerides have been found in elucidating the structure of an allene-containing triglyceride from *Sapium sebiferum* (6), quantitative analysis of triglyceride mixtures (7) and pyrolysis of phosphoglycerides (8). Of special interest is the ion $[M-18]^+$ first described by Barber et al. (3). To our knowledge this is the first observation of an ester function being involved in the formation of this ion. In the present study elucidation of the cracking pattern was attempted by deuteration on successive carbon atoms of the triglycerides.

EXPERIMENTAL PROCEDURES

Alkyl mesylates and cyanides were prepared according to the methods described by Baumann and Mangold (9,10). The procedure of Christie and Holman (11) was followed in the malonic ester syntheses. Unless otherwise stated, triglycerides were synthesized by heating glycerol, 25-50% excess fatty acid and *p*-toluenesulfonic acid as catalyst at 80-90 C and 1 mm pressure for 6-7 hr. The triglycerides were isolated by preparative thin layer chromatography (TLC) and recrystallized 2-4 times from petroleum ether. All triglycerides used in this study were chromatographically homogenous.

The mass spectra were measured in a Hitachi RMU6D instrument at 70 eV and at about 1 to $2x10^{-7}$ torr. The direct solid sample insertion system was used for triglycerides and the liquid sample insertion system for compounds of lower molecular weight.

2,2-Dideuterio-Tetradecanoic Acid (I)

Methyl tetradecanoate, 3.52 g, was added to a solution of 80 mg sodium in 5 ml methanol-d_1. The mixture was refluxed for 15 min. The methanol was removed under vacuum and the treatment repeated twice with two portions of 5 ml fresh methanol-d_1. The ester was hydrolized by refluxing for 1 hr after adding a solution of 265 mg sodium in 2 ml ethanol-d_1 and 3 ml deuterium oxide. The mixture was acidified with 3 ml 10 N deuterium chloride and extracted with chloroform. The solvent was distilled under reduced pressure leaving a colorless crystalline solid, 3.24 g (97%). The acid was recrystallized twice from acetone. Melting point 53.5-54 C. [Lit. (12) 54-54.1 C for tetradecanoic acid]. Important peaks in the high mass region of the mass spectrum were m/e 230 M^+, 213 $[M-17]^+$, 187 $[M-CH_2CH_2CH_3]^+$, 186 $[M-CDHCH_2CH_3]^+$ and 185 $[M-CD_2CH_2CH_3]^+$.

Glyceryl Tri-2,2-Dideuterio-Tetradecanoate (II)

Seventy-three milligrams of II was obtained from 42 mg glycerol, 404 mg I using 10 mg *p*-toluenesulfonic acid as catalyst. The labile protons of glycerol had been exchanged with deuterium by shaking with two portions of 1 ml deuterium oxide which subsequently was removed under diminished pressure. Melting point 57.5 C [Lit. (13) 57 C for glyceryl tri-

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tetradecanoate]. Isotopic purity, based upon RCO^+: 90.3% d_2, 6.1% d_1, 3.5% d_0.

2,2-Dideuterio-Tetradecanol (III)

An amount of 1.66 g I was dissolved in 25 ml anhydrous ether and added slowly to a suspension of 1.7 g lithium aluminum hydride in 150 ml dry ether. The mixture was refluxed for 5 hr. Moist ether was added and the slurry filtered through celite. The solvent was removed leaving 1.35 g (87%) as a white solid. The product was chromatographically homogenous and melted at 37.5-38 C [Lit. (14) 37.62 C for tetradecanol]. No parent peak was observed in the mass spectrum but an intense peak, m/e 198, corresponded to $[M-18]^+$.

2,2-Dideuterio-Tetradecyl Mesylate (IV)

Methanesulfonyl chloride, 1.71 g, was added dropwise to a cooled (O C) solution of 1.25 g III in 15 ml dry pyridine. The mixture was stirred 5 hr at room temperature and worked up as described by Baumann and Mangold (9). Recrystallization from petroleum ether gave 1.09 g (63%) melting at 44 C [Lit. (9) 44-45 C for tetradecyl mesylate]. The TLC revealed one spot only. Its mass spectrum exhibited no parent peak but a strong peak at m/e 198 agreeing with $[M-CH_3SO_3H]^+$.

2,2-Dideuterio-Tetradecyl Cyanide (V)

A solution of 1.02 g IV in 20 ml dimethyl sulfoxide was added to 0.62 g potassium cyanide. The mixture was stirred at 120 C for 2 hr and worked up as described by Baumann and Mangold (10). The slightly yellow oil, 745 mg (95%) had an appropriate mass spectrum (15) having m/e 225 and m/e 224 corresponding to M^+ and $[M-1]^+$.

3,3-Dideuterio-Pentadecanoic Acid (VI)

The nitrile V, 745 mg, was refluxed in a mixture of 2 ml 90% potassium hydroxide and 2 ml ethanol for 58 hr. Acidification with hydrochloric acid and extraction with chloroform gave, after removal of the solvent, a colorless crystalline residue which was recrystallized from acetone. Yield: 543 mg (74%), mp 53 C [Lit. (16) 52.1 C for pentadecanoic acid]. The mass spectrum revealed peaks at m/e 224 and m/e 199 corresponding to M^+ and $[M-CH_2CD_2CH_3]^+$.

Glyceryl Tri-3,3-Dideuterio Pentadecanoate (VII)

After reacting 228 mg VI with 18.5 mg of glycerol using 15 mg catalyst 134 mg (86%) VII was isolated. Melting point 54 C [Lit. (13) 54 C for glyceryl tripentadecanoate]. Isotopic purity of RCO^+: 94.8% d_2, 5.2% d_1.

4,4-Dideuterio-Hexadecanoic Acid (VIII)

Diethyl malonate, 3 g, was added to a solution of 200 mg sodium in 25 ml anhydrous ethanol. The mixture was refluxed for 1 hr and 1.01 g IV was added, and the solution refluxed for 2 hr and left at room temperature overnight. The pink solution was filtered and most of the solvent removed under vacuum. Water was added and the esters extracted with chloroform. The solvent was distilled under reduced pressure leaving an orange oil which was hydrolized by refluxing for 2 hr in a mixture of 5 ml 90% potassium hydroxide and 20 ml ethanol. The mixture was left at room temperature overnight, acidified with hydrochloric acid and extracted with ether. Removal of the ether left a white crystalline residue which was decarboxylated by heating at 150 C for 2 hr. Traces of acetic acid formed from malonic acid were removed under vacuum. The residue was recrystallized thrice from acetone giving 366 mg (41%) melting at 62.5 C [Lit. (14) 62.67 C for hexadecanoic acid]. The mass spectrum showed m/e 258 M^+ and m/e 213 $[M-CH_2CH_2CD_2H]^+$ as prominent peaks in the high mass region.

Glyceryl Tri-4,4-Dideuterio Hexadecanoate (IX)

From the reaction mixture consisting of 18 mg glycerol, 192 mg VIII and 14 mg catalyst, 39 mg IX were isolated. Melting point 66 C [Lit. (13) 65.6 C for glyceryl trihexadecanoate]. Isotopic purity of RCO^+: 96.8% d_2, 0.6% d_1, 2.5% d_0.

3,3-Dideuterio-Pentadecanol (X)

Unused portions of VI (415 mg), VII (110 mg), mono- and diglycerides from the preparation of VII were pooled and reduced with 400 mg lithium aluminum hydride in 50 ml dry ether to yield X. The mixture was refluxed for 3 hr and worked up as described for III. The crude product, 630 mg, melted at 43.5 C [Lit. (14) 43.84 C for pentadecanol]. The chromatographically homogenous product had an appropriate mass spectrum, m/e 212 corresponding to $[M-18]^+$.

3,3-Dideuterio-Pentadecyl Mesylate (XI)

From 620 mg X and 1 g methanesulfonyl chloride in 5 ml pyridine, 520 mg (63%) XI was prepared. The melting point was 49-50 C (51 C for pentadecyl mesylate, Baumann, personal communication). The mass spectrum exhibited a peak at m/e 212 corresponding to $[M-CH_3SO_3H]^+$.

5,5-Dideuterio-Heptadecanoic Acid (XII)

Using the same amounts of sodium, diethyl

malonate and solvent, 520 mg XI were used in a malonic ester synthesis as outlined for VIII. The product was crystallized twice from acetone giving 245 mg (53%) XII. The mass spectrum indicated the presence of an impurity, m/e 300. The methyl ester was prepared by reacting the acid overnight with methanol containing 5% hydrochloric acid. The ester was extracted with chloroform and crystallized from petroleum ether. Melting point 28.5 C [Lit. (17) 28.6 C for methyl heptadecanoate]. The ester was hydrolized by refluxing for 2 hr in a mixture of 2 ml 90% potassium hydroxide and 20 ml ethanol. Acidification with dilute HCl, extraction with ether and recrystallization from acetone gave 148 mg XII. Melting point 60.5-61 C [Lit. (14) 61.19 C for heptadecanoic acid]. The mass spectrum revealed prominent peaks at m/e 272 M^+, m/e 229 $[M-CH_2CH_2CH_3]^+$ and no peak at m/e 300.

Glyceryl Tri-5,5-Dideuterio Heptadecanoate (XIII)

Sixty-four milligrams of XII, 5.7 mg glycerol and 6 mg *p*-toluenesulfonic acid gave, after purification, 26 mg XIII which melted at 64 C [Lit. (13) 64 C for glyceryl triheptadecanoate]. Isotopic purity of RCO^+: 94.3% d_2, 3.7% d_1, 1.9% d_0.

5,5-Dideuterio-Heptadecanol (XIV)

Hydride reduction of XII, XIII and mono- and diglycerides which had been recovered from the preparation of XIII yielded 181 mg XIV. Total weight of the starting materials was 202 mg. The initial product which appeared to be chromatographically pure melted at 53-53.5 c [Lit. (14) 53.31 C for heptadecanol]. The mass spectrum exhibited a major peak at m/e 240 and a minor one at m/e 239 corresponding to $[M-H_2O]^+$ and $[M-HDO]^+$.

5,5-Dideuterio-Heptadecyl Mesylate (XV)

From 180 mg XIV and 700 mg methanesulfonyl chloride in 5 ml dry pyridine 234 mg (100%) XV was prepared. The chromatographically homogenous product melted at 58.5 C which corresponds to a value interpolated from melting points of hydrogen homologs (9). No parent peak was found in the mass spectrum, but an intense peak was located at m/e 240 agreeing with the mass calculated for $[M-CH_3SO_3H]^+$.

5,5-Dideuterio-Heptadecyl Cyanide (XVI)

To 234 mg XV in 10 ml dimethyl sulfoxide was added 280 mg potassium cyanide and treated as outlined for V. A slightly yellow oil, 139 mg (74%), was not further purified or

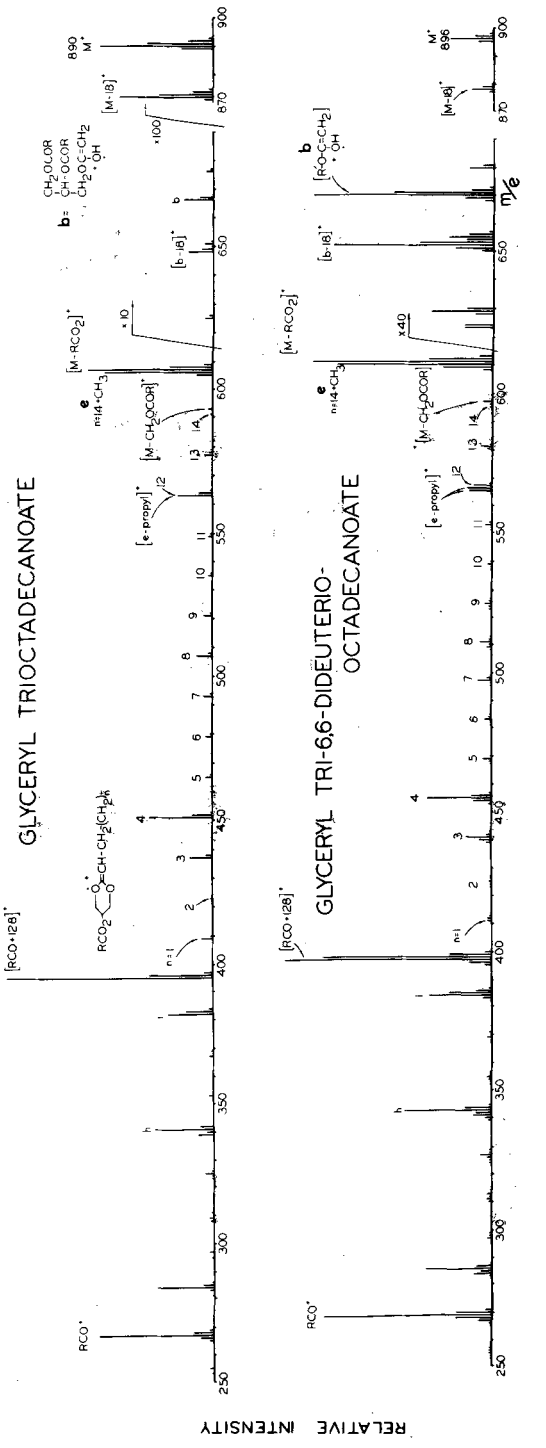


FIG. 1. High mass regions of the mass spectra of a saturated triglyceride and its deuterium-labeled counterpart. Changes in intensity scale refer to total scale magnification.

characterized, but it appeared to be more than 98% pure judging from TLC.

6,6-Dideuterio-Octadecanoic Acid (XVII)

The nitrile XVI, 139 mg, was hydrolyzed as described for VI. Crystallization from acetone gave 102 mg (68%) XVII melting at 68.5 C. Recrystallization gave 48 mg melting at 69.5-70 C [Lit. (14) 69.42 C for octadecanoic acid]. The mass spectrum displayed a strong parent ion at m/e 286 and two ions at m/e 242 and 243 corresponding to $[M-CHDCH_2CH_3]^+$ and $[M-CH_2CH_2CH_3]^+$, respectively, the former being the stronger.

The corresponding methyl ester was prepared by keeping the acid recovered from the mother liquors in 5% hydrochloric acid–methanol at room temperature overnight. The mixture was worked up as described above. The ester crystallized from acetone giving 65 mg melting at 37.5 C [Lit. (18) 37.78 C for methyl octadecanoate]. An intense parent ion at m/e 300 and ions at m/e 269 $[M-OCH_3]^+$, 257 $[M-CH_2CH_2CH_3]^+$ and 256 $[M-CHDCH_2CH_3]^+$ were found in the mass spectrum. The ion at m/e 256 was more intense than that at m/e 257.

Glyceryl Tri-6,6-Dideuterio Octadecanoate (XVIII)

After reacting 55 mg XVII with 5.2 mg glycerol in the presence of 9 mg catalyst, 4.5 mg of pure XVIII was isolated. The crystalline product melted at 72.2 C and at 71.5-72 C when mixed with authentic glyceryl trioctadecanoate. The latter compound melted at 72 C as reported (13). Isotopic purity of RCO^+: 87.7% d_2, 8.7% d_1, 3.6% d_0.

Perdeuterio-Glyceryl Trioctadecanoate (XIX)

In the presence of 8 mg *p*-toluenesulfonic acid 14 mg glycerol-d_8 (ICN, -CD 99 atom %) was reacted with 351 mg octadecanoyl chloride. The product was purified by column chromatography on Florisil, preparative TLC and by three recrystallizations from petroleum ether yielding 10 mg of needles at 71.5 C and 71.5-72 C when admixed with glyceryl trioctadecanoate. The latter melted at 72 C as reported (13).

2-Deuterio-Glyceryl Trioctadecanoate (XX)

After purification, 95 mg (31%) XX were obtained by reacting 988 mg octadecanoic acid with 32 mg 2-deuterioglycerol in the presence of 2 mg catalyst. The melting point, 72 C, was not depressed when mixed with authentic glyceryl trioctadecanoate whose melting point was 72 C as reported (13). Isotopic purity of $[RCO+74]^+$: 96.8% d_1, 3.1% d_0.

2-Deuterio-Glycerol (XXI)

Dihydroxyacetone, 179 mg, was added to a solution of 51 mg sodiumborodeuteride in 2 ml deuterium oxide and the mixture kept at room temperature for 21 hr. Water, 20 ml, was added and the solution filtered through an ion exchange column (1 x 10 cm) consisting of a mixture of anion (IR-45) and cation (IRC-50) exchange material. The resins had been activated with 3% sodium hydroxide and 2 N hydrochloric acid, respectively, and washed to neutrality with distilled water. The resulting aqueous solution was distilled under vacuum leaving a colorless liquid, 154 mg (83%). The mass spectrum revealed no parent peak but the m/e 61 peak of glycerol was shifted to m/e 62 $[CDOH-CH_2OH]^+$. Isotopic purity, 95% d_1, was based on m/e 61, 62.

RESULTS AND DISCUSSION

The reports by Dinh-Nguyen et al. (19,20) on the mass spectra of fatty acid methyl esters were useful in explaining the spectra of triglycerides. Using ^{13}C and deuterium labeling, their studies revealed that four types of reactions contributed to the fragmentations of methyl esters:

(a) Simple cleavage of the alkyl chain giving rise to ions of type a

ion a $\left[CH_3O\text{-}\overset{O}{\overset{\|}{C}}\text{-}(CH_2)_n\right]^+$

(b) Expulsion of part of the chain plus one hydrogen atom resulting in ions of type a.

(c) Exchange of hydrogen atoms between position 2 and positions 5, 6 and 7.

(d) McLafferty rearrangement (2,3 cleavage with transfer of one hydrogen from position 4 giving an ion of type b where R is CH_3).

ion b $\left[R\text{-}O\text{-}\overset{OH}{\overset{|}{C}}=CH_2\right]^+$

These same reactions have been observed to occur in the fragmentation of triglycerides.

McLafferty Rearrangement (21)

All triglycerides studied displayed ions corresponding to 2,3 cleavage with transfer of one hydrogen, comparable to ion b in which R is the glycerol moiety plus two acyl groups. Only when the 2 or 4 positions were labeled with deuterium (II and IX) did this ion retain deuterium atoms in the rearrangement. This agrees with the report of Dinh-Nguyen et al. (19) who studied methyl esters. Most of the deuteriums were apparently retained when located in the 2 positions (II). This is com-

TABLE I

Ions Related to Loss of Water. Abundances Relative to Ion b, the McLafferty Rearrangement Product

Triglyceride of	M+1	M	M-1	M-17	M-18	M-19	M-20	b+1	b	b-1	b-17	b-18	b-19	b-20
18:0	15	25	7	12	21	3	-	50	100	11	39	86	11	-
2,2-d_2-14:0 (II)	9	25	9	13	34	13	4	43	100	43	73	137	64	37
3,3-d_2-15:0 (VII)	8	16	4	10	25	4	1	35	100	14	42	107	27	8
4,4-d_2-16:0 (IX)	10	22	7	7	17	17	5	45	100	22	18	38	98	50
5,5-d_2-17:0 (XIII)	11	22	5	7	19	5	-	42	100	12	32	69	19	12
6,6-d_2-18:0 (XVIII)	10	25	9	6	13	2	-	56	100	17	42	92	22	6
18:0, 2-d-glycerol (XX)	10	18	5	5	9	2	1	45	100	11	41	91	9	-
18:0, glycerol-d_8 (XIX)	9	17	3	4	13	1	-	60	100	-	52	70	-	-

patible with earlier findings (19,20) indicating that extrusion (reaction type b) or exchange (type c) did not precede or interfere with the McLafferty rearrangement to any significant degree.

$[M-18]^+$, $[b-18]^+$

Barber et al. (3) pointed out that the transition $M^+ \rightarrow [M-18]^+$ was accompanied by a prominent metastable peak indicating ionic fragmentation rather than thermal cracking. Our spectra revealed also a second transition $b \rightarrow [b-18]^+$ with its corresponding metastable peak (Fig. 1). These fragmentations are unique because water is lost from an ester. Re-examination of the spectra of diesters of 1,2-ethane diol and 1,3-propane diol (22) revealed that loss of water also occurs with these esters. Spectra of wax esters (Aasen et al., unpublished data) like octadecyl octadecanoate do not exhibit this ion, $[M-18]^+$, probably because competing modes of fragmentations are favored.

Since the spectra of triglycerides labeled in the glycerol moiety (Table I) showed loss of ordinary water, the hydrogen atoms involved must originate from the acyl moieties. Table I shows that the hydrogen atoms in 2 and 4 positions are involved. The spectrum of IX showed that H_2O, DHO and D_2O were lost in the ratio of about <1:70:30, calculated from the relative populations of $[b-18]^+$, $[b-19]^+$ and $[b-20]^+$. The spectrum of II revealed loss of H_2O, DHO and D_2O in the ratio of about 60:30:10. An enol mechanism with 1,4 elimination similar to that suggested by Williams et al. (23) for the formation of [cyclohexanone-18]$^+$ is proposed for triglycerides, where R is the glyceryl diacyl moiety:

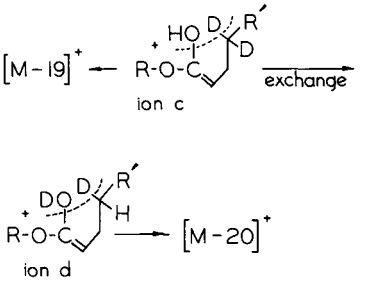


TABLE II

Relative Abundances of Ions [RCO+128]+ and Neighboring Ions in the Spectra of Some Deuterated Triglycerides

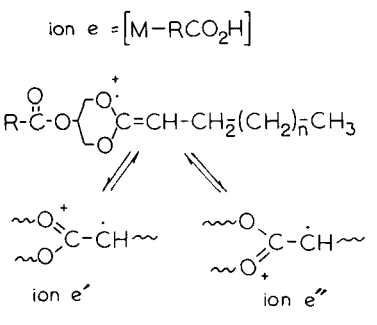
Triglyceride of	RCO+127	RCO+128	RCO+129	RCO+130	RCO+131
18:0	1	100	28	4	1
2,2-d_2-14:0 (II)	4	100	53	11	2
3,3-d_2-15:0 (VII)	1	5	16	100	25
4,4-d_2-16:0 (IX)	9	100	26	6	1
5,5-d_2-17:0 (XIII)	9	100	38	6	1
6,6-d_2-18:0 (XVIII)	11	100	81	20	3
6,7-d_2-18:0[a]	15	100	64	15	2
9,10-d_2-18:0[a]	20	100	29	6	1
18:0, 2-d-glycerol (XX)	1	6	100	27	4
	RCO+132	RCO+133	RCO+134	RCO+135	RCO+136
18:0, glycerol-d_8 (XIX)	7	100	36	5	1

[a]See Reference 1.

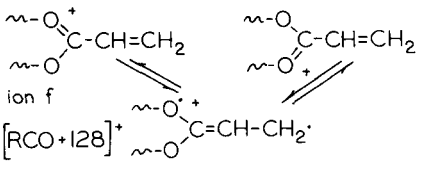
Deuterium-hydrogen exchange occurring between the enol-oxygen and C4 might account for the loss of D_2O. The spectrum of II showed that H_2O was lost to a greater extent than DHO and D_2O, indicating competition by reaction c, or extrusion according to reaction b, or both. The loss of water from acyclic alcohols has been found (24,25) to be almost exclusively 1,4-elimination via a hexagonal transition state as shown in ions c and d.

$[RCO + 128 + 14n]^+$

Barber et al. (3) observed that the spectra of all triglycerides displayed a rather intense ion, 128 m/e units heavier than the RCO^+ ion. High resolution measurements (1) indicated that $C_6H_8O_3$ accounted for this increment. Close inspection of the less abundant ions found at higher m/e values revealed that peaks recurred at intervals of 14 m/e units, suggesting the series $[RCO + 128 + 14n]^+$. The ion $[M - RCO_2H]^+$ is represented by $[RCO + 128 + 14n + CH_3]^+$ in which the terminal methyl group has been added to the final member of the series. Several structures might be drawn for this ion, but e appears simplest, is resonance stabilized, and is most consistent with results for the deuterium labeled compounds.



Each member of the series is thought to be formed by simple homolytic cleavage of the alkyl chain of e (reaction a). The high abundance of the first member $[RCO + 128]^+$, might be explained by allylic homolytic cleavage which is energetically favored (26) giving a resonance stabilized ion, f.



Two other ions, $[RCO+170]^+$ and $[RCO+184]^+$ were usually more intense than others of the series, and the latter was the stronger of the two. This might be rationalized by forming five- or six-membered rings by pairing the electron indicated in e′ or e″ with the unshared electron at C6 or C7. Except for compound II, all the deuterium labeled compounds produced ions agreeing with e (Table II). Deuteriums in all 3 positions of acyl groups effected a shift of two m/e units giving the series $[RCO+130+14n]^+$. Labeled 4 positions resulted in the series $[RCO+128+16+14(n-1)]^+$. Deuteriums farther out in the acyl group, i.e., 5,5; 6,6; 6,7 or 9,10 caused one or two intervals of 16 or 15 m/e units rather than 14. The m/e values of the irregular intervals agreed with postulated ion e having deuteriums in the appropriate positions.

The spectrum of the exception, II, exhibited a stronger $[RCO+128]^+$ ion than the expected $[RCO+129]^+$. The explanation offered for this anomaly is partial loss of deuterium due to exchange with hydrogen (reaction c). It is seen in Table II that the $[RCO+129]^+$ ions of XIII and XVIII and glyceryl tri-6,7-dideuterio-octadecanoate showed increased abundances compared to those of compounds not having deuterium in 5, 6 or 7 position. This suggests that hydrogen in position 2 is replaced by deuterium from position 5, 6 or 7 analogous to what happens in methyl esters (19,20). The spectrum of II shows that most ions are accompanied by intense satellites making it difficult to tell the fate of the deuteriums originally located in the 2 position of the acyl chains. Thus the ions at m/e 384, 398 and 412 representing replacement of one hydrogen with one deuterium in position 5, 6 and 7, respectively, appear to be enriched with deuterium, but so are many other ions. The evidence presented elsewhere (19,20) and above for the interexchange phenomenon suggests that the deuteriums are not transferred by complicated rearrangements to other parts of the molecule, but are largely confined to their original acyl group. Morrison et al. (5) found that little or no scrambling took place between the glyceryl residue and the fatty acid chains in the spectra of deuterated glyceryl 1,3-dioctadecanoates.

The explanation offered for the higher abundance of $[M-18]^+$ than $[M-19]^+$ (Table I) in the case of II was that exchange (reaction c) to a large extent preceded the expulsion of water. The data of Table II indicate that exchange also precedes the formation of ion e, leaving the possibility that exchange occurs already in M^+ and b^+ (see discussion of McLafferty rearrangement above). It was suggested that IX lost some D_2O because exchange had occurred between 4 position and the enol oxygen. This is, however, not reflected in Table

TABLE III

Mass Interval Corresponding to Loss of Propyl Groups From Ions Derived From Deuterium Labeled Methyl Esters and Triglycerides[a]

Position labeled	Propyl group lost: Methyl esters (19,20)	Propyl group lost: Triglycerides
None	43	43
2,2	43,44[b],45	43,44,45[b]
3,3	45	45
4,4	45(44)[c]	45
5,5	43	43
6,6	43,44[b],45	43,44[b],45
6,7	43,44[b]	43,44[b]
9,10	43	43

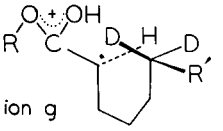
[a]With methyl esters the loss is from the molecular ion, and with triglycerides loss of propyl is from ion *e*.

[b]Tallest peak in the cluster.

[c]A discrepancy exists between references 19 and 20. Reference 19 agrees with our findings that m/e is lost from VIII and IX.

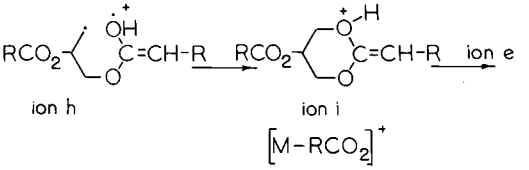
II by increased intensity of $[RCO+129]^+$, indicating that the enol side of the keto-enol equilibrium is favored.

In the case of maximum interchange, i.e., between 2 and 6 positions, a favorable (27) hexagonal transition state might occur (ion g).



ion g

Absence of detectable metastable peaks makes it difficult to propose an unambigous fragmentation pathway leading to e which is formally obtained by M^+ expelling a neutral carboxylic acid molecule. Because triglycerides labeled in the glycerol moiety retained the deuterium, a mechanism differing from a concerted McLafferty rearrangement must operate. A two-step mode of fragmentation is suggested. Following the loss of an acyloxy radical the enol form of the ester (ion h) may cyclize to ion i which represents the major ion $[M\text{-}RCO_2]^+$. Loss of a hydrogen yields e.



ion h ion i

$[M-RCO_2]^+$

Albeit formation of e might be envisaged using the keto form of h, the enol form is favored for the following reasons: (a) In view of the mechanism for the loss of water, the enol form is probably present. (b) The enol oxygen would be in closer proximity to the radical site than the keto oxygen. (c) The double bond of e is already present. (d) The enol form of a compound is ionized more easily (28) than is the keto form.

A rather intense ion at m/e 563 in the spectrum of glyceryl trioctadecanoate was originally thought to be a member of the series $[RCO+128+14n]^+$. However, the m/e values of the corresponding ions in spectra of labeled compounds disproved this. The differences in m/e values between e and this ion for various labeled triglycerides coincided (Table III) with the loss of a propyl group. This is comparable to observations (19,20) made on long chain methyl esters labeled in comparable positions. Thus it appears that extrusion (reaction b) of the three methylene groups adjacent to the carbonyl function plus one hydrogen took place in the acyl group of ion e. The clusters found in the spectra of compounds labeled in 2, 6 and 7 positions are consistent with hydrogen-deuterium exchange (reaction c) between these positions. Since major ions such as [RCO-propyl+128]$^+$, [RCO-propyl+170]$^+$ and

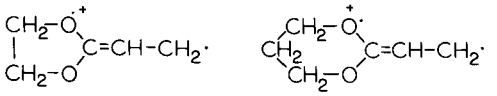
TABLE IV

Relative Abundances of Ions p and Neighboring Ions Formed From Some Deuterium Labeled Triglycerides

Triglyceride of	RCO+115	RCO+116	RCO+117	RCO+118
18:0	100	59	9	0
2,2-d_2-14:0 (II)	17	47	100	50
3,3-d_2-15:0 (VII)	100	62	9	2
4,4-d_2-16:0 (IX)	100	100	27	6
5,5-d_2-17:0 (XIII)	100	57	14	3
6,6-d_2-18:0 (XVIII)	100	66	25	2
18:0, 2-d-glycerol (XX)	15	100	65	12
	RCO+120	RCO+121	RCO+122	RCO+123
18:0, glycerol-d_8 (XIX)	100	62	20	4

[RCO-propyl+184]$^+$ are absent, it appears that simple cleavage (reaction a) of the extrusion product does not take place.

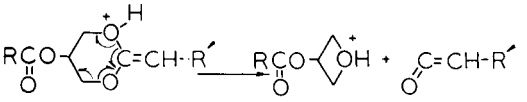
In the mechanisms for formation of ions e, m and p six-membered ring structures have been assumed. Ethanediol diesters, if they fragment by mechanisms analogous to triglycerides, should produce ions with five-membered rings. The mass spectra (22) of long chain diesters of ethanediol exhibited a series of m/e 99+14n, of which m/e 99 was intense, suggesting that a five-membered structure is possible (ion j), similar to that postulated for alk-1-enyl ether esters of diols (29). The spectra of long chain diesters of 1,3-propanediol (22) contained a similar series beginning with m/e 113, suggesting a six-membered ring. Unfortunately, with triglycerides the ions [RCO+128]$^+$ can be written with a five-membered ring involving two adjacent ester linkages or a six-membered ring involving the 1,3-ester linkages. These cannot be presently distinguished.



ion j m/e 99 ion k m/e 113

[RCO + 74]$^+$

The proposed structure, m for this characteristic ion is consistent with accurate mass measurements and with retention of deuterium when the glyceryl moiety is labeled (1).



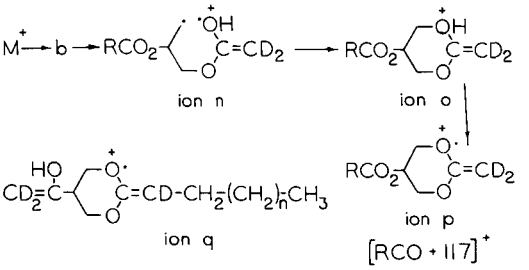
ion l $[M-RCO_2]^+$ ion m $[RCO + 74]^+$

A feasible mechanism for the formation of this ion is loss of a substituted ketene from [M-RCO$_2$]$^+$. The origin of the proton attached to the ether-oxygen is unknown. [RCO+74]$^+$ was never altered when the acyl group was labeled in various positions. Exchange of the deuteriums might account for II not displaying [RCO+74+1]$^+$ in its spectrum.

[RCO + 115]$^+$

Two possible structures, p and q, were distinguished by means of labeling. Structure q could arise via a McLafferty rearrangement of M$^+$ followed by loss of a carboxylic acid molecule, analogous to the reaction h → i → e. Because deuterium atoms in 2 position are retained (18,19) in a McLafferty rearrangement, this sequence applied to II should yield an ion q in which three deuterium atoms are retained, equivalent to RCO+116. However, in structure p, 4 deuterium atoms would be retained, equivalent to [RCO+117]$^+$. The latter was found to be the case as is shown in Table IV.

The following mechanism which is somewhat similar to the proposed pathway leading to e is put forward although there are no metastable peaks to support it.



ion n ion o ion q ion p $[RCO + 117]^+$

Except for IX the intensities of the [p+1]$^+$ ions are about twice the calculated abundance (21-25%) of p's isotopic peak, implying coincidence with a second ion. In the case of IX [p+1]$^+$ is greatly enhanced. This might be due to increase of the second ion, or to retention of some of the dueterium procured in the McLafferty rearrangement. If the latter is the case, the increase of the isotopic peak might come about via the keto-enol equilibrium of b. This would also account for some loss of deuterium in II reflected in the increased [p-1]$^+$ ion.

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