The Identification of Radiolytic Decomposition Products from Tributyrin

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ABSTRACT AND SUMMARY

In order to facilitate the detection of radiolytic products of triglycerides which may be of higher molecular weight than their precursor, a low molecular weight triglyceride, tributyrin, was selected as a model system, and gel permeation chromatography was used to effect their separation. The irradiation treatment was conducted under vacuum at 50 Mrad. Radiolytic products were collected by a precolumn technique for the highly volatile compounds; a combination of cold finger distillation and gel permeation for the less volatile fractions and finally gel permeation chromatography of the residue after distillation for the higher molecular weight compounds. A large number of compounds expected on the basis of previous work were identified in the present work from irradiated tributyrin. In addition, the techniques employed permitted the identification of several new compounds. These include butanetriol triesters, erythritol tetraesters, and other polyglycol polyesters.

INTRODUCTION

In previous work (1,2), a relatively low molecular weight triglyceride, tricaproin, was selected to study the higher molecular weight compounds produced by irradiation. This facilitated the identification of not only the short chain compounds typical of specific radiolytic cleavage in the triglyceride molecule but also several other compounds which are considered to result from the combination of the free radicals formed. It was evident, however, that some of the expected larger recombination products were not observed. These compounds were difficult to detect by the techniques used because of their high molecular weight.

In the present work it was decided to select a model triglyceride of even smaller molecular weight and to employ additional methods to facilitate their separation and identification. In this manner, it was possible to identify new radiolytic compounds by using tributyrin as the substrate and size exclusion chromatography for prefractionation.

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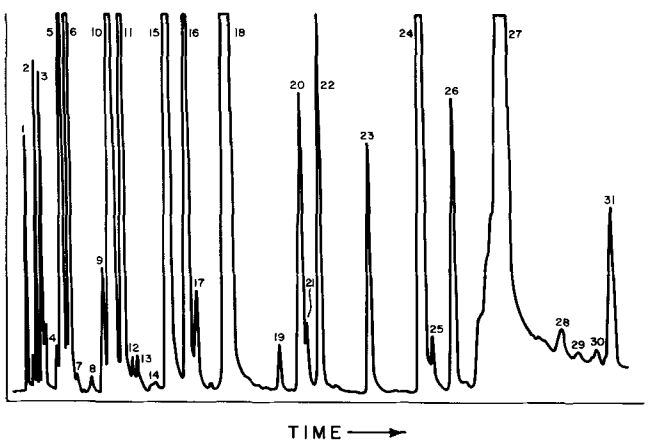


FIG. 1. Gas Chromatogram of the products obtained by cold finger distillation at 10 C of irradiated tributyrin. 700 ft x 0.03 in. open tubular column coated with OV-101, temperature programmed from 70-200 C at 2 C/min. Flow rate, 12 ml/min.

EXPERIMENTAL PROCEDURES

Materials

Tributyrin was purchased commercially and purified by cold finger distillation. Esters of butyric acid were prepared by mixing butanoyl chloride with the appropriate alcohols. Oxopropanediol dibutyrate and hexanediol dibutyrate were synthesized according to Mattson and Volpenhein (3) from butanoyl chloride by reaction with glyceraldehyde and hexanediol, respectively.

Erythritol tetrabutyrate was prepared by reacting erythritol with butanoyl chloride; and 3,3'-oxy-di(1,2-propanediol) tetrabutyrate by oxidizing the di-allyl ether with H_2O_2 and osmic acid according to McClosky and McClelland (4). The resulting hydroxy compound was then acylated with butanoyl chloride.

Ethanediol and propanediol dibutyrate; 1,3-dibutyrin; 1-formo-2,3-dibutyrin; 1-propiono-2,3-dibutyrin and 2-oxo-1,3-propanediol dibutyrate were obtained from Dr. Robert Jensen of the University of Connecticut.

Irradiation

Samples, 5 g each, were sealed in glass ampoules under vacuum (20 Hg) and irradiated at room temperature with gamma rays from the Cobalt 60 source at the Natick Laboratories. Preliminary results showed that formation of radiolytic products was qualitatively independent of dose between 6 and 50 Mrad. Therefore, irradiation was carried out at 50 Mrad to obtain larger amounts of radiolytic products for analysis.

Collection and Fraction of Radiolytic Products

The volatile compounds were collected with the aid of the precolumn and the cold finger distillation (CFD) techniques described earlier by Nawar et al., (5). In this study, however, the distillation was done at relatively low temperatures, i.e., 10 C and 40 C, to avoid collection of tributyrin on the cold finger.

Gel permeation (size exclusion) chromatography (GPC) as described by Vjdi (M. Jajdi, private communication, 1973) was useful as a prefractionation method for both the volatiles and the high molecular weight compounds. The liquid chromatograph used was a Waters Associates, Model ALC-202 401 equipped with a 16-ft 60 Å Poragel and an 8-ft, 100 Å Poragel column connected in series and employing both ultraviolet (254 nm) and refractive index detectors. Fractionation was carried out with chloroform as the mobile phase at a flow rate of 2 ml/min and an inlet pressure of 800-900 psi.

Gas Chromatography

A Perkin-Elmer Model 900 flame ionization gas chromatograph was used with the following columns: 6 ft x 1/8 in. packed with 3% PO-17 (Pierce Chemicals Co.), 3% OV-25, 3% SE-30, 15% Carbowax 20M, and 10% DEGS + 2%

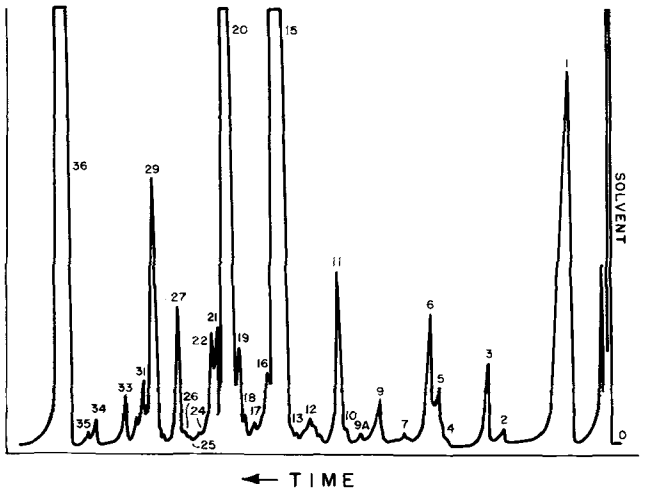


FIG. 2. Gas chromatogram of the products obtained by cold finger distillation at 40 C of irradiated tributyrin. 9 ft x 1/8 in. column packed with 3% PO-17 on 80/100 mesh chromosorb, temperature programmed from 70-270 C at 8 C/min. Flow rate, 15 ml/min.

H_3PO_4 liquid phases on 80/100 mesh chromosorb; as well as 500 ft OV-17, 500 ft Carbowax 20M, and 700 ft OV-101 capillary columns (0.03 in. ID).

GC-MS Computer System

The combination system consisted of a Bendix Model 2200 GC flame ionization gas chromatograph, a Dupont Model 21-491 double deflection mass spectrometer, and a Hewlett Packard 2116B computer equipped with a Disk Drive System, Model 7900A. Mass spectral data acquired on-line with the Hewlett Packard computer during chromatographic elution were transferred to a Digital Equipment Corporation, Model PDP 15/76 Laboratory Data Automation Computer to aid in component identification.

RESULTS

Based on previous work with tricaproin (1,2) the radiolytic compounds are expected to vary widely in molecular weight, i.e., from methane to dimeric or polymeric compounds larger than the original substrate. To allow for adequate analysis of such a wide range of compounds, four different prefractionation techniques were used, each of which optimized the separation and identification of a different range of radiolytic compounds.

Precolumn Collection

The irradiated sample was maintained at 40 C, and the highly volatile fraction was collected on a precolumn as described earlier (5). Carbon dioxide, acetylene, ethane, propane, propene, butane, and pentane were the major compounds identified in this fraction.

CFD at 10 C

The volatiles collected by this technique were separated on the OV-101 capillary column with additional data obtained from analysis on the OV-17 and the carbowax 20 M capillary columns. A typical GC trace of this fraction is shown in Figure 1, and a list of the compounds identified is given in Table I, Column A.

CFD at 40 C and Fractionation by GPC

After cold finger distillation at 40 C for 1 hr of a 20 g sample of irradiated tributyrin, the distillate was taken up in 10 ml chloroform and further fractionated by GPC. A 2 ml portion of the chloroform solution was used for each

TABLE I

Summary of Compounds Identified Among the Radiolysis Products of Tributyrin

A. CFD at 10 C		B. CFD at 40 C and GPC		C. GPC of residue after CFD		
Compound	Peak no. in Figure 1	Compound	Peak no. in Figure 2	Compound		[a]Peak no. in Figure 4
Ethanal	1	Butyric acid	1	[b]Pentano-dibutyrin		
2-Propanone	2	Butyl butyrate	2	b1,2,4-Butanetriol tributyrate		
Iso-butanal	8	Ethanediol dibutyrate	15	Hexano-dibutyrin		1
Propanol	9	1,2-Propanediol dibutyrate	15	Heptano-dibutyrin		2
Butanal	10	1,3-Propanediol dibutyrate	19	Erythritol tetrabutyrate		6
Hexane	11	1,3-Propanediol dibutyrate	20	3,3′-Oxy-di(1,2-propanediol) tetrabutyrate		13
Cyclobutanone	15	1,2-Propanediol dibutyrate	21			
2-Pentanone	16	1-Oxo-2,3-propanediol dibutyrate	23			
Pentanal	17	Methoxypropanediol dibutyrate	25			
Methyl butyrate	18	2-Ethyl-1,3-propanediol dibutyrate	26			
Vinyl butyrate	19	1,2-Hexanediol dibutyrate	27			
3-Hexanone	20	2-Oxo-1,3-propanediol dibutyrate	28			
Ethyl butyrate	22	Dibutyrin	29			
Iso-Propyl butyrate	23	Formo-dibutyrin	31			
4-Heptanone	24	1-Propoxy-2,3-propanediol dibutyrate	33			
Propyl butyrate	26	Aceto-dibutyrin	34			
Butyric acid	27	Propiono-dibutyrin	36			
Butyl butyrate	31	Tributyrin				

[a]Fraction DE in Figure 3.
[b]Found in Fraction CD shown in Figure 3.

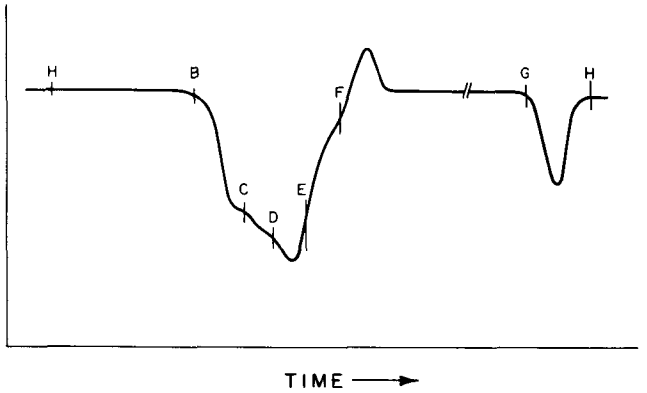


FIG. 3. Gel permeation chromatogram of the products obtained from irradiated tributyrin by extraction after removal of volatile compounds by vacuum distillation. Refractive index detector.

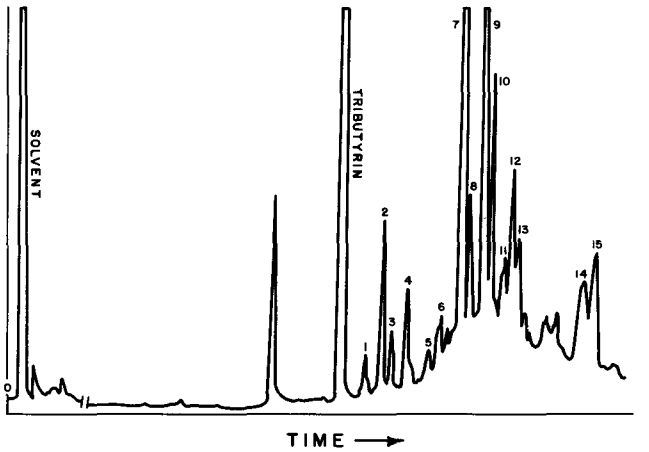


FIG. 4. Gas chromatograpm of the components in gel permeation fraction D-E (Fig. 3) from irradiated tributyrin. 9 ft x 1/8 in. column packed with 3% PO-17 on 80/100 mesh chromosorb, temperature programmed from 65-300 C at 8 C/min. Flow rate, 20 ml/min.

injection into the GPC column.

Several fractions were collected upon elution from the GPC column and examined by GC-MS, but only three fractions were used for identification. The data from the other fractions were not useful because of either overlapping, or in some cases, high volatility resulting in loss of components upon concentration. All three fractions gave essentially the same general qualitative GC pattern. However, the advantage of using GPC fractionation in this case is that it provides fractions with different quantitative ratios of the individual components. This permits better resolution for the various fractions taken for GC separation. The radiolytic compounds contained in the cold finger distillate were thus identified using gas chromatography and mass spectrometry as previously described. GC analysis of this fraction is shown in Figure 2, and a list of the compounds identified is given in Table I, Column B.

The structures of 1- and 2-oxopropanediol dibutyrates were confirmed by their reduction to the corresponding dibutyrins. The reduction was done by adding a few mg of $NaBH_4$ to 20 ml of the cold finger distillate. After 2 min at 20 C, a 1 μl sample was taken from the top layer and analyzed by gas chromatography.

High Molecular Weight Radiolytic Compounds

For the purpose of this presentation, the term "high molecular weight radiolytic compounds" refers to those compounds which elute after tributyrin on the GC column used.

CFD could not be used in this case because of the relatively greater volatility of the substrate itself compared to that of the high molecular weight radiolytic compounds. Trapping the GC column effluent was tried, but due to the formation of aerosols, column bleeding and low efficiency, it was found not practical. Gel permeation proved useful for the separation of the relatively high molecular weight compounds.

In order to concentrate these compounds, the sample was irradiated at a high dose. The sample was irradiated with successive 20 Mrad doses, and volatiles were removed by CFD at 40 C after each treatment. The residue which contained high molecular weight compounds in tributyrin was dissolved in chloroform to make a 10% solution of which a 2 ml sample was fractionated by the gel permeation column.

Several fractions were collected from the irradiated tributyrin residue (Fig. 3) and analyzed by GC. Fraction A-B appeared to contain extremely high molecular weight compounds which could be detected only with a short column at high temperature and high flow rate. Fraction B-C also contained high molecular weight compounds overlapping with those in fraction C-D. Fraction E-F contained essentially tributyrin. Fractions F-G and G-H contained compounds which normally elute before tributyrin on this

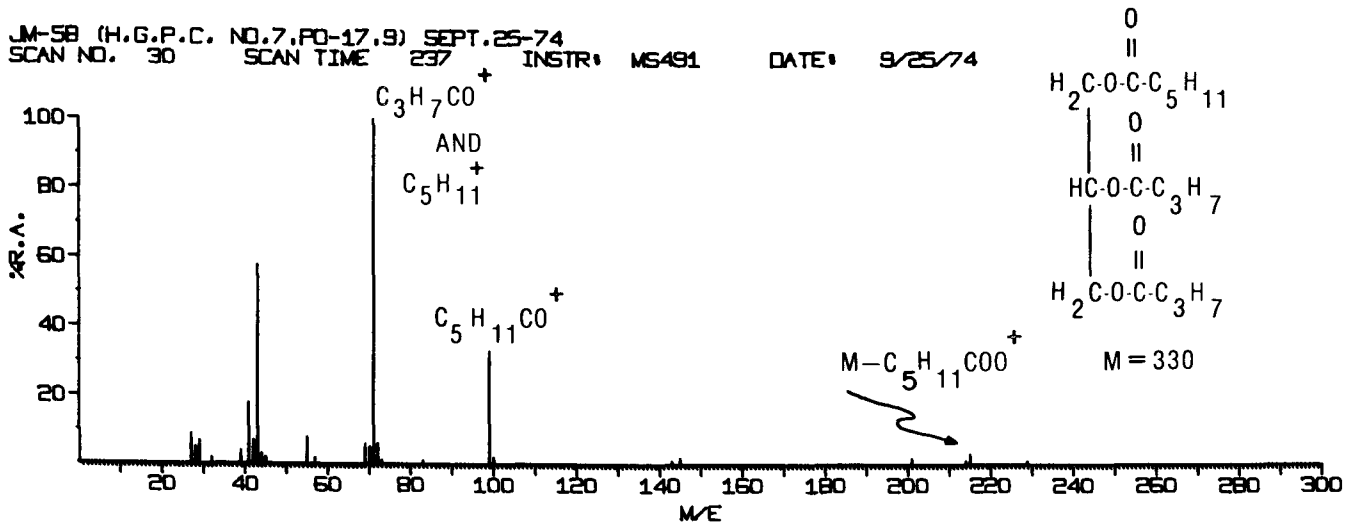


FIG. 5. Mass spectrum of a component separated as peak number 1 in Figure 4 and identified as hexano-dibutyrin.

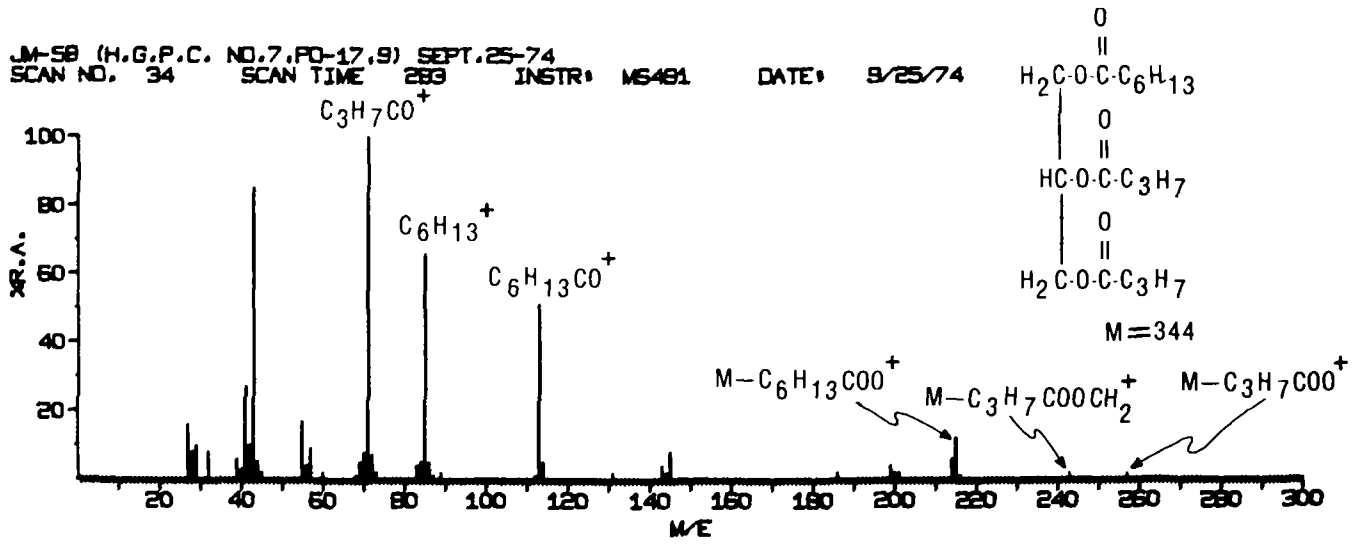


FIG. 6. Mass spectrum of component separated as peak number 2 in Figure 4 and identified as heptano-dibutyrin.

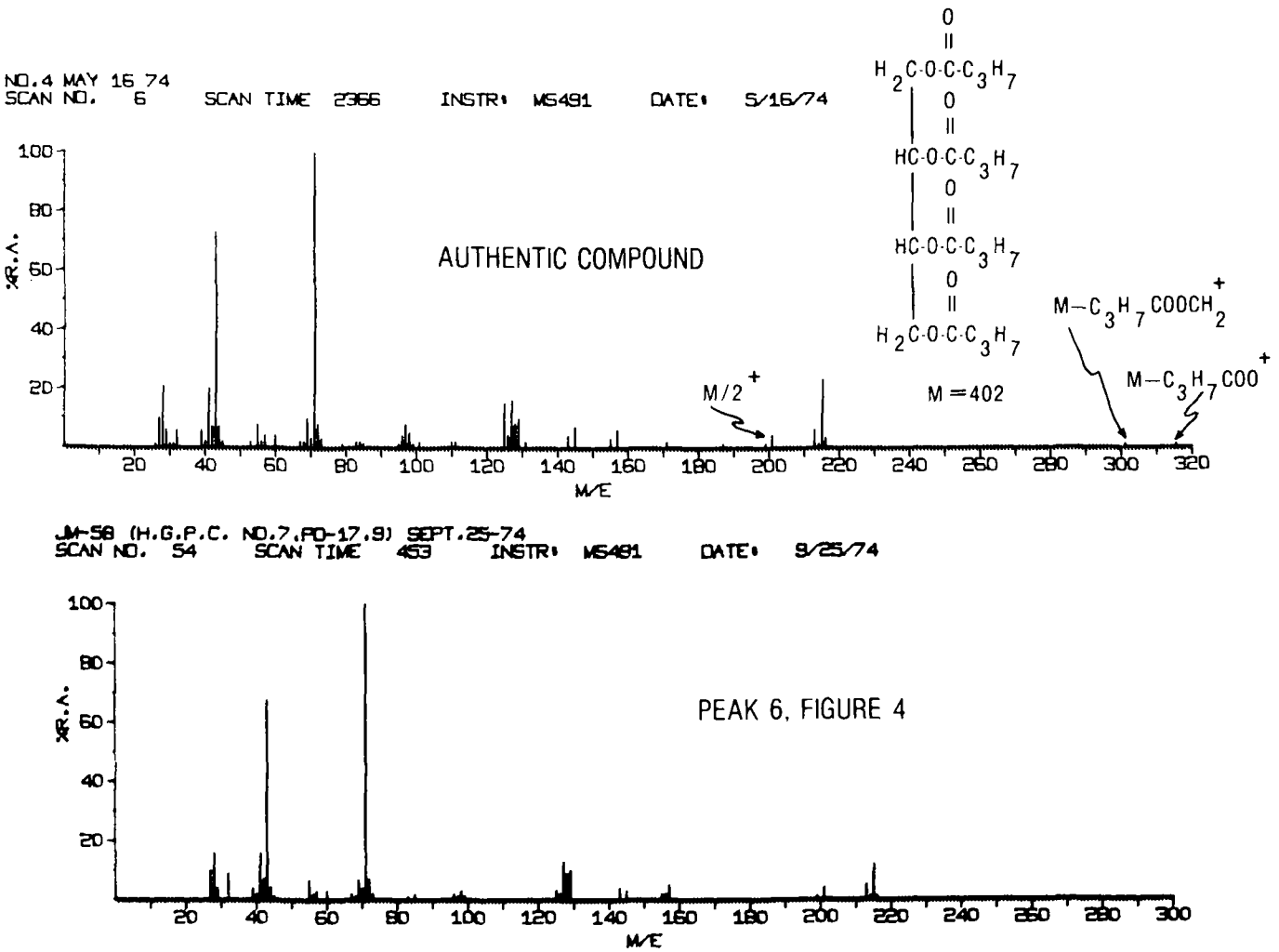


FIG. 7. Mass spectrum of the component separated as peak number 6 in Figure 4 and of an authentic sample of erythritol tetrabutyrate.

column and which have been discussed avoe.

Therefore, only two fractions (i.e., C-D and D-E) provided useful information. Both had essentially the same GC pattern but with quantitatively different ratios. Figure 4 shows the GC analysis of fraction D-E. Only a few compounds in this fraction were identified (Table I, Column C). These compounds were identified as follows.

Pentano-dibutyrin: Identification of this compound is based only on comparison of its retention time with the synthesized compound. Since it elutes immediately after the tributyrin peak, it could not be analyzed by mass spectrometry due to similarities in the spectrum.

1,2,4-Butanetriol tributyrate: The elution of this compound overlaps with pentano-dituyrin. Its identification

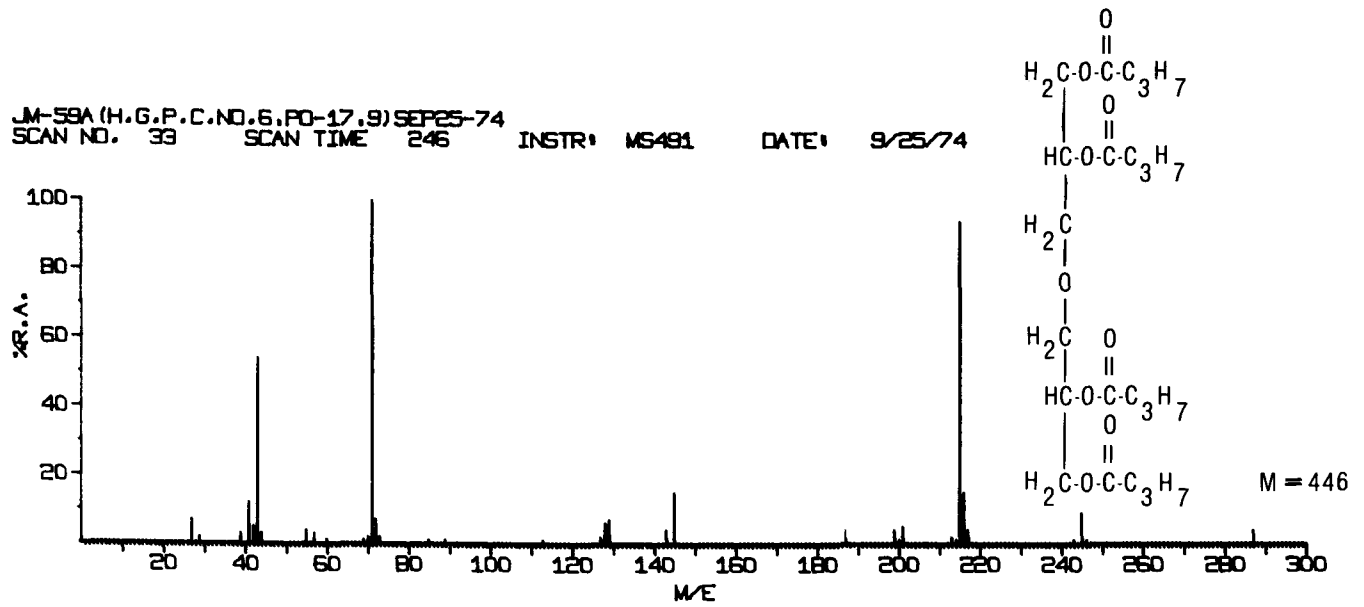


FIG. 8. Mass spectrum of a component separated as peak number 13 in Figure 4 and identified as 3,3'-oxy-di(1,2-propanediol)tetrabutyrate.

likewise is based only on comparison of retention time with the authentic sample.

Chromatographic peaks for the above two compounds can not be seen in Figure 4 since they are concentrated in fraction C-D.

Hexano-dibutyrin: Peak No. 1 in Figure 4 corresponds to this compound. Identification is based on retention time and mass spectral data.

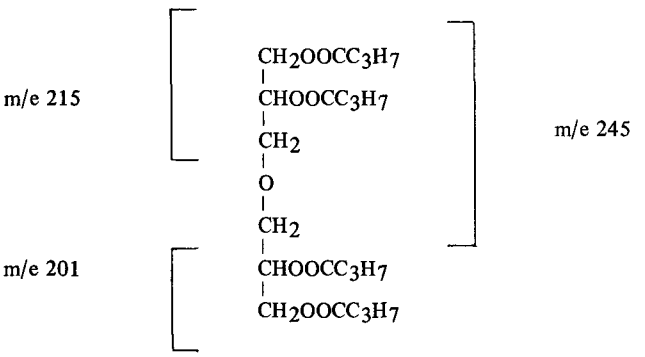
The mass spectrum of hexano-dibutyrin is shown in Figure 5. The spectrum agrees well with that of the authentic compound obtained by GC-MS under similar conditions. Although the parent ion and some of the other expected ions in the high mass range are not observed, the structure of the molecule is easily deduced from the similarity of its spectrum to tributyrin and related dibutyrins and from the appearance of specific ions. Thus, the peak at m/e 99 corresponds to the ion $C_5H_{11}CO^+$. A peak at m/e 215 is due to an ion formed by loss of the larger acyloxy fragment from the parent ion. Both fragments $C_5H_{11}+$ and $C_3H_7CO^+$ contribute to m/e 71. The rest of the mass spectral pattern is similar to that of tributyrin.

Heptano-dibutyrin: Peak No. 2 in Figure 4 corresponds to this compound. The retention time and mass spectrum agree well with those of the synthesized compound. The mass spectrum of heptano-dibutyrin (Fig. 6) exhibits peaks at m/e 85 and 113 corresponding to the fragments $C_6H_{13}^+$ and $C_6H_{13}CO^+$, respectively. The peak at m/e 215 is due to the ion $M\text{-}C_6H_{13}COO^+$, and the peak at 257 is due to $M\text{-}C_3H_7COO^+$. Other fragments are similar to those observed in the mass spectra of tributyrin and other dibutyrins.

Erythritol tetrabutyrate: The mass spectra of an authentic sample of erythritol tetrabutyrate and of the component eluted as peak No. 6 in Figure 4 are shown in Figure 7. The peak at m/e 201 arises from symmetrical cleavage of this compound. Small peaks at m/e 315 and m/e 301 represent the ions $M\text{-}C_3H_7COO^+$ and $M\text{-}C_3H_7CO_2CH_2^+$. The peaks at m/e 71, m/e 43, and other fragments are common to those found in tributyrin fragmentation.

Since the ion fragments observed, except for m/e 315, may also be expected in the spectrum of tributyrin itself (e.g., m/e 301-M-1), identification of this compound is predicated strongly on the correspondence of ion abundances as well as agreement of retention times with those of authentic compounds on several capillary columns.

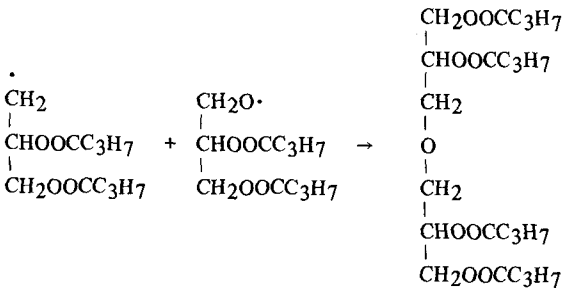
3,3'-Oxy-di(1,2-propanediol)tetrabutyrate: Peak 13 in Figure 4 gave a mass spectrum identical to that of authentic 3,3'-oxy-di(1,2-propanediol) tetrabutyrate. The peaks at m/e 201 and m/e 215 (Fig. 8) correspond to dibutanoyloxyethyl and dibutanoyloxypropyl radical ions, also found in tributyrin and 1,2-propanediol dibutyrate. The ion at m/e 245 corresponds to the fragment $M\text{-}201^+$. Other fragments are similar to those found in the mass spectrum of tributyrin. The fragmentation may be depicted as follows.



DISCUSSION

In general, the results obtained in this study support the mechanisms proposed for the radiolysis of simple triglycerides by Dubravcic and Nawar (6) and more recently for tricaproin by LeTellier and Nawar (1,2,7). Thus, cleavage of the acyloxy-methylene bond in the tributyrin molecule-ion produces butyric acid, and the propane- and propenediol dibutyrates. The radiolytic products butanal, dibutyrin, the oxopropanediol dibutyrates, and cyclobutanone are formed from cleavage of the acyl-oxygen bond. Splitting at the α-bond of the fatty acid moiety produces propane, propene, and formodibutyrin, while carbon-carbon cleavage along the alkyl chain of the fatty acid results in the formation of ethane, ethene, aceto-dibutyrin, and propiono-dibutyrin. The relative peak size of formo-, aceto-, and propiono-dibutyrate showed lower concentration of propiono-dibutyrin in the sample, confirming earlier observations that cleavages at α- and β-positions are favored relative to those distanced more than two carbon atoms from the carbonyl group. Most of the remaining compounds listed in

Table I can be accounted for by the free radical recombination reactions described by LeTellier and Nawar (2). However, the methodology used here permitted the identification of several additional compounds. Among these are erythritol tetrabutyrate which would result from dimerization of the abundant dibutanoyl-oxyethyl free radical; 1,2,4-butanetriol tributyrate which probably arises from recombination of the butanoyloxy methylene radical with the dibutanoyl-oxypropyl free radical. The compound 3,3′-oxy-di(1,2-propanediol)tetrabutyrate may be formed as follows.



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