Evidence for the Mechanisms of Formation of Radiolysis Products Using a Deutero Labeled Triglyceride

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ABSTRACT AND SUMMARY

Several previous studies of the radiolysis of fats and related compounds such as pure triglycerides, fatty acid methyl esters, and free fatty acids, have led to the hypothesis that radiolytic products result from selective cleavages at specific locations. This hypothesis is confirmed by the results of mass spectrometric analysis of the compounds formed upon gamma irradiation of tributyrin labeled with deuterium in the glycerol moiety. Compounds derived from alkyl, acyl, and acyloxy cleavages are characterized by the absence of deuterium whereas those involving the glyceryl moiety are found to carry the deuterium labels.

INTRODUCTION

In 1966 Merritt and co-workers (1) reported the identification of homologous series of hydrocarbons from the irradiation of various lipid systems including methyl stearate, methyl oleate, and tristearin, and concluded that radiation products are primarily the result of direct bond cleavage of carbon-carbon bonds along the fatty acid chains. Dubravcic and Nawar (2) investigated the effect of high-energy radiation on the simple triglycerides containing the C12, 14, 16, 18, 16:1, 18:1, and 18:3 fatty acids. They identified series of normal alkanes as well as certain long chain aldehydes and methyl esters. Based on quantitative data they speculated that the principal cleavages of glyceride molecules occur in the vicinity of the carbonyl groups giving rise to relatively large amounts of the hydrocarbons containing one and two carbon atoms less than the glyceride fatty acid and, in addition, some oxygen-containing compounds of the same carbon numbers as the fatty acid. More recently, in a detailed study with tricaproin, LeTellier and Nawar (3) proposed a general mechanism for the radiolysis of triglycerides. They concluded that radiolytic cleavage occurs preferentially at five locations in the triglyceride molecule: at the acyloxy-methylene bond resulting in the formation of the free acid and propanediol or propenediol diesters; at the acyl-oxy bond producing the aldehyde, 2-alkyl cyclobutanone, diglycerides, and oxo-propanediol diesters; at the alpha and beta bonds of the fatty acid producing the n-1 and n-2 hydrocarbons and triglycerides; and at a C-C bond in the glycerol moiety producing the ethanediol diester and the methyl ester. Random homolytic cleavage of C-C bonds along the fatty acid chains is responsible for the formation of smaller amounts of shorter chain hydrocarbons. The same authors (4) further suggested that certain ketones, esters, longer chain hydrocarbons, glycerol ether diesters, and alkanediol diesters are formed by recombination of the free radicals resulting from the above-mentioned cleavages.

In the preceding paper (5) irradiation of the low molecular weight triglyceride, tributyrin, has been reported. In addition to the radiolytic compounds expected on the basis of previous work, the identification of additional recombination products was possible. Most of the compounds found, however, could be explained on the basis of the general concepts proposed earlier by LeTellier and Nawar (3,4).

The present work provides the confirmation for these proposed mechanisms by irradiating tributyrin labeld with deuterium such that the molecule contains five deuterium atoms in the glyceryl moiety, and analyzing the decomposition products by mass spectrometry.

EXPERIMENTAL PROCEDURES

Labeled tributyrin was obtained from R. Jensen of the University of Connecticut, Storrs, CT. The structure of this compound was confirmed by mass spectrometry as:

$$\begin{array}{l} D_2COOCC_3H_7 \\ | \\ D\text{-}COOCC_3H_7 \\ | \\ D_2COOCC_3H_7 \end{array}$$

As shown in Figure 1, the ions at m/e 43 for $[C_3H_7]^+$ and m/e 71 for $[C_3H_7CO]^+$ did not change in the labeled compound while those at m/e 215 for $[M\text{-}C_3H_7COO]^+$, m/e 201 for $[M\text{-}C_3H_7COOCH_2]^+$, and m/e 131 for $[RCOOCH_2CH(OH)]^+$ have shifted to m/e 220, m/e 204, and m/e 134, respectively.

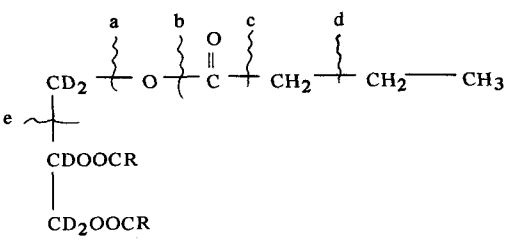
Methods of irradiation, separation, and identification were identical to those used in the preceding paper (5).

RESULTS AND DISCUSSION

The radiolytic products found to contain the deuterium label are listed in Table I. For each compound the major shift in m/e corresponding to the presence of one or more deuterium atoms in its mass spectral fragments is given.

Primary Cleavage Products

The fact that in experiments with deuterated tributyrin the resulting butyric acid did not carry the label while the propanediol and propenediol diesters each contained five and four deuterium atoms, respectively, is proof of the splitting at the acyloxy-methylene bond (a):



Splitting at the acyl-oxygen bond (b) is confirmed by

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TABLE I

Mass Spectral Shift in the Fragmentation of Radiolytic Compounds of Deuterium Labeled Tributyrin as Compared to Those from the Unlabeled Compound

Radiolytic product	m/e in Unlabeled tributyrin	Responsible ion	m/e in Labeled tributyrin	Suggested structure for radiolytic product from labeled tributyrin
Methyl butyrate	103	M + 1	105	$C_3H_7COOCD_2H$
	74	McLafferty rearrangement	76	
Ethyl butyrate	116	M	119	
	101	M - CH_3	104	$C_3H_7COOCD_2CDH_2$
	88	McLafferty Rearrangement	91	
Vinyl butyrate	114	M	117	$C_3H_7COOCD{=}CDH$
	27	$CH_2{=}CH$	30	
Isopropyl butyrate	89	$C_3H_7COOH_2$	70	$C_3H_7COOCD(CD_2H)_2$
	45	CH_3CHCH_3	48	
Butyl butyrate	145	M + 1	147	
	116	$C_3H_7COOC_2H_5$	118	$C_3H_7COOCD_2(C_3H_7)$
	101	$C_3H_7COOCH_2$	103	
2-Pentanone	86	M	88	$C_3H_7COCD_2H$
	43	CH_3CO	45	
1,2-propanediol dibutyrate	172	M-C_2H_4O	174	
	144	$C_3H_7COCH_2OC(OH){=}CH_2$	146	
	129	M-C_3H_7COOH	134	
	128	M-C_3H_7COOH	133	D_2CH
	113	Loss of RCOOH + 3,4-cleavage	118	D-$COOCC_3H_7$
	115	$C_3H_7COOC_2H_4$	118	$D_2COOCC_3H_7$
	100	Loss of RCOOH + McLafferty	105	
Propenediol dibutyrate	143	M-C_3H_7CO	147	D_2C
	127	M-C_3H_7COO	131	$COOCC_3H_7$
				$D_2COOCC_3H_7$
Ethanedioldibutyrate	144	$C_3H_7COCH_2OC(OH){=}CH_2$	146	
	115	M-C_3H_7COO	118	$C_3H_7COOCD_2$
	114	M-C_3H_7COOH	117	$C_3H_7COOCDH$
	86	Loss of RCOOH + McLafferty		
1,2-hexanediol dibutyrate	171	M-C_3H_7COO	176	D_2C-C_3H_7
	157	M-$C_3H_7COOCH_2$	160	D-$COOCC_3H_7$
	144	$C_3H_7COOCH_2OC(OH){=}CH_2$	146	$D_2COOCC_3H_7$
2-oxopropanediol dibutyrate	159	M-C_3H_7CO	163	$D_2COOCC_3H_7$
	143	M-C_3H_7COO	147	C=O
	129	M-$C_3H_7COOCH_2$	131	$D_2COOCC_3H_7$
	101	$C_3H_7COOCH_2$	103	
Dibutyrin	201	M-CH_2OH	204	D_2COH
	445	M-C_3H_7COO	150	D-$COOCC_3H_7$
	131	M-$C_3H_7COOCH_2$	134	$D_2COOCC_3H_7$
	101	$C_3H_7COOCH_2$	103	
Formo-dibutyrin	173	M-C_3H_7COO	178	D_2COOCH
	159	M-$C_3H_7COOCH_2$	162	D-$COOCC_3H_7$
	144	$C_3H_7COCH_2OC(OH){=}CH_2$	146	$D_2COOCC_3H_7$
	101	$C_3H_7COOCH_2$		
Aceto-dibutyrin	201	M-CH_3COOCH_2	204	
	187	M-C_3H_7COO	192	$D_2COOCCH_3$
	173	M-$C_3H_7COOCH_2$	176	D-$COOCC_3H_7$
	144	$C_3H_7COOCH_2C(OH){=}CH_2$	148	$D_2COOCC_3H_7$
	131	$C_3H_7COOCH_2CHOH$	134	

the observation that the labeled tributyrin gave rise to a diglyceride containing five deuterium atoms, an oxopropanediol dibutyrate containing four deuterium atoms and cyclobutanone containing none.

While the hydrocarbons were not labeled, each of the triblycerides, formo-dibutyrin and aceto-dibutyrin, contained five deuterium atoms confirming splitting at locations (c) and (d). The fact that methyl butyrate contained

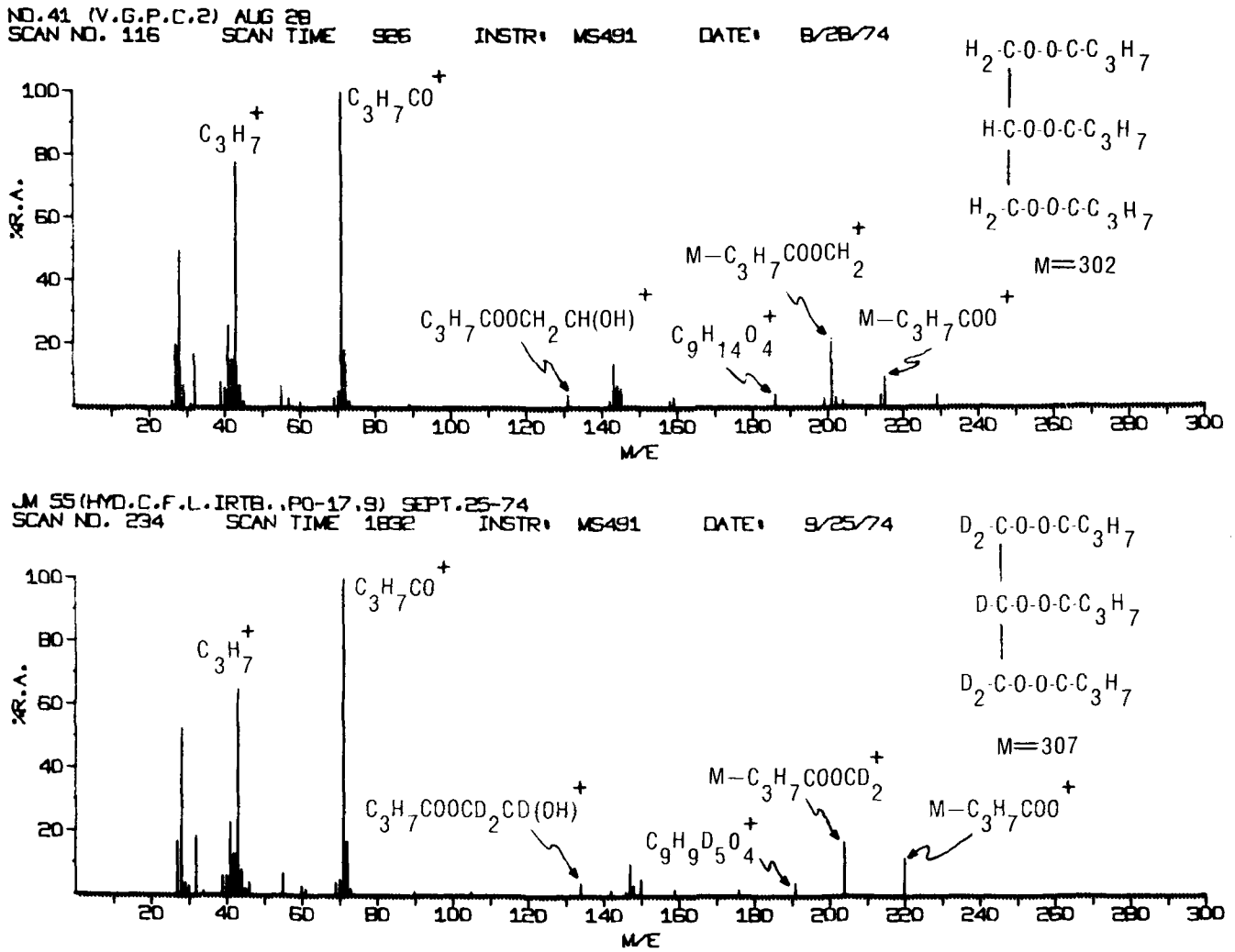


FIG. 1. Mass spectra of tributyrin and deuterium labeled tributyrin.

two deuterium atoms while ethanediol dibutyrate contained three is consistent with radiolytic splitting at location (e).

It can be seen that the above results generally support the mechanisms proposed by LeTellier and Nawar (3) for the formation of the radiolytic compounds by primary cleavage of the triglyceride.

Recombination Products

The following observations are also consistent with LeTellier and Nawar's earlier explanations for the production of recombination products (3).

Propyl butyrate was not labeled and therefore probably arises from recombination of:

$$C_3H_7 + C_3H_7COO \rightarrow C_3H_7COOC_3H_7$$

Likewise, the ketones, 4-heptanone and 3-hexanone, were found not to contain deuterium. Their formation is therefore probably as follows:

$$C_3H_7\dot{C}O + \dot{C}_3H_7 \rightarrow C_3H_7COC_3H_7$$

and

$$C_3H_7\dot{C}O + \dot{C}_2H_5 \rightarrow C_3H_7COC_2H_5$$

On the other hand, 2-pentanone was found to contain two deuterium atoms. The reaction:

$$C_3H_7\dot{C}O + \dot{C}D_2H \rightarrow C_3H_7COCD_2H$$

may be postulated, but the origin of the d^2 methyl radical is not clear.

The mechanism proposed for the formation of butyl butyrate as a recombination product can also be deduced from radiolysis of deuterated tributyrin since the compound contains two deuterium atoms, $C_3H_7COOCD_2CH_2CH_2CH_3$. It probably arises as follows:

$$C_3H_7COOCD_2 + C_3H_7 \rightarrow CH_3COOCD_2C_3H_7$$

The compound 2-oxo-pentyl butyrate is probably formed in a similar manner.

$$C_3H_7COOCD_2 + C_3H_7CO \rightarrow C_3H_7COOCD_2COC_3H_7$$

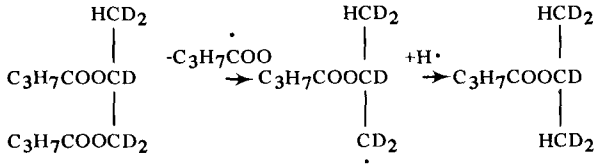
Moreover, the compound 1,2-hexanediol dibutyrate contained five deuterium atoms supporting the reaction:

$$\begin{array}{l} D_2C\cdot \\ | \\ DCOOCC_3H_7 \\ | \\ D_2COOCC_3H_7 \end{array} + \dot{C}_3H_7 \rightarrow \begin{array}{l} D_2C-C_3H_7 \\ | \\ DCOOCC_3H_7 \\ | \\ D_2COOCC_3H_7 \end{array}$$

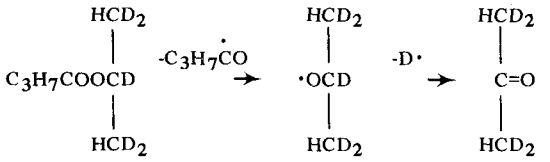
Products of Secondary Cleavages

Iso-propyl butyrate and acetone are examples of secondary radiolytic products formed by mechanisms such as those suggested for corresponding products from tricaproin by LeTellier and Nawar (6). Thus iso-propyl butyrate is

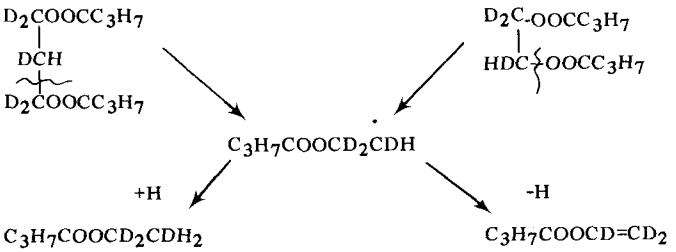
shown by the presence of five deuterium atoms to be formed by further cleavage of propanediol dibutyrate as follows:



Acetone, although produced in low yield, is probably formed in a similar manner by decomposition of isopropyl butyrate, since the compound recovered from deuterated tributyrin showed the presence of four deuterium atoms. Thus, in a simplistic representation:



Since the ethyl butyrate produced from deuterated tributyrin contained three deuterium atoms, the reaction responsible for its formation is probably also due to secondary cleavage as depicted in the scheme below:



As is also shown in this scheme, vinyl butyrate may be formed in a similar manner by loss of hydrogen from the dimethylene butyrate radical.

Data for many other compounds formed in this study provide further evidence for the mechanisms demonstrated by the examples given herein. The evidence cited is for the moment primarily qualitative due to the lack of dose dependency data for the amounts of the radiolytic products. A continuation of this work is concerned with its quantitative aspects.

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