

# Radiolysis of Lipids: Mode of Cleavage in Simple Triglycerides

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## Abstract

The effect of gamma radiation on simple triglycerides was investigated. Trilaurin, trimyristin, tripalmitin, tristearin, tripalmitolein, triolein and trilinolenin were irradiated under vacuum at 6 megarads. The volatile breakdown products were separated by vacuum distillation and identified by gas chromatography and mass spectrometry. Qualitative and quantitative data show that the cleavage in fatty acids essentially follows a specific pattern and is not random. A mechanism of radiolysis is proposed.

## Introduction

MOST STUDIES ON IRRADIATION of fats were carried out by irradiating natural fats in the presence of air. It has been difficult, therefore, to distinguish between radiation-induced oxidative changes and changes resulting directly from exposure to high energy radiation. Studies with simple model systems indicated that the major effect of radiation was the formation of free radicals.

Truby et al. (18) investigated the types of free radicals formed by irradiation of tristearin, stearic acid and linoleic acid. Chipault and Mizuno (4-6) studied the accumulation of peroxides, carbonyl compounds and reducing substances by irradiation of various methyl esters under vacuum, oxygen and nitrogen atmospheres.

Sheppard and Burton (16) and Burton (1) demonstrated that one of the major reactions in alpha-irradiated saturated free fatty acids was decarboxylation producing considerable quantities of the  $C_{n-1}$  hydrocarbons. Similarly Howton and Wu (11) showed that gamma radiolysis of free oleic acid yields polymers and hydrocarbons. They found that the major hydrocarbon produced was *cis*-8-heptadecene.

More recently, Merritt et al. (15) investigated the hydrocarbons produced by radiation of various lipid systems including methyl stearate, methyl oleate and tristearin. They found a series of *n*-alkanes up to  $C_{13}$  and *n*-alkenes up to  $C_{10}$  and several alkynes. They concluded that radiation products are primarily the result of direct bond cleavage and suggested that cleavage by gamma radiation "seems to be random for all bonds within the molecule and is not selective."

The present study was designed to investigate in detail the direct effects of ionizing radiation on model systems of triglycerides.

## Experimental Procedures

### Materials

Saturated triglycerides (95-99% purity) were purchased commercially and crystallized repeatedly from ethyl ether until shown by thin-layer chromatography to be free from diglycerides, monoglycerides and free fatty acids. Unsaturated triglycerides (99% purity) were supplied by Hormel Institute, Austin, Minnesota, and used without purification.

Saturated and unsaturated hydrocarbons (99% purity) were obtained from PolyScience Corpora-

tion, Evanston, Illinois, and aldehydes from K & K Laboratories, Plainview, New York.

### Irradiation

Five-gram samples of the triglycerides were sealed in glass ampoules under vacuum ( $10^{-3}$  torr) and irradiated with gamma rays from the  $Co^{60}$  source at the U. S. Army Natick Laboratories, Natick, Massachusetts. The samples received a dose of 6 megarads ( $4.4 \times 10^4$  rads/min) at 25 C, were placed in dry ice and returned by automobile to the laboratory for analysis.

### Isolation of Radiolysis Products

The products of radiolysis were isolated by vacuum distillation. The products in the boiling point range 150-300 C were collected on a cold finger, essentially as described by de Bruyn and Schogt (8), and were thereafter dissolved in ethyl ether for further analysis. A separate sample was used to collect the lower boiling components on a liquid nitrogen-cooled 4 in.  $\times$   $\frac{1}{8}$  in. alumina-packed precolumn which was subsequently fitted to the gas chromatograph between the injection port and the regular 12 ft column.

### Gas Chromatographic Analysis

The material obtained by cold finger distillation was analyzed on three different columns: a 12 ft  $\times$   $\frac{1}{8}$  in. Carbowax 20M; a 6 ft  $\times$   $\frac{1}{8}$  in. silicone rubber SE 30; and a 200 ft  $\times$  0.02 in. DEGS (LAC 728). The lower boiling components from the precolumn were separated on a 12 ft  $\times$   $\frac{1}{8}$  in. alumina column as described by List et al. (14). Two gas chromatographic instruments were used: an F & M model 810 (F & M Scientific Corp., Avondale, Penn.) and a Varian Aerograph 1200 (Walnut Creek, Calif.). Both were equipped with temperature programming and flame ionization detection systems.

### Mass Spectrometry

The effluent from the gas chromatographic column was admitted through a heated line to a Biemann helium separator and then to the ion source of a Hitachi Perkin-Elmer RMU-6A mass spectrometer (Perkin-Elmer Corp., Norwalk, Conn.). The source was operated at 80 eV and the ionization chamber temperature maintained at 250 C. The collector slit was .15 mm and the electron multiplier operated at 3000 v. A scanning time of 5 sec was employed for sharp GLC peaks and 12 sec for others. The spectra were recorded on a Honeywell 1508 Visicorder.

### Identification of Radiolysis Products

The various components separated by gas chromatography were identified by gas chromatography and mass spectrometry. Agreement of mass spectra and retention times on two or three different stationary phases with those of authentic compounds was used as the criterion for identification. Authentic compounds were available for all the alkanes, the 1-alkenes, the saturated aldehydes up to  $C_{14}$  and all the methyl and ethyl esters. An authentic sample of 1,8-heptadecadiene was obtained through the

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courtesy of D. R. Howton (University of California, Los Angeles). Reference mass spectra of the stearyl, oleyl, linoleyl and linolenyl aldehydes were supplied by R. T. Holman and K. Christiansen (The Hormel Institute, University of Minnesota, Austin).

#### Quantitative Analysis

A known amount of an appropriate internal standard was added to the sample immediately before distillation. The compound to be used as the internal standard was selected on the basis of preliminary analysis to establish its absence in the sample to be analyzed and the suitability of its GLC retention time. For this purpose, 6-dodecyne, n-hexadecane or n-nonadecane were used depending on the glyceride examined. Conversion factors relating GLC peak areas of the identified compounds to that of the internal standard were previously established by distilling known quantities of the authentic compounds (and the internal standard) from a non-volatile lipid medium as recently described by Buziassy and Nawar (2).

### Results

#### Saturated Triglycerides

The radiolysis products of triglycerides were mainly hydrocarbons and some oxygen-containing compounds. Trilaurin, trimyristin, tripalmitin and tristearin yielded the normal alkanes and 1-alkenes listed in Table I. The unirradiated controls for each of these glycerides were free from hydrocarbons. It can be seen that for each irradiated glyceride, four hydrocarbons (underlined) were prominent. In all four triglycerides the dominant alkane has one carbon atom less and the principal alkene two carbon atoms less than the parent fatty acid. In their work on irradiation of free fatty acids, Sheppard and Burton (16) and Howton and Wu (11) have also shown that the major hydrocarbon is the  $C_{n-1}$  compound.

It should be noted that the saturated triglycerides used in this study contained small amounts of contaminant fatty acids, principally the vicinal higher and lower even numbered fatty acids, which were apparently esterified in the triglyceride molecule and could not be separated by recrystallization. As would be expected, the presence of such acids is reflected in the hydrocarbon pattern of the irradiated triglycerides (Table I).

In addition to hydrocarbons, irradiation of each saturated simple triglyceride produced four oxygen-containing compounds: a saturated and an unsaturated aldehyde containing the same numbers of carbons as the fatty acids present in the triglyceride, and also, the corresponding methyl and ethyl ester. All four compounds were absent in the unirradiated controls.

#### Unsaturated Triglycerides

As shown in Table II, irradiation of triolein produces the normal alkanes  $C_1$  to  $C_9$ , the normal 1-alkenes  $C_2$  to  $C_{10}$ , the internally unsaturated alkenes  $C_{11}$  to  $C_{17}$  and the alkadienes  $C_{11}$  to  $C_{17}$ . Examination of the quantitative data presented in Table II reveals that the radiolysis of triolein must have proceeded along a similar pathway to that of saturated triglycerides since the corresponding hydrocarbons are again formed in large quantities. Thus the production of the internally unsaturated heptadecene from triolein is analogous to the forma-

TABLE I  
Quantitative Analysis of Volatiles Formed in Saturated Triglycerides at 6 Megarads, 25 C ( $\mu$ moles/100 g)

Product	Trilaurin	Trimyristin	Tripalmitin	Tristearin
<b>Alkanes</b>				
1 <sup>a</sup>	t <sup>b</sup>	t	t	c <sup>c</sup>
2	t	t	0.1	c
3	0.1	t	0.3	c
4	0.5	0.3	0.9	c
5	0.9	0.5	1.3	c
6	2.6	0.8	1.4	c
7	4.8	1.3	2.6	c
8	3.2	1.8	3.0	1.5
9	3.4	2.1	2.1	2.0
10	5.5	2.1	2.3	2.3
11	131.8	1.8	2.8	1.9
12	0.3	2.6	1.8	1.9
13	2.9	105.8	1.4	2.0
14	0	0.1	19.4	2.8
15	0	3.1	88.4	9.5
16	0	0	1.5	20.7
17	0	0	7.3	85.2
18	0	0	0	0
19	0	0	0	0.7
<b>1-Alkenes</b>				
2	0	0	0	c
3	0.1	t	t	c
4	t	t	t	c
5	t	t	t	c
6	0.2	0	0	c
7	0.6	0.1	0	c
8	1.2	1.1	0	0
9	0.5	0.8	0	0
10	18.1	0.7	0	0
11	5.7	0.6	0	0
12	0.8	10.6	0.3	0
13	0.5	6.8	0.3	0
14	0	0.3	12.7	0.2
15	0	0.2	4.7	0.2
16	0	0	1.1	7.5
17	0	0	0.5	1.8

<sup>a</sup> Carbon number.

<sup>b</sup> Trace (0.05  $\mu$ moles or less per 100 g).

<sup>c</sup> Not determined.

tion of heptadecane from tristearin and the production of hexadecadiene from triolein is analogous to the formation of 1-hexadecene from tristearin. However, triolein yielded more alkadiene than was the amount of 1-hexadecene from tristearin. Apart from the major products from triolein, it appears that there was a slight preferential splitting in the vicinity of the double bond as evidenced by somewhat larger amounts of the  $C_8$  and  $C_{10}$  hydrocarbons. Only one alkyne, hendecyne, could be observed in the radiolysis products of triolein.

Table III shows the major components produced by irradiation of tripalmitolein and trilinolenin. It can be seen that the quantities of the four major hydrocarbons from tripalmitolein as well as their ratios with respect to one another are very similar to those of triolein. Similarly with trilinolenin, it was not surprising to find heptadecatriene and hexa-

TABLE II  
Hydrocarbons in Triolein Irradiated at 6 Megarads, 25 C ( $\mu$ moles/100 g)

	Alkane	1-Alkene	Int. Alkene	Diene
$C_1$	Tr.		$C_{11}$	0.1
$C_2$	Tr.	0.3	$C_{12}$	0.9
$C_3$	0.3	1.4	$C_{13}$	1.6
$C_4$	1.1	1.0	$C_{14}$	0.8
$C_5$	1.5	0.7	$C_{15}$	0.8
$C_6$	0.8	0.8	$C_{16}$	1.2
$C_7$	1.8	1.1	$C_{17}$	41.5
$C_8$	4.0	1.2	$C_{18}$	0
$C_9$	0.1	0.6		9.5
$C_{10}$	0	3.0		0

TABLE III  
The Major Hydrocarbons Formed in Tripalmitolein and Trilinolenin  
at 6 Megarads, 25 C

Compound	$\mu\text{mole}/100\text{ g}$
Tripalmitolein	
C <sub>14</sub> alkene (internal)	1.1
C <sub>15</sub> alkene (internal)	51.8
C <sub>14</sub> alkadiene	73.6
C <sub>15</sub> alkadiene	12.7
Trilinolenin	
C <sub>16</sub> alkatriene	6.0
C <sub>17</sub> alkatriene	36.8
C <sub>16</sub> alkatetraene	32.0
C <sub>17</sub> alkatetraene	8.0

decatetraene in relatively large quantities. The mass spectra of the latter two compounds are given in Fig. 1.

In addition to hydrocarbons, two aldehydes were also produced in each irradiated unsaturated triglyceride: one with the same number of carbon atoms and the same number of double bonds as in the fatty acid and the other with an additional double bond. In contrast to saturated triglycerides, the formation of methyl or ethyl esters could not be established in the radiolysis of unsaturated triglycerides.

### Discussion

It is clear that while a series of saturated and unsaturated hydrocarbons are produced probably by splitting carbon-carbon bonds along the fatty acid chains, the compounds produced in the greatest quantities are those resulting from preferential cleavages near the carbonyl group. Irradiation of

simple triglycerides results in the formation of the following major radiolytic products ( $n$  being the number of carbons in the fatty acid molecule): C <sub>$n-1$</sub>  hydrocarbon; C <sub>$n-1$</sub>  hydrocarbon with an additional double bond in the terminal position (in addition to whatever the number of double bonds in the parent fatty acid may be); C <sub>$n-2$</sub>  hydrocarbon; C <sub>$n-2$</sub>  hydrocarbon with an additional double bond in the terminal position; C <sub>$n$</sub>  aldehyde; and C <sub>$n$</sub>  aldehyde with an additional double bond.

This pattern is consistent for both saturated and unsaturated glycerides as shown in Fig. 2 and 3. In addition to the above compounds, the methyl and ethyl esters of the C <sub>$n$</sub>  fatty acids are produced by irradiation of the saturated triglycerides.

Figure 4 shows a possible mechanism for the formation of the four major hydrocarbons. Cleavage between carbons no. 1 and no. 2 results in a free radical which may accept a hydrogen atom to give the saturated C <sub>$n-1$</sub>  compound (I), or lose a hydrogen atom to give the compound (Ia). As can be seen in Table I, these two compounds are not produced in equal proportions. The hydrogen gained by the free radical to form the relatively large amount of the  $n$ -alkane may be provided via hydrogen-atom abstraction or by C-H bond homolysis at various sites on the hydrocarbon chains (1,9,11).

Formation of the C <sub>$n-1$</sub>  hydrocarbon may proceed via a preliminary cleavage of the acid from the glycerol part or alternatively by direct homolysis in the esterified fatty acid. The former process could occur by a process analogous to that occurring in thermal degradation of simple esters as described

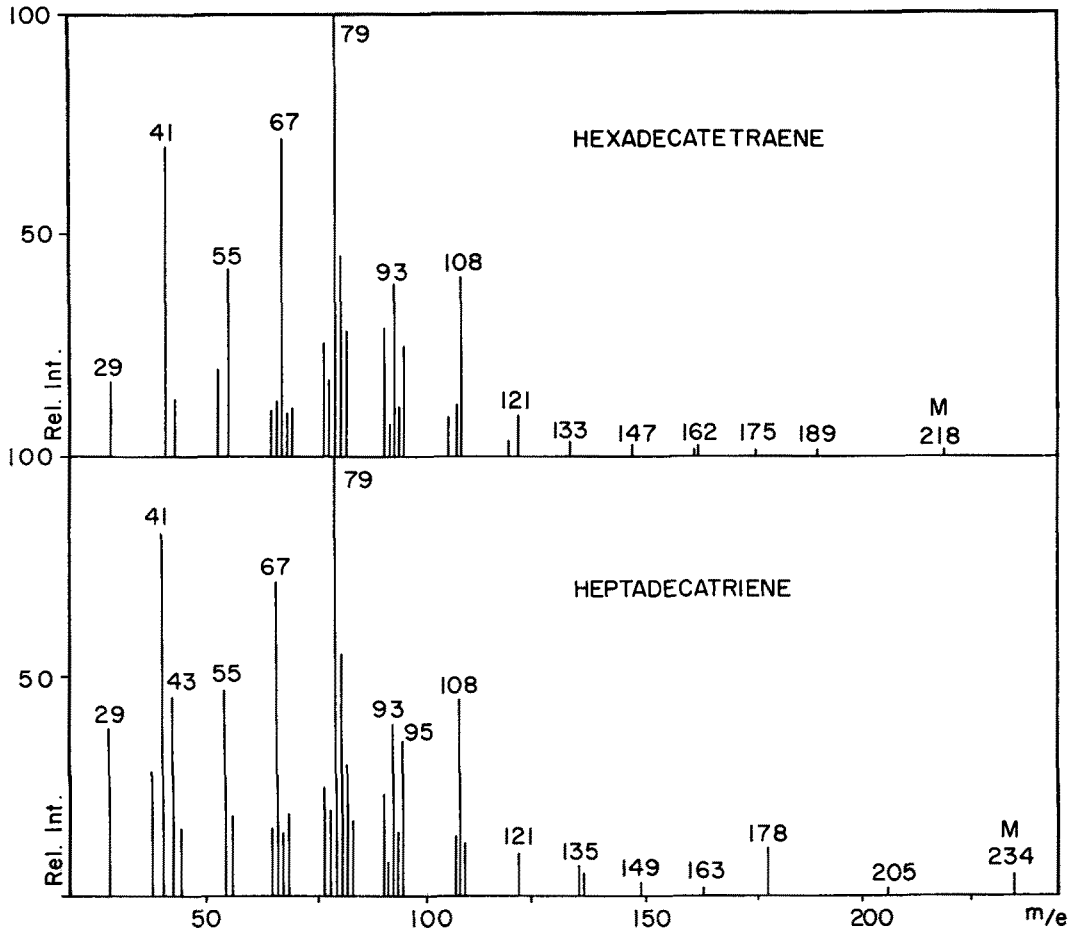


Fig. 1. Mass spectra of GC components identified as hexadecatetraene and heptadecatriene.

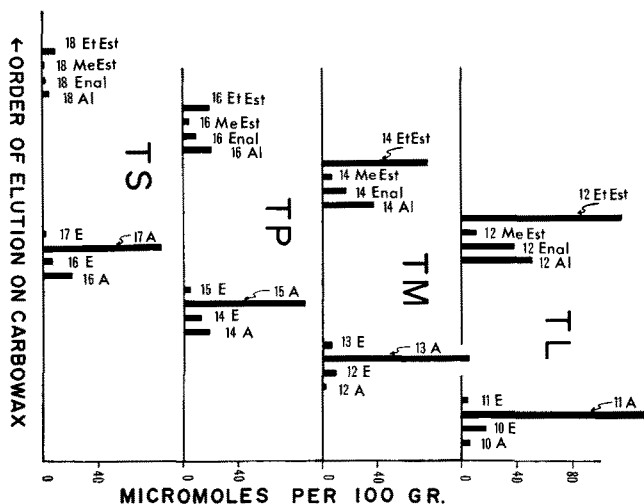
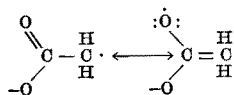


FIG. 2. Major radiolysis products of saturated triglycerides. TL = trilaurin; TM = trimyristin; TP = tripalmitin; TS = tristearin. A = alkane; E = alkene; Al = alkanal; Enal = alkenal; MeEst = methyl ester; EtEst = ethyl ester.

by Hurd and Blunck (12) and of triglycerides as suggested by Crossley et al. (7) or in mass spectrometry of triglycerides (17). On the other hand the  $C_{n-1}$  radical of the acid portion of esters can split away, leaving the carboxyl group attached to the alcohol part, under the mass spectrometry conditions as shown by Williams (19) and when irradiated by ultraviolet rays as proposed by Calvert and Pitt (3).

Cleavage of the fatty acid molecule between carbons no. 2 and no. 3 may result in a free radical which would give the two remaining major hydrocarbons II and IIa (Fig. 4). In this case, the amounts of products produced by loss of hydrogen from the n-2 free radical appear to exceed those formed by hydrogen addition, particularly for the unsaturated triglycerides (Tables II and III). Previous evidence (9) indicates that unsaturated hydrocarbon chains yield less radiolytic hydrogen than saturated systems.

The formation of the olefin from a free fatty acid, detached from the glyceride molecule, may be analogous to the appearance of the rearrangement ion of  $m/e$  60 in the mass spectrometric fragmentation of free carboxylic acids (10). The formation of the  $C_{n-2}$  free radical by homolysis of the glyceride fatty acid chain is an alternative explanation. Such process may be favored by the resonance stabilization of the remaining free radical of the glycerol moiety:



In addition to the four major hydrocarbons, each irradiated simple triglyceride contained aldehydes of the same carbon number as the glyceride fatty acid. The formation of aldehydes can be explained by a radiolytic cleavage of the acyl-oxygen linkages and subsequent hydrogenation. A similar interpretation was given by Khatri et al. (13) to explain the presence of n-alkanals in irradiated milk fat. It is interesting to note that the same paper reports the presence of 2-alkanals ( $C_5$ ,  $C_6$ ,  $C_9$ , and  $C_{12}$ ) in irradiated milk fat.

The formation of the methyl and ethyl esters observed in this study may indicate a cleavage in

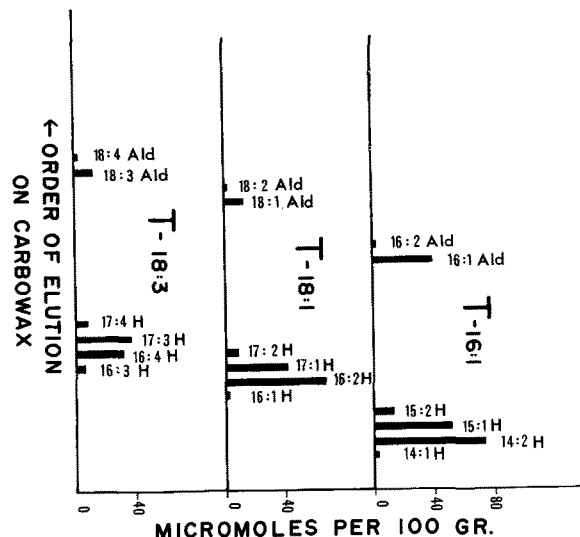


FIG. 3. Major radiolysis products of unsaturated triglycerides. T-16:1 = tripalmitolein; T-18:1 = triolein; T-18:3 = trilinolein; H = hydrocarbon; Ald = aldehyde.

the glycerol moiety since irradiation of free fatty acids did not produce these compounds. Some analogy to this mechanism may be found in the observation by Sprecher et al. (17) that mass spectral fragments from a seed oil triglyceride included

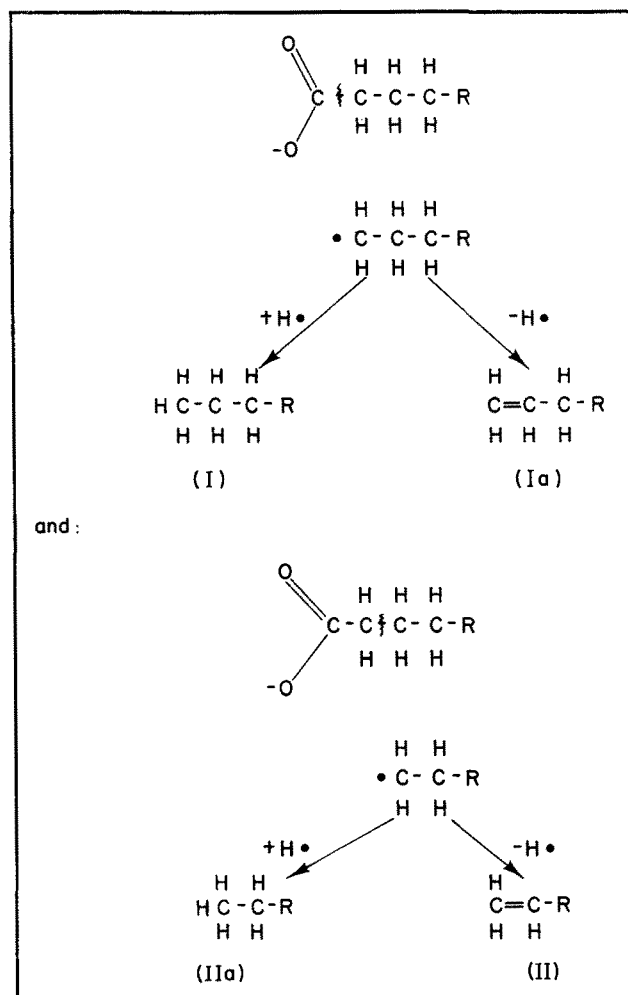


FIG. 4. Reaction scheme for production of major hydrocarbons.

acyloxy-CH<sub>2</sub>- and also acyloxy-C<sub>2</sub>H<sub>4</sub>-ions. No explanation is given at the present time for the absence of esters in irradiated unsaturated triglycerides.

The results clearly show that the radiolysis of fats follows, to a large extent, specific routes which determine the composition of the resulting products. These findings on the specificity of the radiolytic reactions in fats are in agreement with the general concepts maintained by Williams (19). On the other hand, the conclusions reached by Merritt et al. (15), that the radiolytic events are random, should apply only to the relatively small proportion of the lower molecular weight hydrocarbons resulting from the fragmentation along the fatty acid chains.

It is to be understood that other decomposition reactions may occur in addition to those which have been observed in the present investigation. Combination of free radicals, for example, may give dimeric or polymeric compounds which would not be detected under the experimental conditions used.

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