approximately 3% wax. A methanolyzation, followed by gas chromatographic analysis of the fatty acid fraction, showed that besides the fatty acids mentioned in the table there were several unknown components contained in the waxes.

The nomads are said to have collected these fruits in former times and pressed oil out of them, which they used for cooking and medicinal purposes. The oil supposedly has a mild laxative effect. This oil is no longer used today, due to the availability of less work intensive substitutes.

Even though the seeds of the East African savannah bush are not used presently, this oil represents a source of nutrition which could be used in countries with arid regions. If one succeeds in cultivating *Balanites orbicularis* intensively, eliminating the minimal sapogenin content (6,7) and making genetic improvements, the countries with arid regions could build a basis for domestic food production with the help of this new crop.

As in the case of the Canadian rapeseed oil, this seed containing a high source of energy also could be used for fuel. Furthermore there could be the possibility of commercially using this plant's oil as a source of primary energy once other energy sources have been depleted.

ACKNOWLEDGMENTS

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Identification of Adduct Radiolysis Products From Pork Fat

M. VAJDI and **C. MERRITT JR.**, Science and Advanced Technology Laboratory, U.S. Army Natick Research and Development Center, Natick, MA 01760

ABSTRACT

Mass spectrometric evidence is given to show the formation of adduct radiolysis products in pork fat. A variety of adduct radiolysis products were identified. Only the major recombination products are considered and discussed herein. These compounds consist of triglyceride dimers, propanedioldiester-triglyceride adducts, propanedioldiester dimers and branched alkyl substituted triglycerides.

INTRODUCTION

Evidence for the formation of adduct radiolysis products in pure triglycerides has been given in previous studies (1-4). Recently, formation of adduct radiolysis products from ethyl palmitate and ethyl oleate has been reported (5). In these earlier studies, model compounds were chosen in order to elucidate the mechanism for the formation of adducts induced by gamma-irradiation. This study is concerned with the identification of adduct radiolysis products found in pork fat. Based on studies showing a similarity in the formation of other radiolysis products in various meats, viz. beef, chicken and ham (26), similar adducts may be expected to form in all meat fats. The results of this study provide an insight into the nature of the adduct radiolysis products formed in a natural fat and are wholly consistent with the prior knowledge of both the radiation chemistry and mass spectrometry of triglycerides and related compounds.

EXPERIMENTAL

Analysis of the high molecular weight radiolysis products was carried out on a 3 gm sample of ground pork irradiated at 3 Mrads with Co^{60} at −45 C under vacuum (10^{-3} Torr). The irradiated sample was freeze dried and then extracted in a soxhlet assembly using diethyl ether as solvent.

Separation of the appropriate fraction from the extracted fat was achieved by means of a size exclusion liquid chromatographic (SEC) column employing Styragel 60 Å and 100 Å (Waters Associates, Inc.), as the stationary phase (2-4). The fraction containing the adducts was collected and evaporated for further analysis.

Mass spectrometric analysis of the adduct fraction was carried out by means of a solid insertion probe on a Kratos Model number MS50 Mass Spectrometer equipped with a fast atom bombardment (FAB) ionization source accessory.

The sample was applied to the probe both neat and in a matrix of 2,5-dipentylphenol (DPP) and ionized by 6 KV xenon atoms. The spectrum was scanned at the rate of 100 sec per decade over a mass range of 200-1800 with a mass resolution of 1:2000. The matrix was found to enhance the sensitivity of the spectrum, but did not change the ionic composition of the spectrum nor the relative abundances of the ions.

The identity of the characteristics of the spectra obtained from a sample run with and without the matrix under the same spectrometer operating conditions is shown in Figure 1. The sample is a mixture of the radiolytic adducts formed in pure tripalmitin (4) and separated by SEC in the same manner as the pork adduct fraction described above. The portion of the spectrum shown in Figure 1 depicts the cluster of peaks around the predominant propanedioldiester (PDDE) ion, m/z 551, from tripalmitin dimer adduct. The peaks are analogous to those seen in Figure 4 for the PDDE ions corresponding to the triglyceride dimer adducts in the pork fat sample. The com-

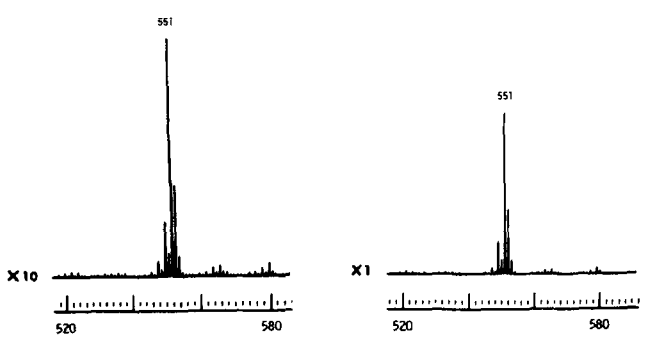


FIG. 1. Portion of a fast atom bombardment mass spectrum in the region of m/z 551 of a sample of tripalmitin dimer adduct. Left, no matrix; right, in a 2,5-dipentylphenol (DPP) matrix.

TABLE I

Comparison of Mass Spectrum Peak Ratios in the FAB Spectra of a Sample of Tripalmitin Dimer Adduct Obtained With and Without a Matrix

Ion pair	No matrix	DPP matrix
549/551	.231	.213
550/551	.099	.107
552/551	.396	.409
553/551	.093	.094

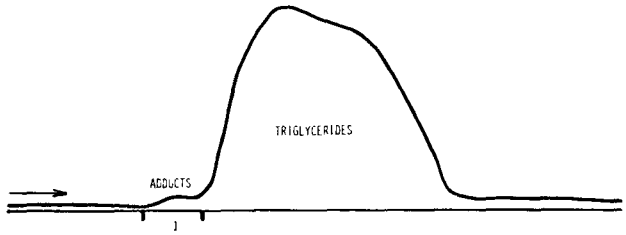


FIG. 2. Size exclusion chromatogram of irradiated pork fat (3 Mr) showing separation of adduct compounds from triglycerides. (See Ref. 4 for chromatographic conditions.)

parison of the spectra obtained with and without a matrix shows clearly that identical spectra are obtained. Moreover, a comparison of the ratios of the relative abundances of the ions given in Table I shows that the quantitative relationships are identical.

Although in some mixtures, particularly those of a polar nature where a matrix may in fact be required to obtain a spectrum, a disparity in ionization behavior may exist between spectra obtained with or without a matrix, in the case of neutral compounds of nearly the same size and having similar structural features it is not unreasonable to expect a similarity in spectral characteristics observed for the triglyceride adducts. Most of the predicted triglyceride dimer adducts (vide infra) differ in size by two methylene groups and at the extreme by only four methylene groups. The average difference in molecular weight is 1.6%, and the range is 3.2%.

In the sample run without a matrix the composition on the surface may be considered the same as that of the bulk of the sample. Moreover, the ionization characteristics of the components must be considered to be nearly the same because of their similarities in size and structure. In the case of the sample ionized in the matrix, the triglyceride adducts were found to be soluble in the DPP, and the composition of the solution is presumed therefore to be uniform. The diffusion characteristics of the solutes, as with other properties, must also be considered similar because of the likeness of size and structure. The assumptions are substantiated by the identities in the mass spectra observed for the pork fat adducts run without a matrix and in DPP. The spectra shown in Figures 4 and 5 are those obtained from the irradiated pork fat sample in DPP, and the data, therefore, were used in their interpretation because of the enhanced intensity of the spectrum peaks. The quantitative relationships of the dimer adducts deduced from the relative abundances of the PDDE ions are predicted on the presumed similarities of physical properties and ionization behavior.

RESULTS AND DISCUSSION

The size exclusion chromatographic separation of the adduct radiolysis products in fat derived from irradiated pork is shown in Figure 2. Fraction 1 containing the adduct radiolysis products was collected and evaporated for direct analysis by the mass spectrometer. Methods for separating the adduct compounds are poorly developed, hence a mass spectrum of the entire mixture was acquired.

Previous studies (8-14) related to fatty acid and triglyceride composition of animal fats have shown that oleic and palmitic acids are the most abundant fatty acids giving rise to the corresponding abundant triglycerides. Stearic and linoleic acids are next in the order of abundance and are present in approximately equal amounts. Consideration of the amounts of these four fatty acids upon computer calculation (15-17) of the probable triglyceride composition of the fat predicts the occurrence of six triglycerides in major abundance. The fatty acid composition of the pork fat and corresponding computed triglyceride composition are shown in Table II. The computed numerical values for

TABLE II

Fatty Acid[a] and Triglyceride Composition[b] of Pork Fat

Fatty acid	Abundance (%)	Triglycerides[c,d] 1-2-3	Probable abundance (%)[e]	Equivalent carbon number
16:0 (P)	24.1	OPO	17.7	48
16:1 (P_O)	3.3	SOO	8.7	50
18:0 (S)	11.8	OPP	8.6	48
18:1 (O)	49.5	SPO	8.4	50
18:2 (L)	11.3	OOL	8.3	46
		OPL	8.1	46
		SOL	3.9	48
		OP_OO	2.5	46
		P_OPO	2.4	46
		SSO	2.1	52
		SPP	2.1	50
		PPL	2.0	46
		SPL	1.9	48
		OLL	1.9	44
		OP_OS	1.2	48
		OP_OL	1.1	44
		SPS	1.0	52

[a]By GC analysis of methyl esters from hydrolysate (16).
[b]By computer calculation of probability (15).
[c]TG's < 1% abundance not listed.
[d]Written as most probable configuration.
[e]Data not given for 63 TG's having <1% abundance comprising 18.1% of the total.

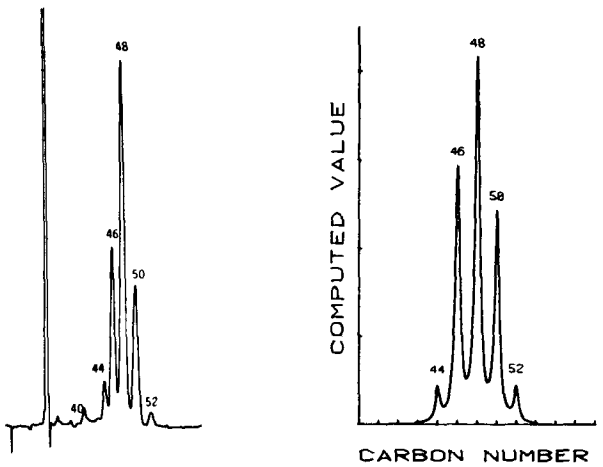


FIG. 3. Comparison of liquid chromatogram (left) of pork fat triglycerides (conditions as in Ref. 15) with computer generated chromatogram (right) from computer predicted composition (data from Table I). Unit separation (R = 1) and Lorentzian distribution are assumed.

TABLE III

Summary of Major Fragmentation Ions Found in the Mass Spectrum of Dimer Triglycerides

	M	M/2	PDDE	M-O	M-P	M-S	M-L
OPO-OPO	1714	857	577	1433	1459		
OPO-OOS	1742	857/885	605	1461	1487[a]	1459	
OPO-SOO	1742	857/885	603				
OPO-OPP	1688	857/831[b]	551	1407[a]	1433		
OPO-PPO	1688	857/831[b]	577				
OPO-OPS	1716	857/859	579	1435[a]	1461	1433	
OPO-SPO	1716	857/859	577				
OPO-LOO	1738	857/881	603	1457			1459
OPO-OOL	1738	857/881	601				
OPO-OPL	1712	857/855	575	1431[a]	1457		1433
OPO-LPO	1712	857/855	577				

[a]Unique ions.
[b]Weak ion.

the composition of the major triglycerides is seen to be verified by a comparison (Fig. 3) of a chromatogram generated from the computer data and an actual liquid chromatographic separation of the pork fat triglycerides.

Although possibly all the triglycerides in the fat are involved in irradiation induced adduct formation, leading to an extremely complex mixture, the number and abundance of such adducts is limited by probability considerations to a few such adducts of a generalized type. Based on the possible combinations of the six major triglycerides and without regard to the permutations resulting from the positional isomerism of the particular fatty acids, 21 adducts are possible. Of these, only six may be expected to occur in measurable abundance because of the overwhelming preponderance of the glycerol 1,3 dioleate 2 palmitate (OPO). (Conventional shorthand notation will be used herein to denote the various triglycerides and adducts.) According to probability calculations based on possible combinations and the relative abundances of the six major triglycerides, the six corresponding adducts are likly to be the various combinations of OPO with itself and with each of the other five abundant triglycerides. These six dimer triglyceride adducts, listed in Table III, have a probability of occurrence of 0.49. The mixed dimer, e.g. PPO-OPS, formed from combinations of triglycerides excluding OPO has a probability of 0.16, and the homologous dimers, e.g. PPO-OPP, have a probability of 0.10. Dimers formed from triglycerides having an abundance less than 8% have a negligibly small probability of occurrence. In fact, no ions are found in the spectrum that may be attributed to a triglyceride dimer except those of the six adducts of OPO and the other triglycerides having abundances of greater than 8% (Table II). Further assumptions to assist in the interpretation of the mass spectrum are made with regard to the positional isomerism of the fatty acids in the triglycerides and in the nature of the adduct bond formation induced by irradiation. These assumptions are made in consonance with generally established facts concerning the structure of triglycerides and the previously observed behavior of triglycerides, and fatty acids and their esters, upon irradiation. In the computer calculations of the triglyceride composition from the fatty acid analysis of the pork fat, the algorithm (17) excluded all simple triglycerides and computed all other possible mixed triglycerides without regard to fatty acid distribution. It was further assumed, however, based on previous studies (8-14), that for purposes of predicting adduct configurations, palmitic acid would be preferentially distributed at position 2 in the triglyceride backbone and the unsaturated fatty acids would reside at positions 1 and 3. Assumptions regarding more detailed configurations of the distribution of fatty acids in the various triglycerides, based largely on the work of Christie and Moore (13). are indicated by the structures given in Tables II and III.

Based on prior studies of model systems, which have included both radiation product analyses (4-6) and in situ ESR studies of irradiated triglycerides and fatty acid esters (7), adduct formation in the irradiated pork fat is presumed to occur in a similar manner mainly by recombination reactions of free radicals induced by radiation in the unsaturated fatty acids, particularly oleate, of the triglyceride. Although adduct formation may be equally possible for fatty acids at position 2 in the triglyceride, for simplicity bonding is assumed to occur with fatty acid moieties at positions 1 or 3 which are considered to have equivalent reactivity.

The presence of the various triglyceride adducts in the pork fat may be imputed to the occurrence in the mass spectrum of the ions corresponding to predicted fragmentation of the postulated structures.

The composite FAB mass spectrum of the adduct radiolysis products in fraction 1 of the size exclusion chromatogram is presented in Figure 4.

The principal ions seen in the low mass region of the spectrum are those commonly attributed in the EI spectra of triglycerides and related compounds to alkyl, acyl and acyloxy cleavages (19-23). It has been observed previously in the FAB spectrum of tripalmitin adducts (4) that fragmentation is closely parallel to that seen in the EI spectra of related compounds such as tripalmitin, triolein, tributyrin and tributyrin dimer adduct. Accordingly, the ions seen in the pork adduct spectrum at m/z 211, 239 and 255 may be attributed to the alkyl ion, $C_{15}H_{31}^{+}$, the acyl ion, $C_{15}H_{31}CO^{+}$, and acyloxy ion, $C_{15}H_{31}COO^{+}$. Corresponding ions derived from the oleate moiety are seen at m/z 237, 265 and 281. Ions of this type are common to all the triglyceride adducts.

Other fragmentation typical of the triglycerides also was observed in the composite spectrum corresponding to all the triglyceride adducts. These ions are related to the fragment ($C_{15}H_{31}CO + 128$) at m/z 367 and a series of corresponding ions [(RCO + 128) + (CH_2)n] which is seen at m/z 381, 395, 409, 437, 451 and 465 (22). Similar patterns

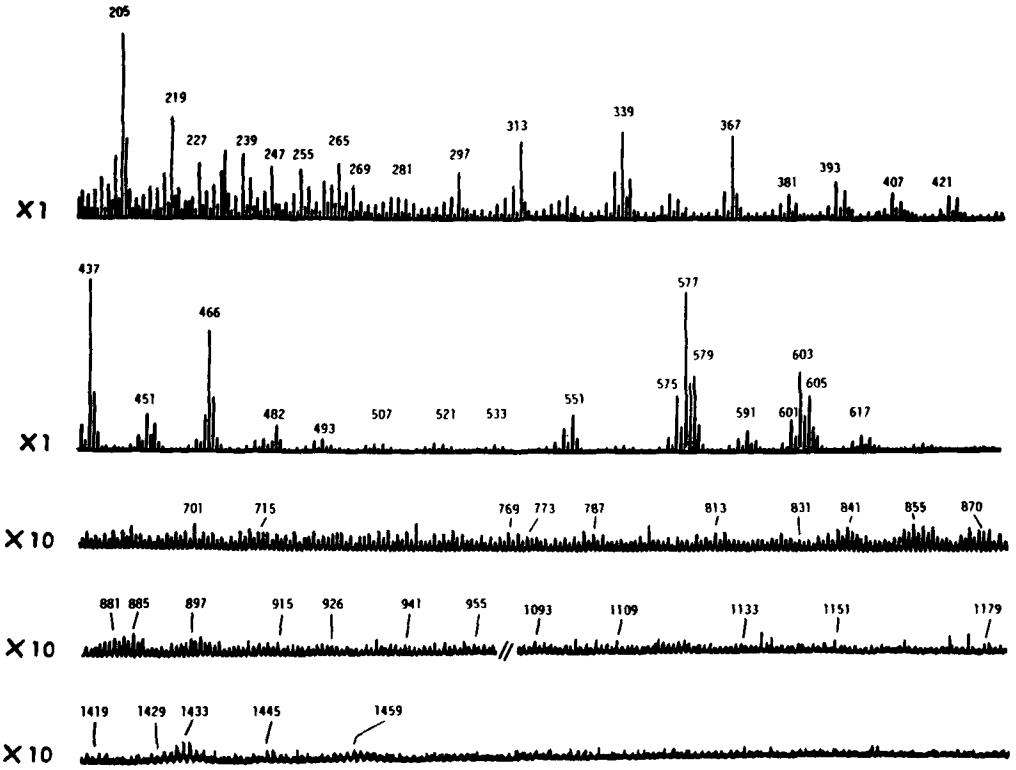
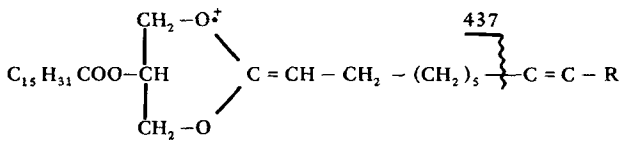


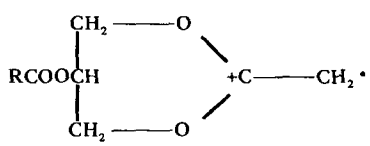
FIG. 4. Fast atom bombardment mass spectrum of fraction from irradiated pork fat containing adduct compounds. Mass scale adjusted to nominal mass taking isotopic mass defects into account.

for the unsaturated fragment $[(C_{17}H_{33}CO + 128) + (CH_2)n]$ give rise to ions at m/z 393, 407, 421, 435, 449 and 463, respectively. The relatively abundant fragment ion at m/z 437 indicates cleavage α to the double bond and confirms the abundance of a type OPO triglyceride resulting in the formation of the aforementioned ion:



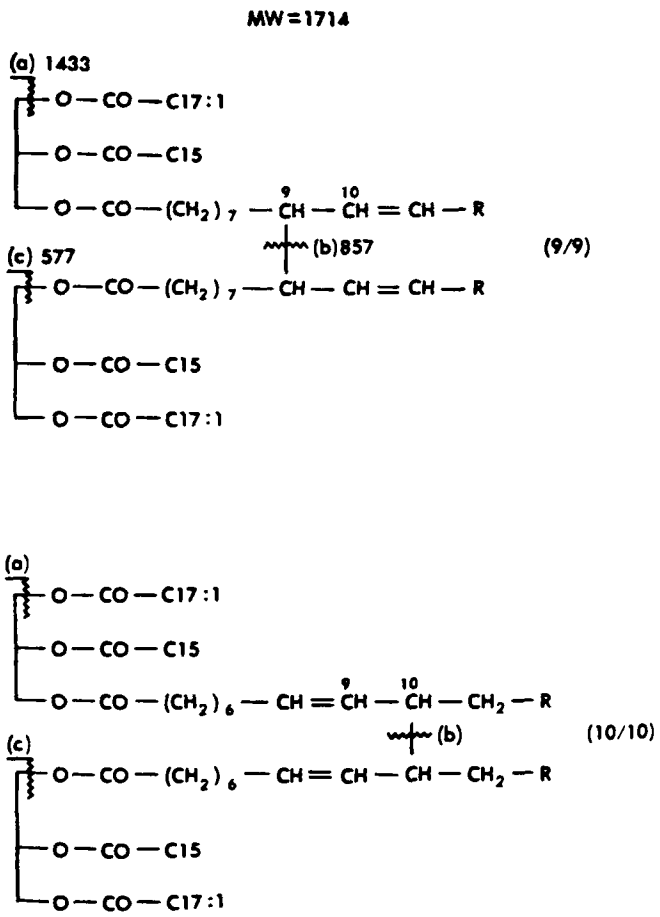
The formation of this ion confirms also the initial assumption that palmitate resides in the 2 position in OPO.

Additional ions related to the triglyceride fragmentations were seen at m/z 368 and 394 for $(C_{15}H_{31}CO + 115 + CH_2)$ and $(C_{17}H_{33}CO + 115 + CH_2)$ arising from the following ion, respectively (22):



Thus, a series of ions of increasing even mass derived from the above structure are seen to be due to fragmentation along the fatty acid chain. Typical fragments corresponding to $(C_{15}H_{31}CO + 74)$ and $C_{17}H_{33}CO + 74)$ ions were observed at m/z 314 and 339.

Identification of the various adducts is accomplished by consideration of the ions seen in the intermediate to high mass regions of the spectrum, i.e. m/z > 500, wherein ions are found to correspond to particular structural features which are typically diagnostic and in some cases unique.



SCHEME 1

Triglyceride Adducts Having Oleate-Oleate Bonding

A typical adduct is the dimer of glycerol 1,3 dioleate 2 palmitate (OPO). A structural diagram of the triglyceride adduct OPO/OPO is shown in Scheme 1. Since the formation of a free radical is equally likely at carbons 8, 9, 10 and 11 of an oleic acid moiety, 10 isomers are possible (5). As seen in the study of the adducts of ethyl oleate (5), the mass spectra of the various isomers would show identical ion fragments in spite of the different sites of crosslinking in the adduct at positions 8 through 11. The representation of the dimer adduct of OPO shown in Scheme 1 depicts, as examples, crosslinking at positions 9-9 and 10-10 of the oleate moiety. It also shows the major cleavages leading to diagnostic ions for the dimer compound.

The dominant ion in the intermediate mass range of the pork adduct spectrum is at m/z 577 corresponding to propanediol palmitateoleate moiety (Scheme 1, cleavage site c). This ion, and the corresponding dioldiester ions derived from the other triglycerides involved in adduct formation, represents one of the most significant ions for characterization of the adducts (vide infra).

Cleavage of the molecule at the bond joining the two oleate chains (M/2) gives rise to an ion at m/z 857 seen in the spectrum in moderate abundance. The parent ions are not observed. The mass range of the mass spectrometer used in this study exceeds m/z 2000 and, accordingly, the fact that no ions of mass greater than about 1530 are seen in the spectrum is due to their low abundance.

Significantly abundant ions due to loss of acyloxy fragments are observed in all spectra of triglycerides (19-23), propanedioldiesters (24,25) and the dimer adducts of tributyrin, tripalmitin (4) and trilinolein (unpublished) data). Thus, loss of oleoyl (M-O) results in a typical ion (cleavage site a in Scheme 1) that is diagnostic for many of the adduct triglycerides. The loss of acyloxy also may occur at other sites of attachment to the triglyceride backbone, and abundant ions also may be observed for loss of palmitoyl (M-P) or linoleoyl (M-L) fragments. The ions expected to be observed for losses of acyloxy fragments are tabulated in Table III. The predominance of these ions is seen more readily in Figure 5 in a spectrum obtained in the high mass range at higher sensitivity than the spectrum shown in Figure 4.

The most abundant ion derived from a triglyceride adduct corresponds to a cleavage of the acyloxy linkage of the fatty acid moiety involved in the crosslinking, i.e. site c. This cleavage results in a 1,2-propanedioldiester ion. In the case of the dimer OPO-OPO (Scheme 1) the ion is m/z 577. In fact, all six of the predicted abundant adducts are of a type in which OPO is one of the triglycerides involved. Accordingly, they all would show a 1-octadecenoyl, 2-hexadecanoyl propanedioldiester ion (OP), m/z 577, which is seen to be the most abundant peak in the FAB spectrum of the pork fat adducts. Several other dioldiester ions are seen in abundance corresponding to the other triglycerides forming adducts with OPO. These are listed in Table IV. Although propanedioldiester ions (PDDE) are of little value in ascertaining the structure of the adduct, since the counter ion (M-PDDE) corresponding to the loss of the propanedioldiester group from the parent ion is of low abundance, they provide significant quantitative information about the relative amounts of the adducts once the structures have been elucidated (vide infra).

Another abundant adduct, OPO-OOS, predicted from probability, has many ions which are isometric with those of OPO-OPO but its presence is established by a unique loss of acyloxy ion, in this case M-P, m/z 1487 (Fig. 5). Other diagnostic ions are seen corresponding to the PDDE ion and a TG ion (M/2; m/z 885). The ions derived from the various cleavages of the several OPO-TG adducts, especially those resulting in unique ions, are given in Table III.

Although adducts of ethyl palmitate (5), tributyrin and tripalmitin (4) all have been isolated and elucidated, the relative abundances of adducts formed from ethyl oleate (5), oleic acid (25), triolein (2), methyl linoleate and trilinolein (unpublished data) are all seen to be substantially greater when a comparison is made of the relative peak areas of fraction 1 in comparable size exclusion chromatograms. These data support the theoretical conclusion that radiation induced adduct formation is more likely to occur at the more reactive site of unsaturation in the oleate moiety. Thus, all of the abundant triglycerides are seen to form adducts with OPO through respective oleate moieties. These adducts are depicted in Figure 6. The mass spectro-

TABLE IV

Relative Abundances of Dioldiester Ions in Mass Spectrum of Pork Fat Adducts[a]

Dioldiester	m/z	A	PDDE/PO	Fraction of TG dimer form yielding PDDE[b]
OP	577	24	–	1.93[c]
OO	603	12	.5	.9[d]
SP	579	11	.45	.9
OS	605	8.5	.35	.70
LP	575	8	.33	.67
PP	551	6	.25	.50
LO	601	5	.21	.42
				Σ = 6.02

[a]From Figure 4.
[b]See Figure 6.
[c]Sum of contribution from forms yielding OP.
[d]Sum of contribution from OPO-SOO and OPO-LOO.

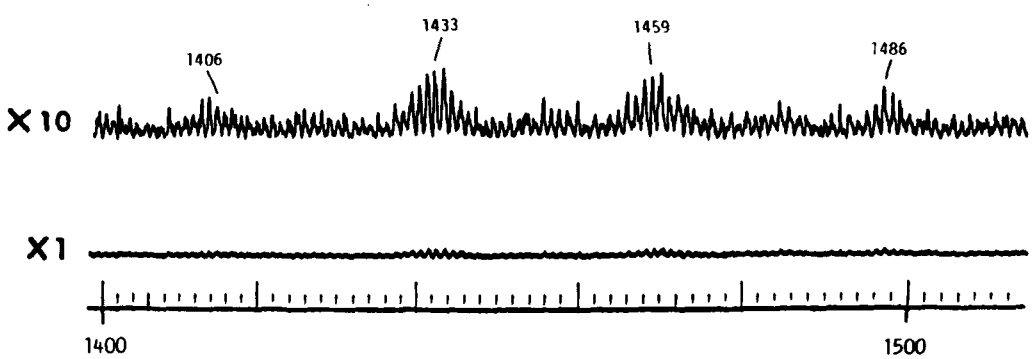


FIG. 5. High mass region of a fast atom bombardment spectrum of fraction from irradiated pork fat containing adduct compounds.

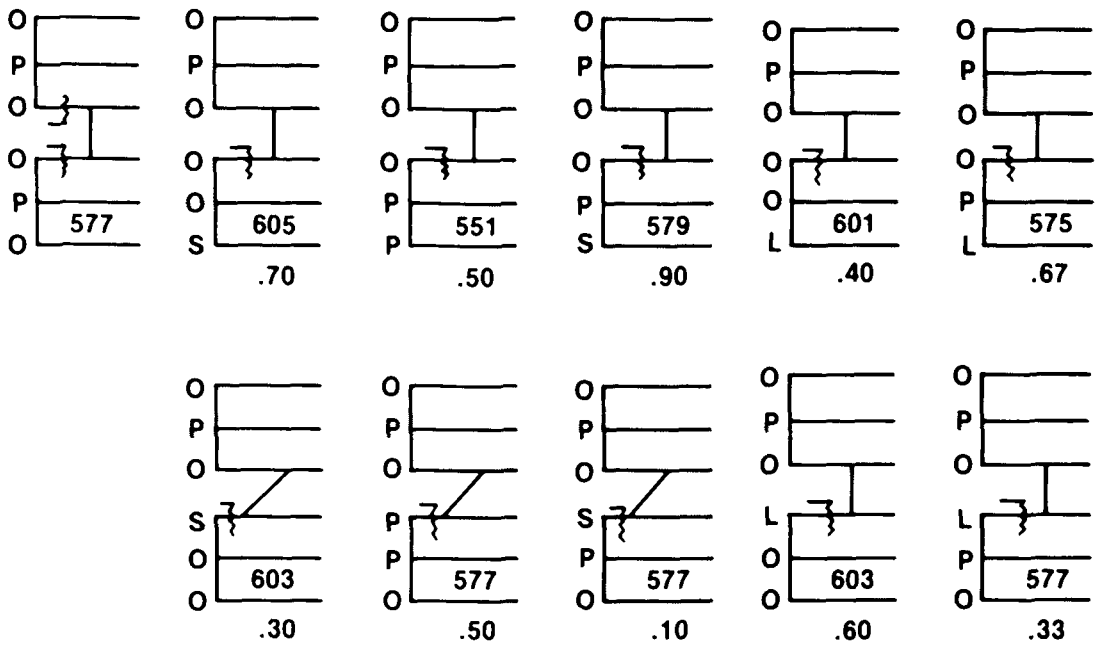


FIG. 6. Abbreviated schemes for dimer triglyceride adducts showing alternative configurations and resulting dioldiester ions. Decimals denote estimated fractions of the relative amount of each component.

metric data giving evidence of their occurrence are given in Tables III and IV.

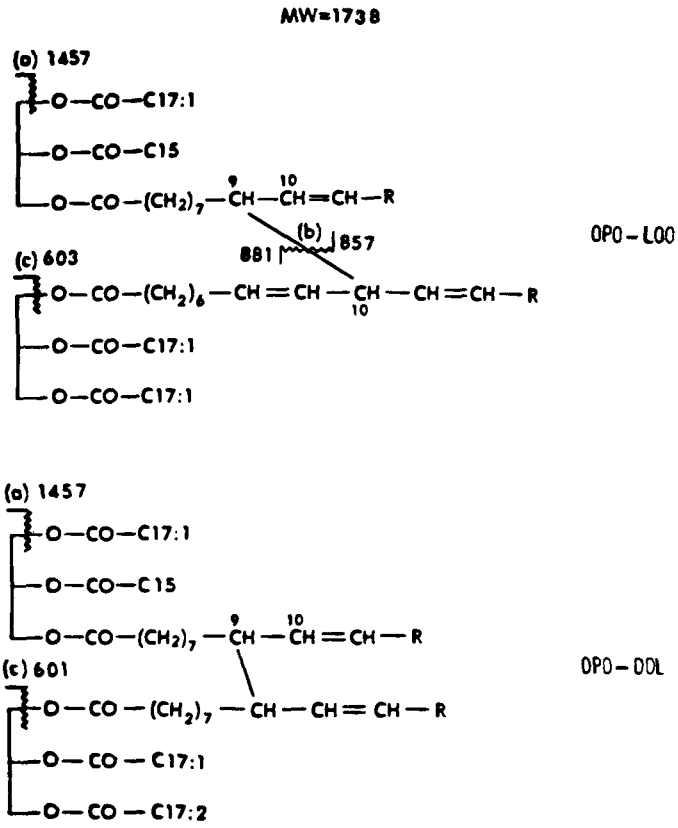
Triglyceride Adducts Having Oleate-Linoleate Bonding

There are two abundant triglycerides in pork fat containing linoleate, OOL and OPL. Although the mass spectrum of the radiation adduct fraction shows ions corresponding to oleate-oleate dimers of OPO and OOL, and OPO and OPL, the reactivity of the free radical site in linoleate at position 10 suggests that adducts having an OPO-LOO and an OPO-LPO configuration also are likely. Consideration of the principal mass spectrometric fragmentation ions for the respective structures (Scheme 2) shows that most of the ions of the two alternative structures are isometric. Only the ions corresponding to the dioldiester fragments, viz. oleate-oleate, m/e 603, vis a vis oleate-linoleate, m/e 601, are different. The propanediol 1-oleate, 2-linoleate, ion, m/e 601, is unique for an oleate-oleate adduct of OPO-OOL, but the propanediol 1,2 dioleate ion, m/e 603, corresponding to OPO-LOO, also may arise from an alternative configuration of an adduct of OPO and SOO in which an oleate-stearate linkage exists in lieu of the preferred oleate-oleate crosslink.

Consideration of the expected mass spectral fragments from OPO-OPL vis a vis OPO-LPO leads to similar conclusions. All the ions are isometric, i.e. for the alternative configurations, for an adduct of OPO and OPL (the M-O ion, m/z 1431, is unique for such an adduct) except as above for the dioldiester ions. Thus, the 1-linoleate, 2-palmitate dioldiester ion, m/z 575, is unique for an oleate-oleate coupled adduct, but the alternative 1-oleate, 2-palmitate dioldiester ion, m/z 577, is attributable as well to all the OPO adducts and an alternative configuration of an adduct of OPO and OPP (vide infra).

Triglyceride Adducts Having Crosslinks Between Oleate and Saturated Fatty Acid Moieties

Saturated fatty acid moieties, e.g. in tributyrin and tripalmitin (4), have been shown to form adducts. Although crosslinks between two saturated fatty acid moieties in pork fat triglycerides are quite unlikely (most of the palmitate, ca. 85-95%, is in position 2), an oleate-palmitate



SCHEME 2

or an oleate-stearate crosslink is possible. From the previous studies (4,5), crosslinking in a saturated fatty acid moiety is seen to occur at the carbon atom α to the carboxyl group. In a mixed adduct with an oleate, therefore, the crosslink would be expected between carbon 2 of the saturated moiety and carbons 8, 9, 10 or 11 of the oleate. An example of such a structure is seen in Scheme 3. The occurrence of this configuration cannot be confirmed, however, by any unique ion. The M-DG ion at m/z 1095 is too weak to be definitive. Likewise, a similar alternative configuration for

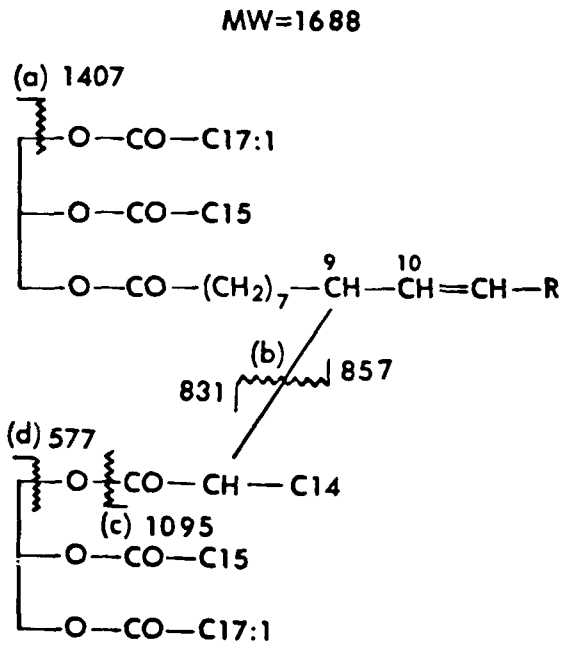
the dimer of OPO and OOS, viz. OPO-SOO, cannot be confirmed by the presence of any unique ions. The existence, however, of oleate and palmitate or stearate crosslinks may be strongly inferred by the quantitative relationships of the dioldiester ions.

The dioldiester ions are among the most abundant in the spectrum and can be measured, therefore, with reasonable accuracy. The data are summarized in Table IV. The origin of the several dioldiester ions from the various dimer adducts of OPO with the other triglycerides in their variegated configurations is depicted in Figure 6. Five of the six adducts are seen to occur in two possible configurations forming different dioldiester ions.

The relative abundance of the several dioldiester ions may be presumed to be proportional on a molar basis to the amounts of the triglyceride adducts from which they are derived (see Experimental). Each adduct may produce a dioldiester ion by a cleavage in either of the triglyceride moieties comprising the adduct. Since OPO is common to all the adducts and the chance of cleavage is random, the OP dioldiester ion has a probability of being twice as abundant as each of the other dioldiester ions. If an adduct exists in two forms, i.e. all except OPO-OPO, the sum of the respective dioldiester ions derived from each form must equal unity. On this basis the relative abundance of each dimer may be calculated from the relative abundance of the dioldiester ion. The results are given in Table IV and depicted in Figure 6.

There are no unique ions found for the dimer OPO-OPO (Table III), and all the postulated fragmentations lead to ions which are isometric with ions formed similarly in the other triglycerides. The presence of OPO-OPO is confirmed, however, by the amount of the PDDE ion.

The propanedioldiester ions are not, of course, unique to the triglyceride dimer adducts. The other adduct types described in the following sections, e.g. PDDE-TG adducts, Scheme 4, also yield the same PDDE ions, but their abundance in the spectrum is governed by the same probability that determines their abundances in the spectrum resulting from the dimer adducts. The relative abundances of the PDDE ions is, therefore, unchanged by the presence of adducts other than the dimer type.

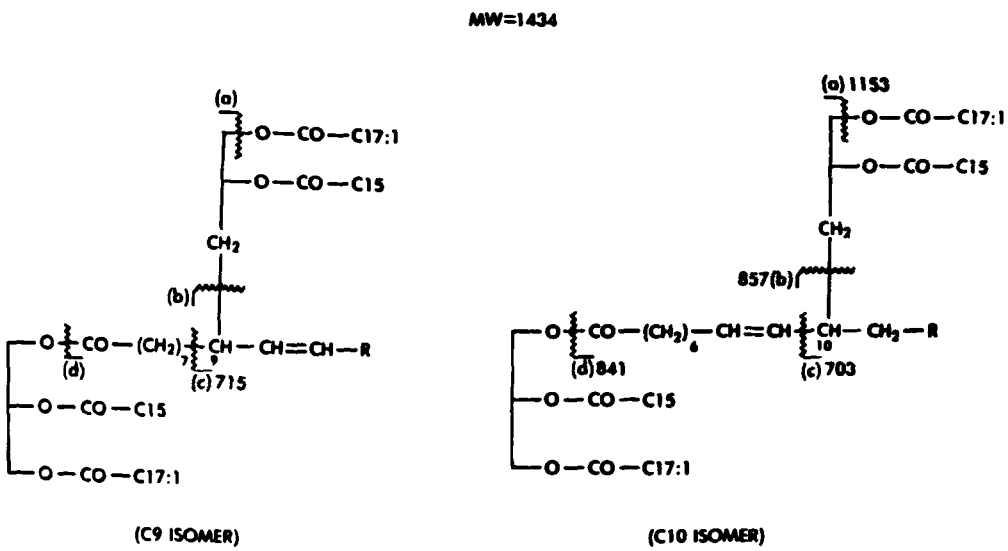


SCHEME 3

Adducts of the Propanedioldiester-Triglyceride Type

Prior studies (4) have shown that in addition to the radiation induced formation of dimer triglycerides, adducts of a propanedioldiester moiety to the triglyceride also are formed. Since one of the major reactions induced in a triglyceride by radiation is a cleavage resulting in an acyloxy and a propanedioldiester free radical, the recombination of the propanedioldiester radical with a triglyceride radical (formed in diverse ways as described in prior work [4,5]) is to be expected. In pork fat, owing to the preponderance of triglycerides containing oleate and palmitate, viz. OPO, OPP and OPS, the most abundant dioldiester moiety unquestionably is propanediol 1-oleate, 2-palmitate (OP). The most abundant of such adducts are, of course, those of OP with the six most abundant triglycerides. These are summarized in Table V together with the mass spectral data providing evidence of their occurrence.

Scheme 4 presents an example (OP-OPO) of the structure of a typical dioldiester-triglyceride adduct. The molecular ion, m/z 1434, is seen in the spectrum. The M-O ion (site a), however, is not seen. The most abundant ion arising from these structures is the M-DG ion (site d). This cleavage yields m/z 841 which is characteristic of the adduct type.



SCHEME 4

TABLE V

Major Fragmentations of Recombination Products of Type OP-TG[a]

Adduct combination	M	Fragmentation site (a) m/z M-O	Fragmentation site (b) m/z	Fragmentation site (c) m/z C9	Fragmentation site (c) m/z C10	Fragmentation site (d) m/z
OP-OPO	1434	1153[b]	857	715	703	841
OP-OPP	1408	1127[b]	831	715	703	841
OP-OOS	1462	1181[b]	885	715	703	841
OP-OPS	1436	1155[b]	859	715	703	841
OP-OOL	1458	1177[b]	881	715	703	841
OP-OPL	1432	1151	855	715	703	841

[a]See Scheme 4.
[b]Not seen in the spectrum.

TABLE VI

Major Fragmentations of Recombination Products of Type OP-PDDE

Adduct combination	M	Fragmentation site (a) M-O m/z	M-P m/z	M-S m/z	M-L m/z	Fragmentation site (b) m/z
OP-PO	1154[a]	873	899	–	–	591
OP-PP	1128	847[a]	873	–	–	565[a]
OP-SO	1182[a]	901[a]	927[a]	899	–	619
OP-PS	1156[a]	875[b]	901[a]	873	–	593
OP-OO	1180	897	925[a]	–		617
OP-PL	1152[a]	871	897	–	873	589
OP-OL	1178[a]	895[b]	923[b]	–	899	615

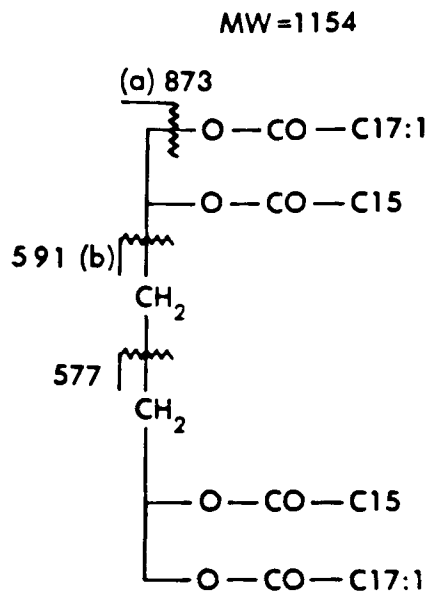
[a]Not observed.
[b]Weak peak.

The ions formed by cleavage at site b are not significant, but are ubiquitous to the spectra of nearly all the triglyceride adducts. In the adducts of type OP-OPO, addition of propanedioldiesters to triglyceride can occur equally at carbons 8, 9, 10 and 11 of the oleate chain. Scheme 4 shows only the addition of adducts at carbons 9 and 10. The diagnostic ions observed at sites a and d for both isomeric structures are the same. Two unique ions are observed for each structure at site c, m/z 715 (C_9 isomer) and m/z 703 (C_{10} isomer). The relative abundance of these ions in the spectrum (Fig. 4) indicates that both structures are present in equal amounts.

Similar ions are found in the spectrum indicating the occurrence of adducts of OP with other triglycerides, as seen in Table 5. The molecular ions are seen for all the adducts of OP with the triglycerides (Fig. 5). The M-O ion, however, is not seen for any of the adducts except for that of OP-OPL. Strong ions corresponding to cleavages at sites c and d giving rise to m/z 701 and 839 suggest that the adducts of OP with OOL and OPL may have a PDDE-linoleate bond.

Recombination Adducts of Type OP-PDDE

Adduct compounds of this type result from recombination of two propanedioldiester moieties. Since the OP propanedioldiester free radical is the most abundant of the type, one would expect to observe recombination of this free radical with itself and others yielding a variety of recombination adducts. Scheme 5 shows a representative mass spectral fragmentation pattern for the OP-PO recombination product. Most of the molecular ions are not seen. Some of the M-O (site a) peaks are seen, but unfortunately serve only to identify the type of adduct, since several of the ions, resulting from loss of acyloxy groups, are isometric. A typical fragmentation for $(PDDE)_2$ adducts,

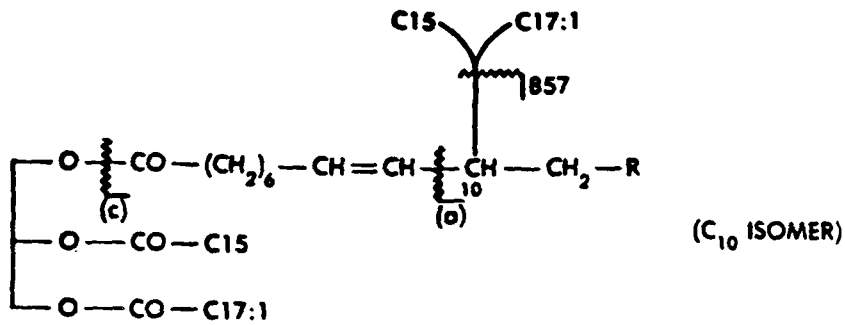


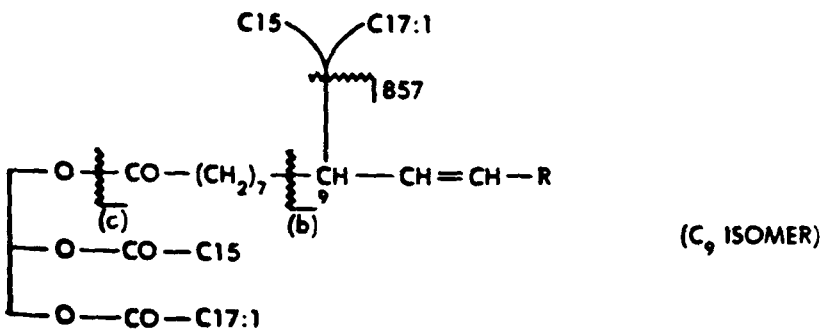
SCHEME 5

observed previously in irradiated tributyrin and tripalmitin (4), is a cleavage at site b. These ions are unique and occur in moderate abundance. The site b ions are sufficient to verify the occurrence of the adducts. Characteristic fragmentations arising from combinations of the various dioldiesters with OP are presented in Table VI.

Alkyl-Triglyceride Adducts of Type OPO-(C_{15}/$C_{17:1}$)

Addition of the two most abundant alkyl free radicals (C_{15} and $C_{17:1}$) to various triglycerides will result in a variety of recombination products. Addition of these radi-





SCHEME 6

TABLE VII

Fragmentation Ions Related to Alkyl Recombination Products

Adduct combination	Attachment of alkyl groups to oleic acid chain (Scheme 6)			
	Fragmentation site (a) Carbon 10 m/z		Fragmentation site (b) Carbon 9 m/z	
	C15	C17:1	C15	C17:1
OPO	337	363	349	375
OPP	337	363	349	375
OSO	337	363	349	375
OPS	337	363	349	375
OOL	337	363	349	375
OPL	337	363	349	375

cals to OPO is shown in Scheme 6. Attachment of either alkyl radical ($C_{15}H_{31}$ and $C_{17}H_{33}$) to carbon 8, 9, 10 and 11 of the oleate chain on the triglyceride molecule would give rise to a variety of isomeric adduct radiolysis products. Four of the possible compounds arising from these additions at carbon 9 and 10 are presented in Scheme 6 and discussed here. The mass spectral data are given in Table VII.

Little can be deduced from the pertinent ions observed in the spectrum. Ions corresponding to cleavage at sites a and b in Scheme 6 are observed in significant abundance, but since they are isometric for all the putative alkyl-triglyceride adducts they serve only to indicate the presence of the adduct type. Ions associated with alkyl radical attachment to other fatty acid side chains, e.g. palmitate, in the triglycerides, although in some cases observed in low abundance were too weak to be considered definitive.

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