Mass Spectrometry of Lipid Molecules[1]

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Abstract

The mechanisms of mass spectrometric fragmentation and their applications to the elucidation of structure of lipids related to fatty acids are reviewed. The mechanism of fragmentation of saturated fatty acids and the mass spectrometric determination of the position of the double bond in unsaturated esters are emphasized. The use of pyrolysis-mass spectrometry for locating double bonds is introduced. Patterns of fragmentation of wax esters and glycerides are also given.

Introduction

THE APPLICATION OF mass spectrometry to characterization of lipid molecules has made considerable progress in the last decade. The mass spectrometric fragmentation of many simple lipids, such as hydrocarbons, fatty acid esters, long-chain alcohols and fatty aldehydes, have been studied extensively mainly because they are sufficiently volatile and stable to be examined, and their simple molecular structure and clear pattern of fragmentation make the interpretation of their spectra an easy task. The knowledge obtained from the study of these simple molecules is useful in characterizing more complex lipids. However, difficulty is encountered with complex molecules of high polarity and molecular weight, such as phospholipids, which do not have sufficient volatility and thermal stability for proper examination.

Spectra of some chemical derivatives have yielded information about molecular structure, not otherwise obtainable from the spectra of the parent compounds. High resolution mass spectrometry facilitates the determination of the chemical composition of ions, even if two different ions have the same mass unit. Therefore it is highly useful for interpretation of spectra of lipid compounds.

Much work has been done on the exploration of the mechanism of the mass spectrometric fragmentation of lipid molecules. This is mainly done by substitution or isotope labeling at the site of interest in the molecule. By this technique many specific reactions of ionized lipid molecules have been studied, leading to the understanding of the pathways of fragmentation. This presentation will discuss primarily the mechanisms of fragmentation of methyl esters of fatty acids as an example, and as a basis of extrapolation to more complex lipids.

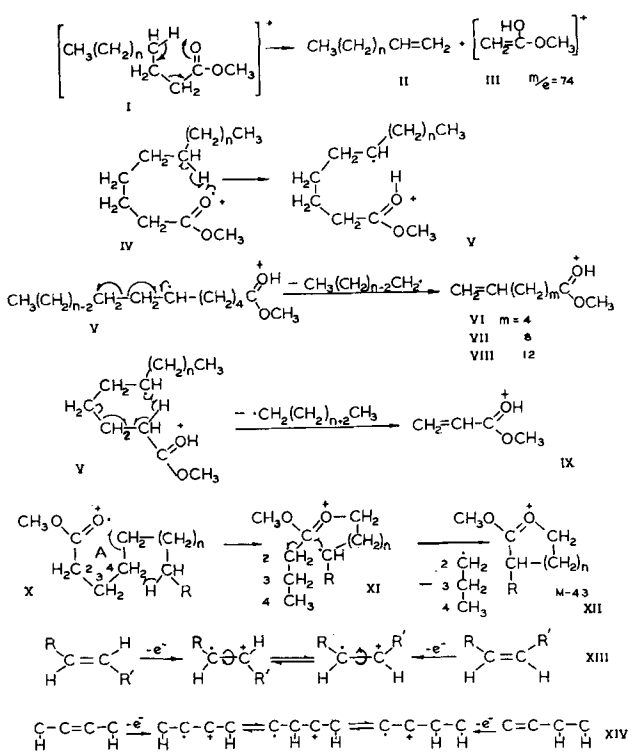
Pattern and Mechanism of Fragmentation of Esters of Saturated Fatty Acids

Esters of saturated fatty acids were among the first organic compounds whose mass spectra were studied in detail. The investigations of the pattern and mechanism of the mass spectrometric fragmentation of fatty acid esters mainly done by Stenhagen and Ryhage (1,2) provided considerable impetus to the application of this physical analytical method to other organic compounds.

Upon bombardment by high energy electrons (70 eV) methyl esters of saturated fatty acids with more than five carbon atoms fragment to give hydrocarbon ions and oxygen-containing ions—esters of lower homologous carboxylic acids fragment differently (3). The hydrocarbon ions result from the breakdown of the hydrocarbon chain of the molecule and are of little diagnostic interest. The oxygen-containing ions give prominent peaks in the spectrum. These include the molecular ion peak (M), the acylium ion formed by loss of a methoxy group (M-31), the peak m/e 74 which is characteristic of most methyl esters, and a series of peaks of ions corresponding to $(CH_2)_nCO_2CH_3^+$. The formation and structure of these ions will be discussed.

In the spectrum of methyl stearate, shown in Fig. 1, the molecular ion peak is rather intense. In comparison with the base peak (most intense peak of the spectrum) the intensity of the molecular peak tends to increase with increasing chain length of the long-chain esters (4,5). The loss of a methoxy group from the molecular ion forms the acylium ion (RCO^+) giving a peak at M-31. In the spectrum of trideuteriomethyl stearate (6) this peak shifts three mass units to M-34 indicating that the piece eliminated is the methoxy group containing three deuterium atoms in this case.

The base peak of the spectrum of methyl stearate (I) is m/e 74. This fragment results from a McLafferty rearrangement, which transfers a γ-hydrogen atom of the acid moiety to the carbonyl oxygen through a cyclic transition state, and cleaves the C2–C3 bond to give olefin II and ion III. The



migration of a γ-hydrogen during this rearrangement was confirmed by labeling with deuterium (7). The spectrum of methyl 4,4-dideuterioeicosanoate has a

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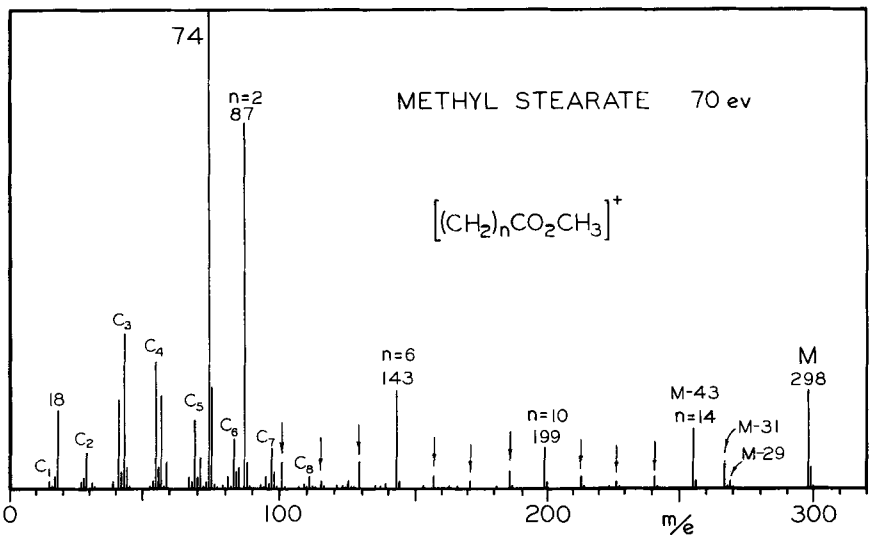
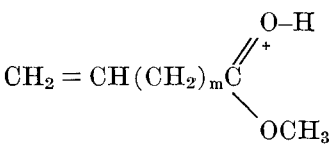


FIG. 1. Mass spectrum of methyl stearate, 70 ev. The principal hydrocarbon fragments are indicated by C_1 through C_8, and the series $(CH_2)_nCO_2CH_3^+$ is indicated by arrows or marked by n = 2, etc.

peak at m/e 75 instead of 74, indicating that a deuterium atom at position 4 (γ to the ester group) is transferred to ion III. In a study of the similar rearrangement in *n*-butyric acid-1-^{13}C, the isotope was retained quantitatively in the rearranged ion (8). This showed that the ion contains the carboxylic carbon atom. Moreover, the spectra of both trideuteriomethyl stearate (6) and methyl 2,2-dideuterioheptadecanoate (7) showed retention of the isotope in the rearranged fragment (III), proving that the ester methyl group and the carbon atom at the 2-position are parts of this ion.

Between the peak m/e 74 and the molecular ion peak is a series of peaks corresponding to the homologous ions $(CH_2)_nCO_2CH_3^+$. These peaks show a peculiar periodicity in their intensities (4). Fragments of n = 2, 6, 10, 14 (m/e 87, 143, 199 and 255, respectively) are more intense than their neighboring homologous peaks (shown by the arrows), and the peaks m/e 87 and 143 are especially intense. The peak m/e 87 is found to undergo hydrogen exchange with the methylene groups at positions 5 or 6 (7), and the intensity of this peak decreases in comparison to that of m/e 143 when the ester is ionized by low energy electrons (13 eV) and in a cold ion source (50 C). Spiteller et al. (9) therefore suggested that the $(CH_2)_nCO_2CH_3^+$ ions of n = 2, 6, 10 etc. have the structure



They postulated that these ions are formed from a common intermediate V, which results from a transfer of a hydrogen atom from position 6 to the ionized carbonyl oxygen of the molecular ion (IV). An intermediate similar to V, but with the hydrogen transferred from position 5 is likely. A homolytic cleavage of the bond between carbons 7 and 8 of the radical ion V gives the m/e 143 fragment (VI). A further migration of a hydrogen atom from positions 2 to 6 in V, and scission of the bond between carbons 3 and 4 forms the fragment m/e 87 (IX). If hydrogen atoms shift through a similar cyclic transition state from carbon 10 to 6 instead of from 2 to 6, followed by a cleavage of the bond between carbons 11 and 12, the resulting fragment will be the ion m/e 199 (VII). Further rearrangements and bond ruptures occurring at positions farther from the ester group give the fragment m/e 255 (VIII) and higher homologous ions.

Among the ions mentioned above, only VI is formed by a single hydrogen shift and bond cleavage. Two or more hydrogen shifts from the same intermediate V are needed for the formation of the other homologous members. Therefore, the energy required for the formation of VI is less than those for VII, VIII and IX. This explains the abundance of m/e 143 (VI) in the spectrum, and also the decrease in intensity of m/e 87 when the ionization energy decreases. According to this mechanism, a fatty acid methyl ester with deuterium labeling in the position 6 should give an ion of m/e 88 instead of 87. In our spectrum of methyl 6,6-dideuterioheptadecanoate (86% isotope purity) shown in Fig. 2, both m/e 88 and 87 are intense peaks. The persistance of the peak m/e 87 means that the proposed intermediate V cannot be the only precursor for the formation of this ion, a considerable amount of hydrogen must be rearranged from 5 or other positions. Moreover, a triplet of m/e 199, 200 and 201 was found in the spectrum rather than the expected single peak at m/e 201 corresponding to the peak m/e 199 of the nondeuterated ester.

The peak at M-43 in the spectra of fatty esters results from a loss of a propyl radical from the molecular ion to form a fragment of the type $(CH_2)_nCO_2CH_3^+$. That the propyl group eliminated was the three methylene groups at positions 2, 3 and 4 plus a hydrogen atom from the rest of the molecule (7), was proven by ^{13}C (2) or deuterium (7) labeling at 2, 3 or 4 positions, or by methyl (7), chlorine or bromine (2) substitutions at the 2-position of the ester molecule. In all cases, the isotopes or substituents were lost together with the C_3-fragments. Budzikiewicz et al. (10) have proposed a mechanism for this expulsion reaction which involves a migration of this C_3-fragment to the carbonyl hydrogen, followed by a bond cleavage. (X, XI and XII) The loss of an ethyl group from the molecular ion (M-29) can be formulated by a similar mechanism, in which ring A in the transition state is a 5-membered ring

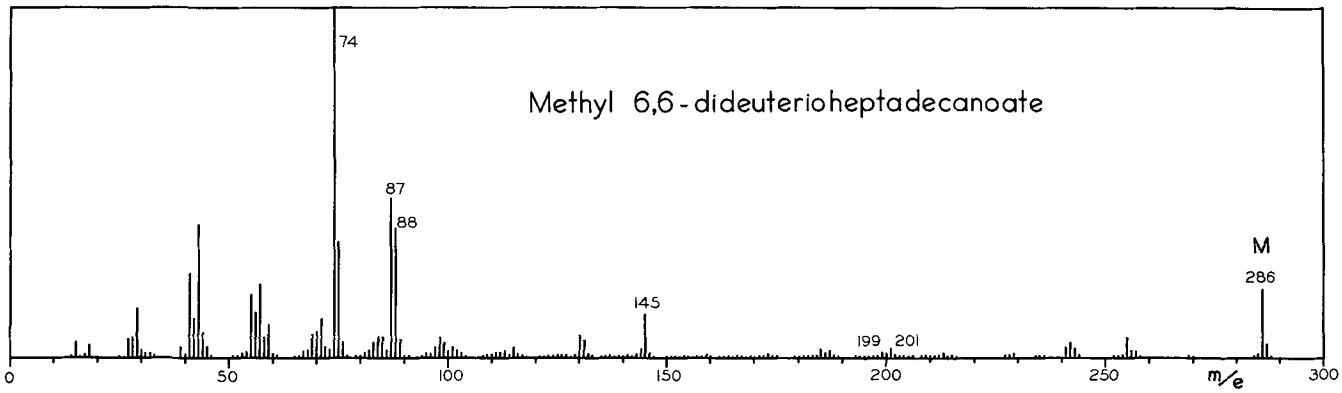


FIG. 2. Mass spectrum of methyl 6,6-dideuterioheptadecanoate.

rather than the 6-membered one in the above scheme. An expulsion of a fragment bigger than a propyl group can also be formulated through a larger transition ring A.

According to the above discussions, hydrogen migration and skeletal rearrangement occur to a certain extent in a long-chain fatty ester molecule during mass spectrometric fragmentation. The ions of structure $(CH_2)_nCO_2CH_3^+$ can be formed either by simple cleavage of a carbon carbon bond of the ester molecule, or by expulsion of a part of the chain plus a hydrogen atom, or by cleavage after single or multiple hydrogen shifts occurring in the molecular ion. Therefore, the hydrogen and carbon atoms in the resulting fragments may not be in the same positions in which they were in the original ester molecule. Which mechanism dominates, and to what extent a mechanism is responsible for the formation of a $(CH_2)_nCO_2CH_3^+$ ion depends on which ion is under consideration. For example, in the spectrum of methyl 18,18,18-trideuteriostearate (Fig. 3) (7) the ion of M-43 (m/e 258) was found to be formed exclusively through the expulsion mechanism. Members of the same series of ions in which $n > 6$ are found to partly consist of ions containing the three deuterium atoms, indicating that a part of them is formed by the expulsion mechanism. As another example of this complication, the spectrum of methyl 11-methyl-nonadecanoate (11) is shown in Fig. 4. In this spectrum, the relatively intense peaks m/e 185 ($C_9H_{18}CO_2CH_3^+$) and 213 ($C_{11}H_{22}CO_2CH_3^+$) resulted by a preferential cleavage of the carbon carbon bonds at each side of the branched carbon, indicating the branch is at carbon 11. If neither skeletal rearrangement nor hydrogen migration had occurred, no peak corresponding to $C_{10}H_{20}CO_2CH_3^+$ should exist, but a small peak of m/e 199 in the spectrum indicates some rearrangement did take place.

Mass Spectrometry of Unsaturated Fatty Acid Esters and Their Derivatives

Though the fragmentation of saturated fatty esters yields ions of high diagnostic value, the spectra of unsaturated esters are complicated, and very little information about the molecular structure can be deduced from their many prominent peaks. Isomeric unsaturated esters have similar spectra (12) except for the α,β-unsaturated esters (12,13). The spectra of the methyl esters of oleic, elaidic and petroselinic

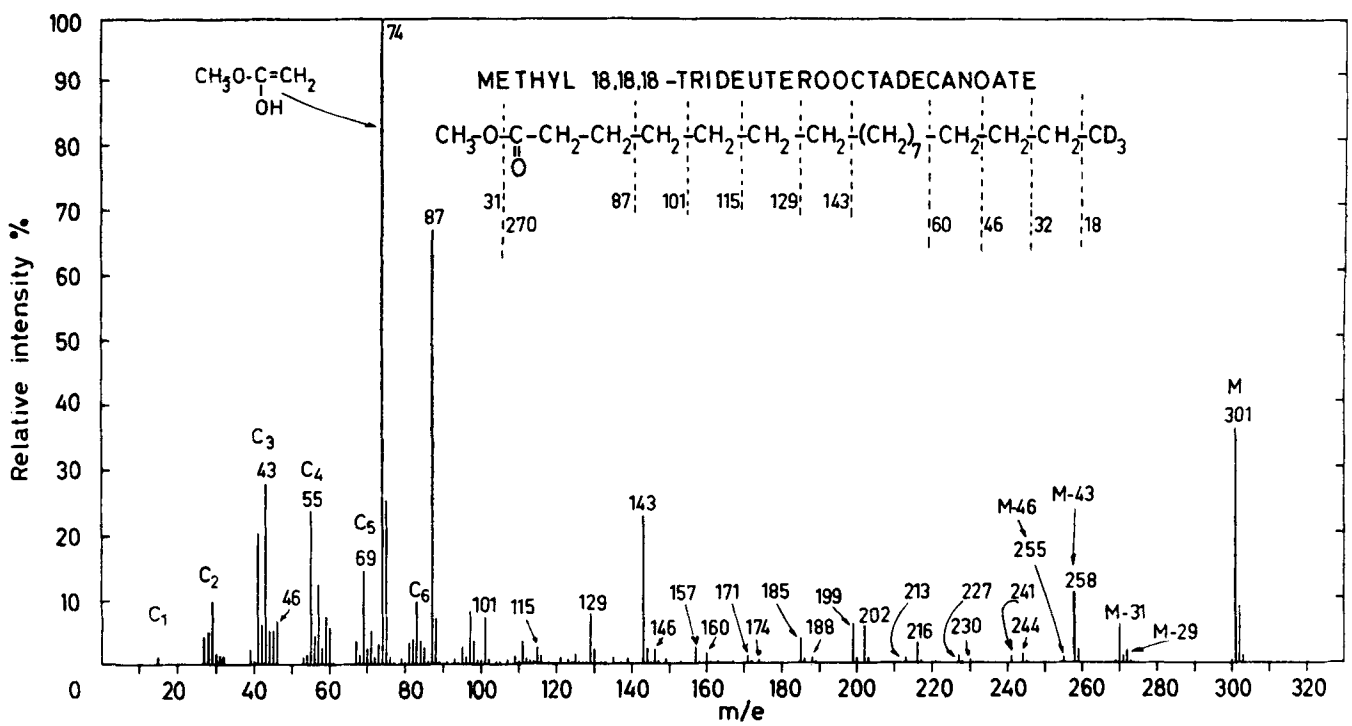
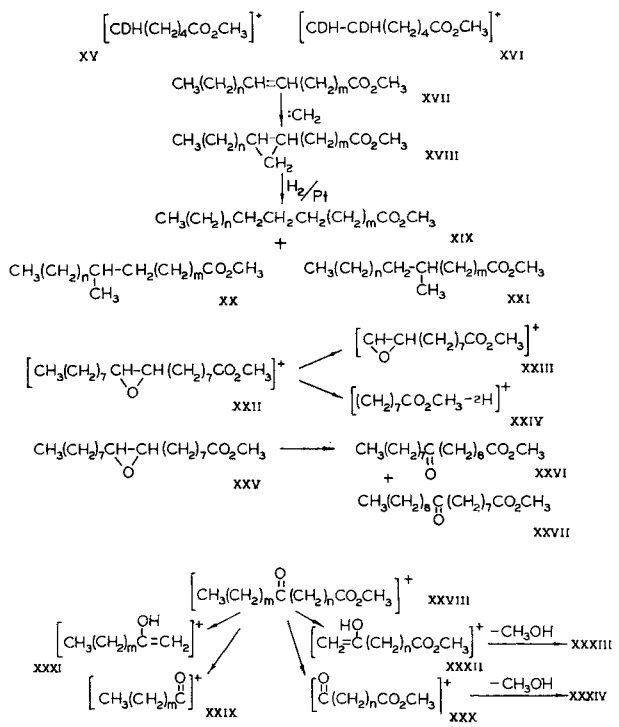


FIG. 3. Mass spectrum of methyl 18,18,18-trideuteriostearate (7).

acids are almost identical, indicating that neither positional nor geometric isomerism have significant effects upon the fragmentation. The lack of geometric difference between *cis*- and *trans*-isomers may be caused by the ionization of the carbon carbon double bond which becomes a single bonded radical-ion after losing one electron from the π-bond. Rotation about the bond is possible, and the two ions from the geometric isomers become equal as is shown in scheme XIII. The double bond in a long-chain unsaturated hydrocarbon or ester is also believed to be mobile upon electron impact; and therefore the mass spectrum cannot reveal the original site of unsaturation. Biemann (14) proposed a sequence of successive shifts of hydride ions and hydrogen atoms in the olefinic chain to explain the delocalization of the ionized double bond as shown in scheme XIV.

Efforts have been made to mark the position of the carbon carbon double bond in an olefinic ester by converting it into a substituted saturated ester. An early approach to this problem was to saturate the double bond with detuerium by reacting the ester with deuteriohydrazine and oxygen (15). The mass spectrum of the saturated ester then gives deuterium-containing ions derived from $(CH_2)_nCO_2CH_3^+$, if the carbon atom which bears the isotope is involved in the fragment. For instance, in the spectrum of methyl 6,7-dideuteriooctadecanoate, which is obtained from methyl petroselinate, the peak of m/e 129 is shifted to 130 (XV) and the 143 to 145 (XVI), indicating that one deuterium is in position 6, and the second one is in position 7 of the compound. Thus, the original double bond was between carbons 6 and 7 in the ester. The practical application of this method is limited, because of the complication caused by



hydrogen and skeletal rearrangements discussed above. Moreover, some $(CH_2)_nCO_2CH_3^+$ peaks in the spectrum are too feeble to be identified, especially when the isotope content of the ester is low.

Addition of a carbene across the carbon carbon double bond of an unsaturated ester forms a cyclopropane derivative of the ester, but the position of this 3-membered ring in the molecule is also impossible to locate by direct mass spectrometry (16,17). Because the carbon carbon linkages in a cyclopropane ring are bent bonds, which are weaker than the other normal carbon carbon δ—bonds in the rest of the chain, the ring opens readily upon electron impact giving a spectrum which is similar to that from an unsaturated ester. Therefore, the position of the ring in the original molecule cannot be deduced from the spectrum, but it can be located by converting the compound into a branched chain ester (18). After catalytic hydrogenation one of the carbon carbon bonds in the cyclopropane ring can be broken to give a mixture of a normal chain (XIX) and two branched chain isomers (XX,XXI), depending upon which one of the three bonds of the ring is cleaved. The positions of the branches in the two isomeric esters can be determined by mass spectrometry (11), and thus the location of the ring in the original ester molecule is deduced. The combination of converting an unsaturated ester to a cyclopropane derivative, which is further converted to isomeric branched esters, offers a way to determine the position of a double bond in an unsaturated fatty ester.

Another method to locate a carbon carbon double bond is to oxidize it to an epoxide (19), which upon fragmentation, forms characteristic ions (XXIII, XXIV) corresponding to cleavages at either side of the ring if it is in the central portion of the hydrocarbon chain. Isomerization of the epoxide ring of XXV to a keto compound by sodium iodide gives a mixture of two isomeric keto esters (XXVI, XXVII) (20). The spectrum of each keto ester (XXVIII) shows six prominent peaks which indicate the position of the keto group. Two result from the cleavage of the bonds at each side of the keto group (ions XXIX and XXX), and two are formed by McLafferty rearrangement occurring at each side of the keto group (ions XXXI and XXXII). The remaining two correspond to the loss of methanol from the ions XXX and XXXII to yield the secondary fragment ions XXXIV and XXXIII, respectively. The hydrogen atom which leaves fragment XXX together with the methoxy group, is one of the labile hydrogen atoms either α to the keto group or α to the carboxyl carbonyl group, and the hydrogen which is lost in the same way from fragment XXXII is the labile enol hydrogen which was originally at the position γ to the keto group (21). The position of the keto group in each of the two isomeric keto esters can be located by the respective sets of characteristic peaks, and each peak from one set should have 14 mass units difference from the corresponding peak of the other set.

Andier and co-workers reported that the position of the carbon carbon double bond in a long-chain unsaturated compound can be located by mass spectrometry of the corresponding dimethyl amino alcohol derivative (22). The double bond is first oxidized to epoxide (XXXVI), and further treatment with dimethylamine gives a mixture of two isomeric dimethylamino alcohols, XXXVII and XXXVIII. In the fragmentation of these, cleavage occurs at the bond between the two carbon atoms bearing the amino and hydroxyl groups, and the spectrum of the mixture shows two intense peaks, corresponding to the fragments containing the amino group from

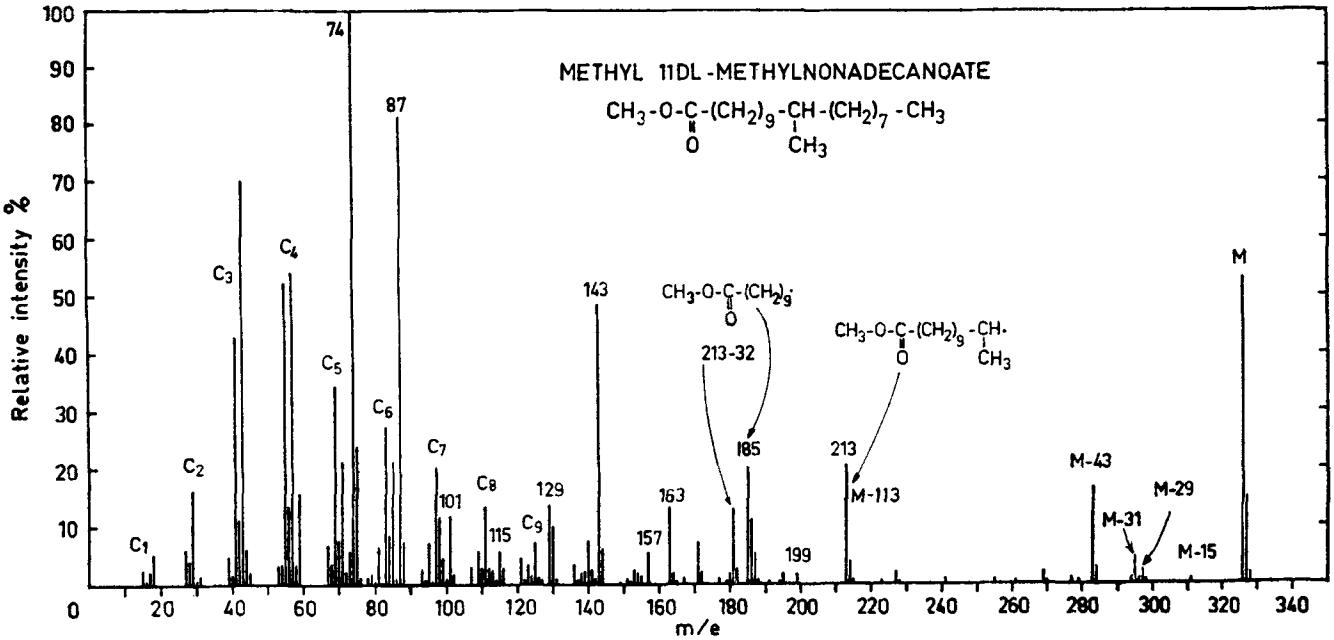
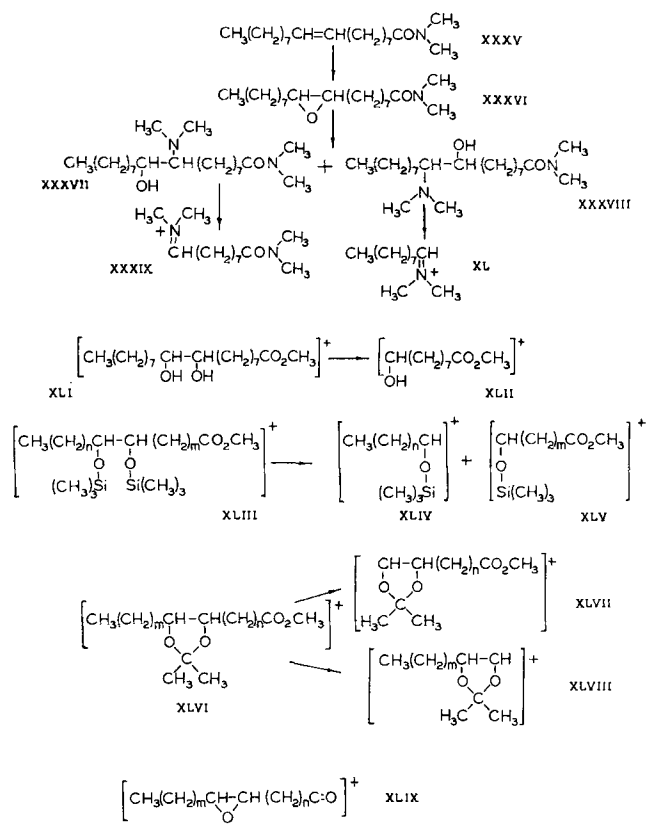


FIG. 4. Mass spectrum of methyl 11-DL-methylnonadecanoate (11).

each of the isomers (XXXIX and XL). The dimethyl amide of oleic acid (XXXV) is given as example.



Hydrolysis of the epoxide ester (XXV) yields a *vic*-dihydroxy ester (XLI), the mass spectrum of which reveals a predominant cleavage of the carbon carbon bond between the two hydroxyl groups (19). Thus, the spectrum of methyl 9,10-dihydroxy stearate has an intense peak at m/e 187 corresponding to ion XLII. This fragment also tends to lose methanol to form another prominent peak at m/e 155. The hydrogen atom which is expelled together with the methoxy group is, to a large extent, the labile hydrogen from the hydroxyl group (21). The di-trimethylsilyl (TMS) derivative of a *vic*-dihydroxy ester has a simple spectrum at 20 eV (23). The two most intense peaks correspond to cleavage between the two carbons bearing the two TMS groups (XLIV, XLV). Di-trimethylsilyl derivatives have the advantage of high volatility making separation by gas chromatography possible.

The condensation of the two hydroxyl groups of a *vic*-dihydroxy ester with one molecule of acetone forms a 1,3-dioxolane derivative XLVI. The mass spectrum of this derivative (24) has two peaks (ions XLVII and XLVIII) which correspond to cleavages of the bonds at either side of the ring. The intensity of the peak corresponding to fragment XLVII decreases with increasing chain-length of the ester. The intensities of the peaks at M-89 (XLIX) in the spectra of the dioxolane derivatives can be used to distinguish between two geometric isomers (24). In the spectrum of the *erythro* isomer, which is derived from a *cis*-unsaturated ester, the peak M-89 is at least twice as intense as that in the spectrum of the *threo* isomer derived from the *trans*-unsaturated ester. This ion (XLIX) results from a loss of one mole of acetone plus a methoxy group from the molecular ion, and it has the same structure as the ion M-31 of the epoxide derivative.

All the derivatives of an unsaturated ester mentioned above show characteristic peaks in the mass spectrum from which the original position of the double bond can be deduced. The spectra of dioxolane derivatives also give information about the geometric configuration of the double bond. However, the application of these methods is restricted to monounsaturated fatty acids. Moreover, these derivatives of α,β- or terminally unsaturated esters usually have fragmentation patterns different from those discussed above, restricting the application to esters with centrally located double bonds.

The recently reported spectra (25) of methyl 9,10,12,13,15,16-hexamethoxyoctadecanoate and methyl 6,7,9,10,12,13-hexamethoxyoctadecanoate derived from α- or γ-linolenic acids, respectively, showed peaks corresponding to the two fragment ions formed by cleavage between carbons of each *vic*-disubstitution.

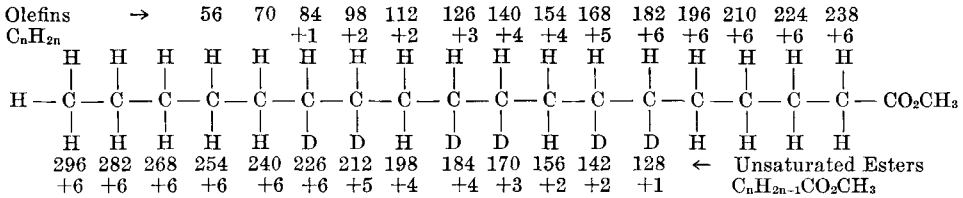


FIG. 5. Pyrolysis of methyl 6,7,9,10,12,13-hexadeuteriostearate.

Each of these primary ions gives a series of peaks due to subsequent losses of methanol, because each of them contains one or more methoxyl groups. These large numbers of primary and secondary fragment-peaks plus peaks resulting from other cleavages make the spectrum rather complicated. The overlapping of some of these peaks makes the location of the positions of the substitutions, and thus the sites of unsaturation in the original acids, even more difficult.

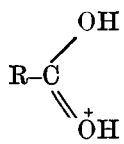
A method combining pyrolysis, gas-liquid chromatography and mass spectrometry was found to be satisfactory for deducing the positions of carbon carbon double bonds in a polyunsaturated ester with unsaturated sites in any position of the hydrocarbon moiety (26). The methyl ester of an unsaturated acid is specifically reduced by deuterated hydrazine and oxygen to the corresponding saturated deuterio ester. This compound is then subjected to pyrolysis in a flow system at 600 C, the pyrolytic products thus obtained are separated by gas-liquid chromatography, and these are identified by mass spectrometry. The principle products are a homologous series of unsaturated esters having shorter chain length than the original ester, plus a homologous series of olefins. The absence or presence of deuterium in a fragment may be used to positively locate deuterium in the ester, because, under the conditions of pyrolysis, expulsion, rearrangement and secondary reactions are minimized. By examining the chain lengths and deuterium contents of both series of fragments, the positions of the deuterium atoms can be determined, and thus the sites of unsaturation in the original unsaturated ester. Figure 5 shows the result of the pyrolysis of methyl 6,7,9,10,12,13-hexadeuteriooctadecanoate which is derived from methyl γ-linolenate.

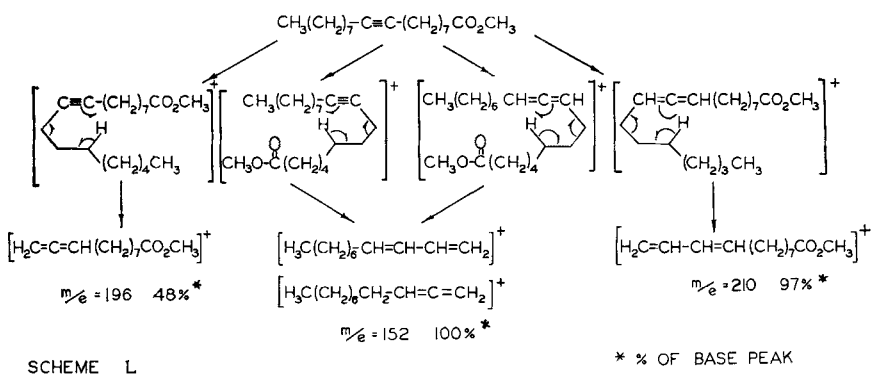
In the spectra of fatty acid esters containing triple bonds, most of the prominent peaks are found to result from cyclic eliminations with hydrogen transfer (27). These rearrangements occur at the position of the triple bond or at an allene linkage which results from the primary isomerization of the triple bond. The fragmentation of methyl stearoleate is given as a typical example in scheme L. This cyclic elimination mechanism has been proven by deuterium labeling studies.

Acetylenic fatty esters with the triple bond close to the ω-terminal or to the ester group show preferential fragmentations through one or two of the four mentioned pathways. Therefore, esters with unsaturation at 2, 3, 4 or terminal positions have spectra different from those having the triple bond at the central positions of the molecule. Similar results have been obtained also from the spectra of a series of isomeric methyl nonynoates (28). In addition to the ions resulting from these four pathways, ions formed by secondary fragmentation of these, or by other types of fragmentation, or by the shift of the triple bond to other positions followed by a cyclic elimination, are all abundant. This complication of the spectra of these compounds causes confusion in the determination of their structures by direct mass spectrometry. However, the position of the triple bond in the ester molecule can be located after deuteration by the method of pyrolysis mentioned above, or by any of the other methods applicable to olefinic esters after a partial reduction of the triple bond to a double bond (29).

Fragmentation of Esters of Fatty Acids and Long-Chain Alcohols

Although the mass spectrum of the ethyl ester of a fatty acid has a fragmentation pattern similar to that of the methyl ester, fatty acid esters of longer chain alcohols fragment quite differently (30). (Fig. 6) Abundant ions arise by simple bond cleavage, or by rearrangements at the ester linkage. For an ester RCO_2R', in which R and R′ are hydrocarbon chains of the acid and alcohol moieties, respectively, the major ions are $RCO_2H_2^+$, RCO^+, $R'-1^+$ and $R'OCO^+$. From these ions the chain lengths of both R and R′ can be determined. If both R and R′ are saturated groups, the peak corresponding to $RCO_2H_2^+$ is very intense, and is often the base peak of the spectrum. In the study of the mass spectrometry of acetates of long-chain alcohols, McLafferty and Hamming (31) formulated ions of this type as





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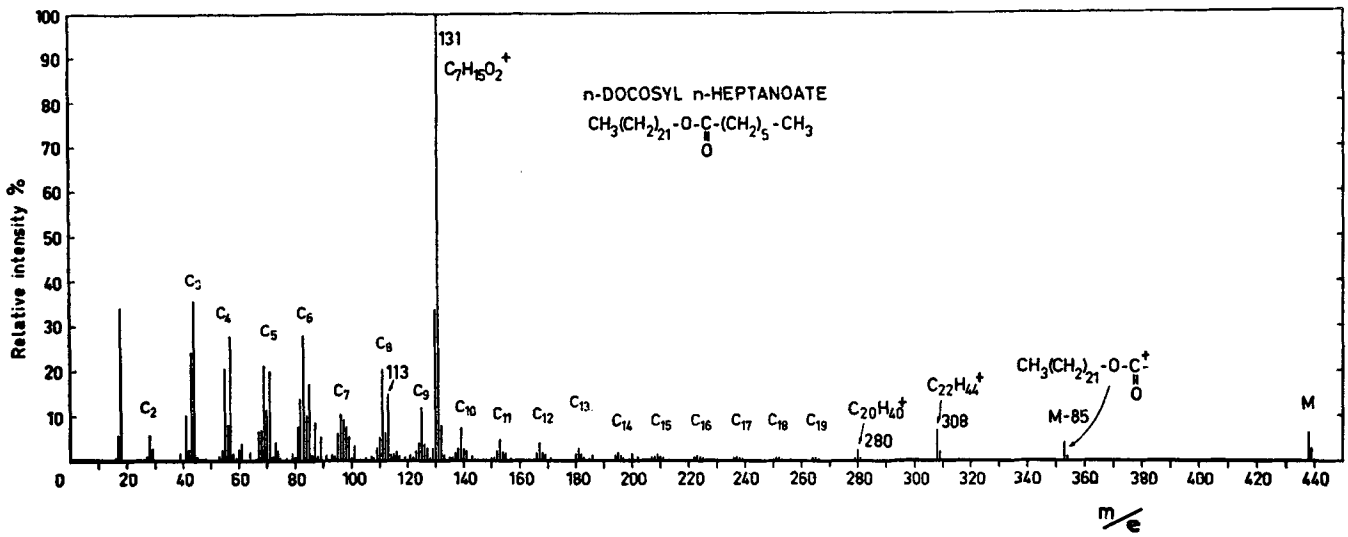

FIG. 6. Mass spectrum of n-docosyl n-heptanoate (28).

and found that one hydrogen is rearranged from the γ-position of the alcohol moiety to the carbonyl oxygen. The second hydrogen of the ion comes mainly from the shift of a hydrogen atom from the γ-position of the alcohol to the alkoxy oxygen, but some δ-hydrogen may also be involved.

Determination of Structure of Glycerides

The two positional isomers of a monoglyceride have almost identical spectra. This is likewise true for the spectra of the two diacetyl derivatives. Therefore, it is impossible to distinguish between 1- or 2-monoglycerides by direct mass spectrometry or via the spectra of the corresponding acetates. Substitution of the two hydroxyl hydrogens in each of the isomeric monoglycerides by two trimethyl silyl groups yields two isomeric di-TMS derivatives. The spectra of these two derivatives were found to differ greatly in the intensities of peaks corresponding to fragments containing TMS groups (32). Thus, the di-TMS derivatives of 1- and 2-monoglycerides are distinguishable by mass spectrometry, and this may become a basis for analysis of these isomers.

A full analysis of the structure of mixed triglycerides by mass spectrometry was reported by Barber et al. (33). The fatty acid moieties can be identified by the most intense peaks in the spectra, which correspond to the elimination of the acyloxy groups from the molecular ion $[M-R^{1,2,3}\ CO_2]^+$ and to the acylium ion peaks $[R^{1,2,3}\ CO]^+$. (Fig. 7) In the case of a triglyceride having three different acid moieties, each of these types of ions gives three peaks in the spectrum. Another set of three peaks of less intensity corresponds to $[M-R^{1,2,3}\ CO_2CH_2]^+$. The ion peak corresponding to the acid moiety at the 2-position of the glycerol $[M-R^2\ CO_2CH_2]^+$ usually is significantly smaller than those from the acid moieties in the 1- and 3-positions. For example, in Fig. 8 the peak $[M-C_{17}H_{35}CO_2CH_2]^+$ (m/e 509) is only one third the size of the other two peaks of the same type which resulted from the corresponding eliminations at positions 1 and 3 (m/e 565 and 537). From these differences in intensities of characteristic peaks, the fatty acid in the 2-position of the glycerol can be identified.

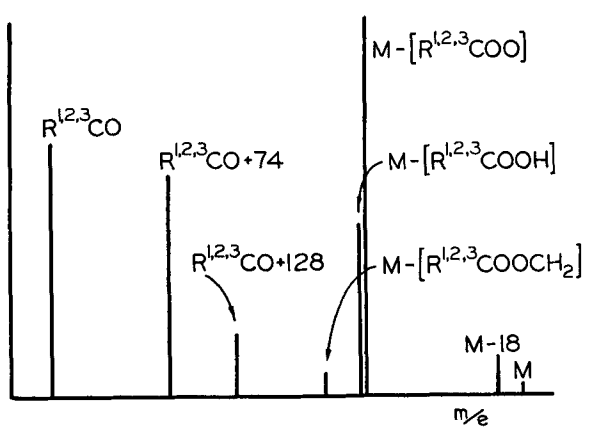

FIG. 7. Cracking pattern of a triglyceride (31).

Two more sets of peaks corresponding to $[R^{1,2,3}\ CO + 74]^+$ and $[R^{1,2,3}\ CO + 128]^+$ are always observed in the spectra, and a peak M-18 is also characteristic of triglycerides. Mass spectra of 2-lauro-1,3-didecoin (34) and a complex triglyceride isolated from the oil of *Sapium sebiferum* (35) are also in agreement with the results discussed above, except that the molecular peak and the M-18 peak in the spectrum of the former were not detectable. This was probably caused by introduction of the sample into the mass spectrometer through a liquid sample system instead of through a direct inlet as was the case of 1-myristo-2-stearo-3-palmitin (33).

Miscellaneous Lipids

Mass spectrometric fragmentation of other lipid related compounds such as aliphatic hydrocarbons (36), long-chain aliphatic alcohols (37), fatty aldehydes (38) and steroids (39) have been studied also. The patterns of fragmentation of model compounds have been used widely and successfully in characterizing unknown lipid compounds. For example, the structures of many branched chain fatty esters from feather waxes (40), from bacterial sources (41), or from dairy products (42,43) have been determined by the aid of mass spectrometry. Complex lipids are usually converted to their simple components, which are then examined by mass spectrometry. However, the characterization of the major components of complex molecules may not indicate the structure of the natural compound. This is an objective to which mass spectrometry has yet to be applied. The combination of gas-liquid chromatography and mass spectrometry

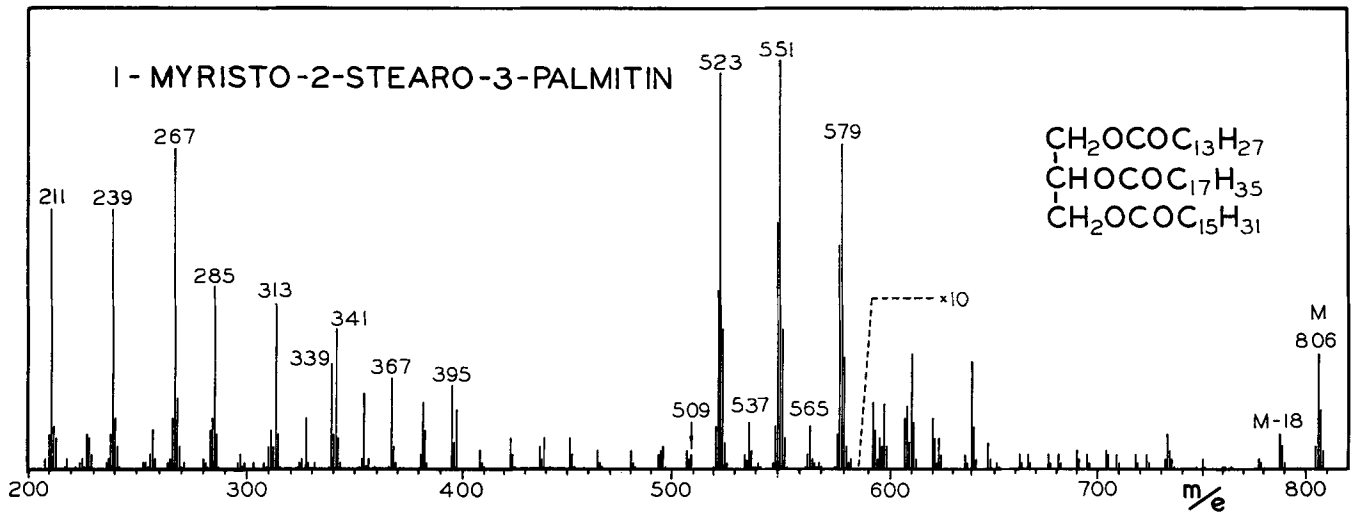

FIG. 8. Mass spectrum of 1-myristo-2-stearo-3-palmitin (31).

is even a more powerful tool for the analysis of natural lipid mixtures than each is alone, for mass spectrometry cannot at this stage be used for analysis of complex mixtures of lipids, but after GLC separation, even minor components can be identified.

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