Mass Spectrometry of Lipids. I. Cyclopropane Fatty Acid Esters[1]

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ABSTRACT

A method was developed for the almost quantitative conversion of unsaturated esters (from monoenes to tetraenes) to cyclopropanes using diiodomethane and a highly active zinc-copper couple. These derivatives are sufficiently volatile for GLC analysis and *cis* and *trans* isomers can be distinguished by this technique. Equivalent chain lengths of the cyclopropane derivatives were measured on polar and nonpolar phases. The mass spectra of the monocyclopropane compounds are very similar to those of the parent unsaturated esters. Those of dicyclopropanes, however, are quite distinctive so that the original structure of the ester can be deduced. Polycyclopropanes give complex spectra which are difficult to interpret in terms of the position of the original double bonds.

INTRODUCTION

ISOMERIC UNSATURATED fatty acid esters, differing in the position of the olefinic center, have identical mass spectra because of the mobility of double bonds under electron impact (1). One of the principal unsolved problems of mass spectrometry as applied to lipid chemistry, therefore, is to find some derivative of a double bond which has a unique mass spectrometric fragmentation pattern to allow the original structure of the ester to be determined. Ideally, this derivative should be sufficiently volatile to be readily eluted from a GLC column, even when prepared from a polyene. The method of preparation should be simple and quantitative so that it can be applied either to small amounts of purified esters or preferably to mixed methyl esters prepared from tissue lipids to allow a GLC mass spectrometer combination to be used. Of the existing methods, deuterohydrazine reduction (2) can only be applied to pure monoenoic esters giving mass spectra which are difficult to interpret. Oxidation to an epoxide, followed by rearrangement to a pair of ketones (3), probably is not quantitative and gives a mixed product too polar for ordinary GLC analysis if more than one double bond is present in the original acid.

The simplest nonpolar symmetrical compound which can be prepared from an olefin is a cyclopropane, so it was decided to investigate methods of preparing such derivatives in the hope that they might have distinctive mass spectra. The reaction of an olefin with diiodomethane and a zinc-copper couple has become a general method for the synthesis of stereochemically pure cyclopropanes since it was first described by Simmons and Smith (4). The reaction is a bimolecular process involving the formation of a stable organozinc intermediate which then transfers a methylene group to a double bond (5,6).

$$2\ CH_2I_2 + 2Zn \longrightarrow (I\ CH_2)_2\ Zn \cdot ZnI_2$$

$$2\ -CH{=}CH- + (I\ CH_2)_2\ Zn \cdot ZnI_2 \longrightarrow$$

$$2\ -\underset{\diagup}{CH}\ \ \underset{\diagdown}{CH}- + 2ZnI_2$$

The yields, however, were not particularly good until LeGoff (7) described the preparation of a much more active zinc-copper couple. A slight modification of LeGoff's method enabled us to achieve quantitative conversion of monoenoic through tetraenoic unsaturated esters to cyclopropanes. A series of cyclopropane esters were prepared and their gas chromatographic and mass spectrometric properties investigated.

A method of synthesizing cyclopropane acids in high yield is also desirable because these have now been found as major components in the lipids of a number of bacterial species (8). They also occur as minor components of certain seed oils (9,10) along with cyclopropene acids. The metabolism of cyclopropane acids in the rat has recently been discussed (11).

EXPERIMENTAL

Materials

Diiodomethane (Fisher Scientific Co.) was redistilled and stored over copper metal before use. Zinc dust was Mallinckrodt analytical reagent grade and cupric acetate monohydrate was Fisher's reagent grade. Most of the methyl esters of the unsaturated fatty acids were supplied by The Hormel Institute, though the methyl linoleate isomers were prepared synthetically as part of another project.

Preparation of the Zinc-Copper Couple

To vigorously stirred nearly-boiling glacial acetic acid (10 ml) in a 25 ml 2-necked flask was added zinc dust (2.0 g). After 1 min,

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cupric acetate monohydrate (0.4 g) in hot glacial acetic acid (10 ml) was added and the mixture stirred approximately 1 min until the blue color disappeared. The hot supernatant liquid was decanted and the couple washed thoroughly with glacial acetic acid (5 × 20 ml) and then with anhydrous ether (5 × 20 ml). Fresh couple was always prepared immediately before use and no attempt was made to store it.

Preparation of the Cyclopropane Derivatives

The general procedure for the preparation of the cyclopropanes was as follows, taking the cyclopropane derivative of methyl linoleate as an example. A condenser and dropping funnel were fitted to the flask containing the zinc-copper couple in anhydrous ether (10 ml). To this was added a solution of diiodomethane (4 ml and methyl linoleate (0.2 g) in ether (5 ml) at such a rate that the solution was kept at reflux by the heat of reaction. When the addition was complete, the solution was refluxed overnight under nitrogen. At the end of this time, the ether solution was decanted from the unchanged couple and washed 3 times with cold hydrochloric acid (1N) and 3 times with water before drying over sodium sulfate. The ether was removed and the excess diiodomethane distilled off at 100C at 0.5 mm Hg. To remove polar by-products, the crude material was chromatographed on a column of florisil (20 cm × 1 cm) and the required cyclopropane ester eluted with 100 ml of a petroleum ether: ether mixture (70:30). Traces of unchanged unsaturated esters were removed by preparative TLC on silica gel plates (0.5 mm thick) impregnated with silver nitrate (12) with petroleum ether:ether (90:10) as the solvent system. The other cyclopropane esters were prepared in a similar manner, the amounts of the various reagents being adjusted according to the number of double bonds in the ester and the quantity of ester available. GLC analysis showed that 95–100% reaction occurred in all cases and overall yields of 70–80% were obtained.

Gas Chromatography

Gas chromatographic separations were made on an 8 ft × ¼ in. glass column packed with 20% ethylene glycol succinate (EGS) and 2% phosphoric acid on Gas-Chrom P (80–100 mesh) in a gas chromatograph with a β-ionization detector, at a temperature of 180C and argon flow rate of 70 ml/min. A Beckman GC-2A instrument with flame ionization detector was also used with a 6 ft × ⅜ in. aluminum column packed with 20% Apiezon L (Ap L) on Gas-Chrom P (80–100 mesh) at a temperature of 220C and helium flow rate of 50 ml/min. Relative retention times were recorded as equivalent chain lengths (ECL) (13).

Mass Spectrometry

The mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6D single focusing instrument. A liquid injection system was used with a suboven heating the sample to 160C to produce the vapor. Spectra were produced at a standard ionization potential of 80eV and then at the lowest voltage which gave a countable spectrum, usually 6–12eV.

Elemental Analyses

Elemental analyses of some of the cyclopropane derivatives, selected at random, were carried out and found to be satisfactory. Clark Microanalytical Laboratories, Urbana, Illinois, performed the estimations.

RESULTS AND DISCUSSION

GLC of the Derivatives

The cyclopropane derivatives of all the unsaturated esters, even those of the tri- and tetraenoic esters, were sufficiently volatile to be readily examined by GLC. The ECLs of each ester on both polar and nonpolar columns (EGS and Ap L) were determined and are recorded in Table I. The average increment in ECL over that of the normal saturated ester is approximately +1.5 on EGS or +0.9 on Ap L per *cis* cyclopropane ring in the molecule. For a *trans* cyclopropane ring, it is approximately +0.85 on EGS or +0.55 on Ap L. The

TABLE I

Cyclopropane Derivatives and Equivalent Chain Lengths On EGS and Apiezon L

Derivative of:	ECLs: EGS	ApL
Me 10-undecenoate	13.70	11.20
Me palmitoleate	17.57	16.85
Me oleate	19.46	18.77
Me elaidate	18.80	18.52
Me petroselinate	19.56	18.93
Me petroselaidate	18.92	18.57
Me vaccenate	19.57	18.88
Me 11-eicosenoate	21.31	20.85
Me erucate	23.38	22.80
Me nervonate	25.54	24.86
Me linoleate	21.07	19.60
Me *trans,trans*-linoleate	19.90	18.95
Me 4,7-octadecadienoate	20.90	19.68
Me 5,8-octadecadienoate	20.83	19.56
Me 10,13-octadecadienoate	21.05	19.63
Me *trans,trans*-9,11-octadecadienoate	20.02	18.12
Me linolenate	22.95	20.58
Me arachidonate	25.68	23.01
Me hydnocarpate	20.05	19.19

cyclopropane derivatives of related *cis* and *trans* fatty acid esters, e.g., methyl oleate and elaidate or methyl *cis,cis*-linoleate and *trans, trans*-linoleate, are readily separable on both polar and nonpolar GLC columns, so the reaction has potential as a method of estimating *trans* double bonds. The derivative from the single conjugated ester, methyl *trans,trans*-9,11-octadecadienoate has remarkably low ECL values, particularly on Ap L.

Mass Spectrometry

Mass spectra were recorded for each ester at both high and low ionization potentials. Larger amounts of sample are necessary for low voltage spectra, but these are often remarkably distinctive and emphasize the primary or larger fragments and minimize secondary degradations. For some substances measurement of spectra at intermediate voltages facilitated counting and indicated the approximate appearance potentials of certain unique peaks in the spectra.

Monocyclopropanes

The mass spectra of the cyclopropane derivatives of methyl oleate and elaidate have already been described by Wood and Reiser (11). They noted no differences in the spectra of the two isomers which, in turn, were very similar to those of the parent esters. Our findings are similar and our interpretation of the spectra differs only in minor details. In Figure 1, the mass spectra of methyl oleate and its cyclopropane derivative at 80eV are compared. The only difference immediately apparent is that the molecular weight of the derivative is 14 more than that of the parent ester. The characteristic peaks described by Stenhagen et al. (1) for monounsaturated esters are found in both, i.e., at m/e = M-32 (loss of methanol from the ester function), m/e = M-74 (loss of the ester group plus one carbon from the chain) and m/e = M-116 (loss of the ester group plus four carbons from the chain). The hydrocarbon peaks at the low end of the spectra are also virtually indistinguishable. When the relative intensities of the ions corresponding to $(C_nH_{2n+1})^+$, $(C_nH_{2n})^+$, $(C_nH_{2n-1})^+$, $(C_nH_{2n-2})^+$, and $(C_nH_{2n-3})^+$, in the spectra of both were plotted against number of carbons and compared, almost no difference was found. The low voltage spectra are equally similar. Peaks which might be expected by fragmentation on either side of the cyclopropane rings at m/e = 113 and 197 or m/e = 153 and 157 are no more prominent in the spectrum of the cyclopropane derivative than in that of the parent ester.

The spectra of the other monocyclopropane esters are likewise similar to those of the parent monoenoic esters at both high and low ionization potentials. Also, geometrical isomers cannot be distinguished. The only exception is the

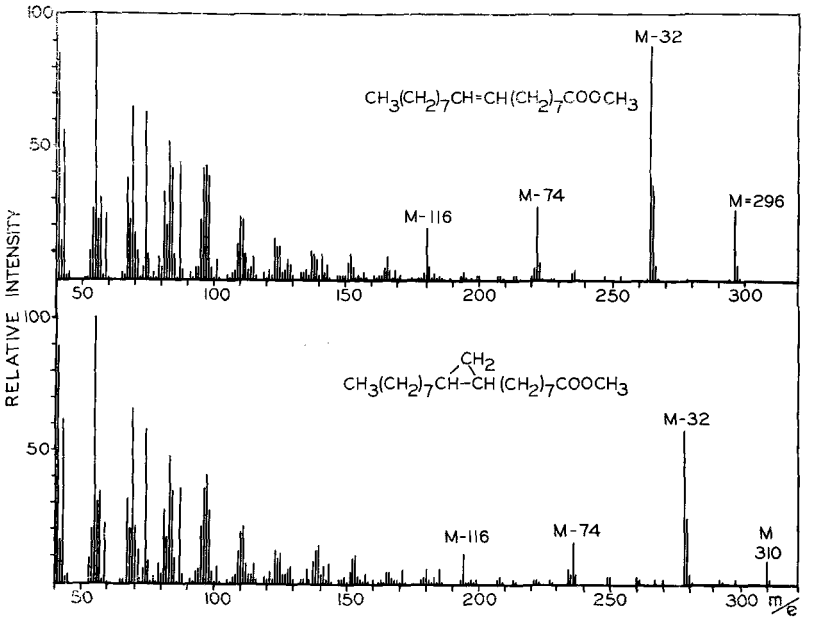
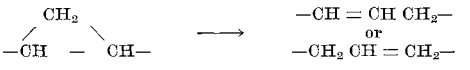


FIG. 1. Mass spectra of methyl oleate and its cyclopropane derivative at 80eV.

low voltage (8eV) spectrum of the cyclopropane derivative of methyl erucate, in which m/e = 226 or M-100 is the base peak and is probably formed by the loss of a rearrangement ion including the ester group and three carbons from the chain.

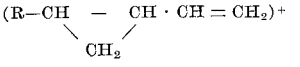
To explain the similarities between the spectra of the monocyclopropanes and those of the monoenoic esters from which they were derived, we postulate an immediate and complete cleavage between the two carbon atoms in the cyclopropane ring which originally constituted the double bond. This gives, in effect, a monounsaturated ester, one carbon atom longer, i.e.:



This then exhibits the mass spectral fragmentation pattern expected of a monoene. Thus, the position of the cyclopropane ring cannot be deduced.

Dicyclopropanes

The mass spectra of the cyclopropane derivatives of the dienoic esters are much more distinctive, particularly those of the series containing the vinyl methylene rhythm. In Figure 2, the spectra of the derivative from methyl *trans,trans*-linoleate at high (80eV) and low (6eV) ionization potentials are compared. This compound was chosen for illustrative purposes because it contains all the features of interest noted in the spectra of the other esters of this type. At 80eV, the base peak is at m/e = 149, and this peak is the base peak for all these diene derivatives with the exception of the cyclopropane derived from methyl *cis,cis*-linoleate itself, in which it is insignificant. The most prominent peaks occur at m/e = 224 (especially at low voltages) and at m/e = 192 and 138. The molecules, therefore, appear to cleave preferentially to give fragments with two carbon atoms attached to a cyclopropane ring. A completely analogous pattern is found with the other dicyclopropanes. In the high and low voltage spectra of the cyclopropane derivative of methyl 10,13-octadecadienoate, there are very distinctive peaks at m/e = 238 and 124. In those of the 5,8 isomer, there are prominent peaks at m/e = 168, 194 and 248; and in those of the 4,7-isomer, large peaks are found at m/e = 154 and 208. In each case, ions of the form:



appear to be particulary stable though, of course, the ions may not remain in this form but may exist as conjugated or cyclic structures. If, in fact, the presence of a grouping of this kind does confer stability on an ion,

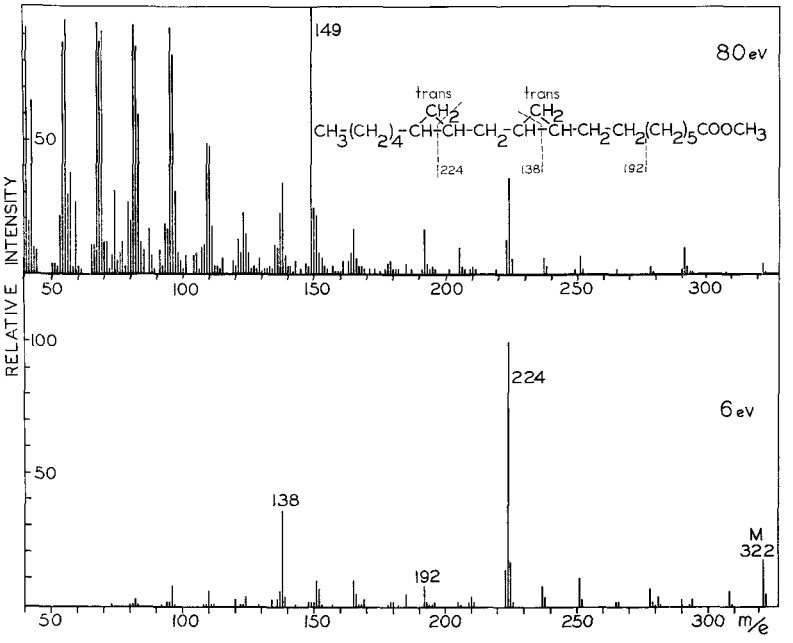
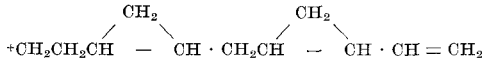


FIG. 2. Mass spectra of the dicyclopropane derivative of methyl *trans,trans*-linoleate at 80eV and at 6eV.

it might be postulated that the peak at m/e = 149 is equivalent to:



though, possibly some rearrangement occurs.[2] Formation of this ion requires a double cleavage of the molecule, so it would be expected at high rather rather than low ionization potentials. In a study of the effect of variation of the ionizing voltage on this ion, it remained the base peak down to about 20eV when it began to decline sharply until at about 10eV it virtually disappeared.

The mass spectra of the cyclopropane derivative of the conjugated ester, methyl *trans, trans*-9,11-octadecadienoate, also has some unusual features (Fig. 3). At a high voltage (80eV), little of interest is apparent at higher mass numbers though, at the other end of the spectrum, the peaks at m/e = 45, 59 and 73 are much larger than usual. These are not particularly significant in the spectra of the parent ester or of any of the other dienes or their derivatives. They must contain at least one oxygen atom, but it is difficult to see how the presence of cyclopropane rings influences the disruption of the carboxyl end of the molecule. In the low voltage spectrum (6eV), the base peak is the parent ion, which may imply that the two cyclopropane rings rearrange readily to give a conjugated diene, for in the low voltage spectrum of the ester from which this was derived, the parent ion is the only one found. However, a large peak occurs at m/e = 266, corresponding again to cleavage giving two carbons attached to a cyclopropane ring. There are also peaks which could be attributed to cleavage at nearly every bond in the rings.

[2] Evidence for a strong conjugative effect of cyclopropyl groups with adjacent carbonium ion centers or double bonds has recently been obtained by nuclear magnetic resonance spectrometry. See Deno, N. C., H. G. Richey, Jr., J. S. Liu, D. N. Lincoln and J. O. Turner, J. Am. Chem. Soc. *87*, 4533 (1965), and Pittman, C. V., Jr., and G. A. Olah, Ibid. *87*, 5123 (1965).

Polycyclopropanes

The mass spectra of the cyclopropane derivatives of polyunsaturated esters are much less predictable than the spectra of the dicyclopropanes. This can be seen from Figure 4 where the mass spectra of the cyclopropane derivative of methyl arachidonate at high and low ionization potentials are compared. In the high voltage spectrum, the only unusual feature is a prominent peak at m/e = 270 which probably results from some rearrangement after cleavage between the 4th and 5th carbons in the original chain. At low voltages, the base peak is at m/e = 266, corresponding to a break between the 3rd and 4th rings in a manner contrary to the theory that the most stable ions are those

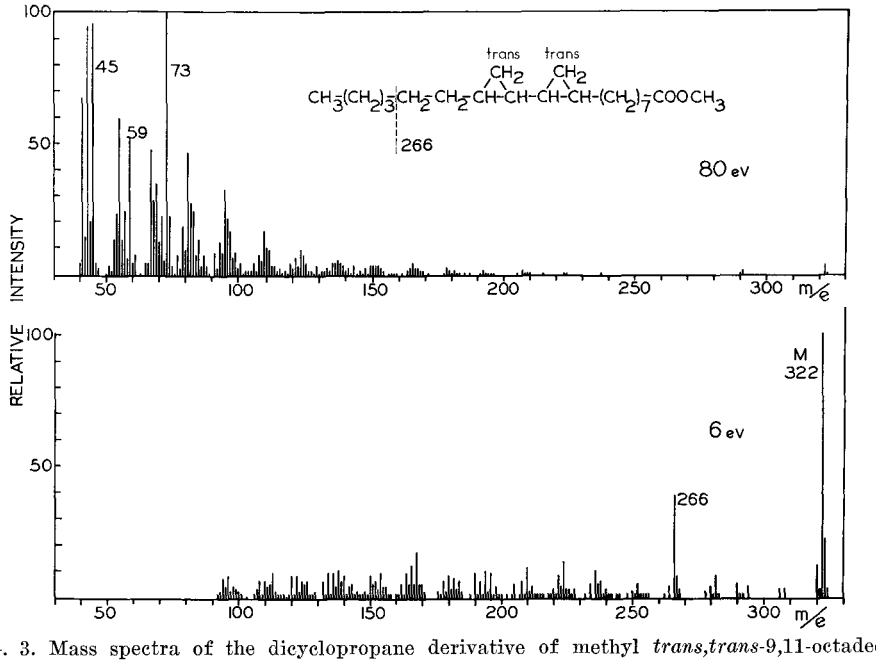


FIG. 3. Mass spectra of the dicyclopropane derivative of methyl *trans,trans*-9,11-octadecadienoate at 80eV and 6eV.

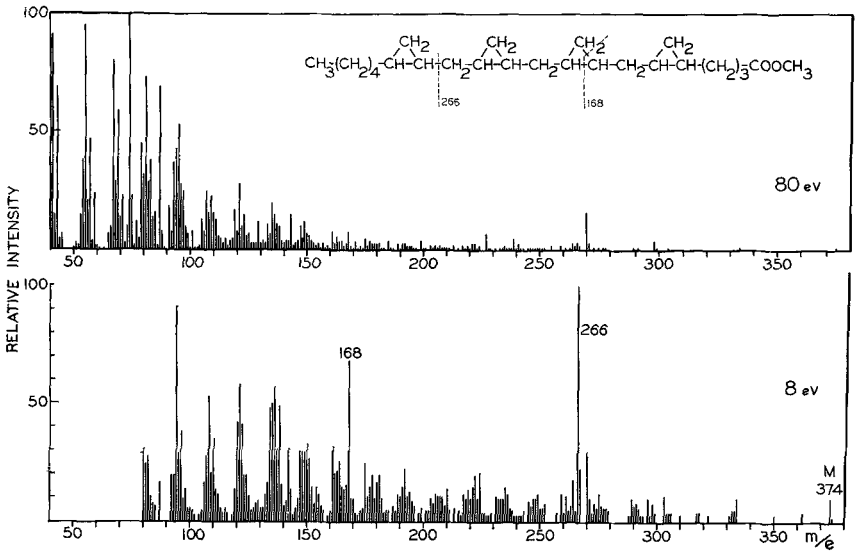


FIG. 4. Mass spectra of the tetracyclopropane derivative of methyl arachidonate at 80eV and 8eV.

with two carbon atoms attached to a cyclopropane ring. There is a large peak at m/e = 168, resulting from fragmentation in the second ring in the prescribed manner, though there are also peaks which could be interpreted as due to cleavage at almost any position in the rings or the chain.

The spectra of the cyclopropane derivative of methyl linolenate are only slightly less complicated than those of the tetracyclopropane compound above. At 80eV, there are no prominent peaks of high mass number. In the low voltage spectrum (6eV), however, the base peak is at m/e = 224, corresponding to cleavage in the second ring in the expected manner; though the next largest peak, at m/e = 136, must result from a break between the 1st and 2nd rings contrary to expectations. In fact, there is a large number of other significant peaks which could occur by fragmentation at any of the bonds in or between the cyclopropane rings.

Mass spectra of all the parent esters and the cyclopropane derivatives listed in Table I have been measured and are available upon request.

CONCLUSION

The reaction of unsaturated esters with diiodomethane and the highly active zinc-copper couple, described above, provides a useful synthesis of cyclopropane esters in high yields. These derivatives are sufficiently volatile to be eluted from a GLC column under moderate conditions and *cis*- and *trans* isomers are easily distinguished.

The method did not furnish a derivative which could be used to locate double bonds in all unsaturated esters by mass spectrometry. Monocyclopropanes have mass spectra very similar to those of the parent esters. Dicyclopropanes from dienoic esters with a single methylene group between the double bonds have unique fragmentation patterns which allow the positions of the original double bonds to be determined. This may possibly be extended to conjugated dienes though if the double bonds were separated by more than one methylene group this might not hold. Polycyclopropanes give complex spectra which are difficult to interpret in terms of the position of the cyclopropane rings, though the reaction could at least be used to confirm the number of double bonds in the molecule.

In the mass spectra of the di- and polycyclopropanes, there are a number of peaks for which the assistance of high resolution mass spectrometry will be necessary before the structures of the ions which they represent can be discussed with any confidence.

ACKNOWLEDGMENTS

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