separated. The tube was opened, the water was removed, and four additional milliliters of amine was distilled under vacuum into the tube, which was again sealed and heated at 175° for 1 day. No further reaction was evident, so the tube was opened and the contents distilled, b.p. 67° (1 mm.). V.p.c. analysis showed the distillate to contain about 60% ketimine and 40% ketone. Pure samples of the former were obtained by preparative vapor phase chromatography.[27]

(27) NOTE ADDED IN PROOF. A similar exchange has been observed for compounds such as p-trifluoromethylbenzophenonimine: D. Y. Curtin and J. S. Smith, private communication. We thank Professor Curtin for informing us of this unpublished work.

Mass Spectra of O-Isopropylidene Derivatives of Unsaturated Fatty Esters

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The mass spectra of O-isopropylidene derivatives of diols obtained by stereospecific oxidation of a number of unsaturated fatty esters are interpreted, and are shown to be useful for the characterization of positional and geometrical isomers of the unsaturated esters. Deuterium and oxygen-18 labeling are used for the elucidation of fragmentation mechanisms. A simple preparation of oxygen-18 labeled diols is described.

Introduction

During the past decade, systematic studies of the mass spectra of long-chain esters[1] have contributed greatly to the basic understanding of the modes of decomposition these molecules undergo upon electron impact. Principally because of this work, mass spectrometry has become a useful tool for the structure elucidation of these compounds,[2] but has proved far less useful in studies involving unsaturated fatty esters, since positional and geometrical isomers give very similar spectra, unless the double bond is in close proximity to the carbonyl group.[1,3] Since catalytic deuteration of double bonds leads to extensive isotope incorporation and scrambling,[4] an early approach to this problem involved specific reduction of the double bond with deuterium by treatment with deuteriohydrazine,[5] but was complicated principally by partial H–D exchange.[6a,b] More recent approaches involve oxidation of the olefin to the epoxide, followed by ring opening to isomeric mixtures of ketones[6b] or N,N-dimethylamino alcohols.[7] While

the mass spectra of the mixtures of products may then be used to determine the position of the original double bond, geometrical isomers are not distinguishable.

During the preparation of O-isopropylidene derivative of unsaturated fatty esters for gas chromatographic studies,[8] mass spectra of the products revealed the presence[8] of peaks corresponding to cleavages of bonds α to the 1,3-dioxolane ring. In addition, some intensity differences were noticed between *erythro* and *threo* isomers. It therefore appeared that mass spectrometry may be potentially useful in the complete structural characterization (of both positional and geometrical isomers) of unsaturated fatty esters, *via* their O-isopropylidene derivatives. We have therefore examined in detail the modes of mass spectrometric fragmentation of these compounds, derived from a number[9] of unsaturated fatty esters. This
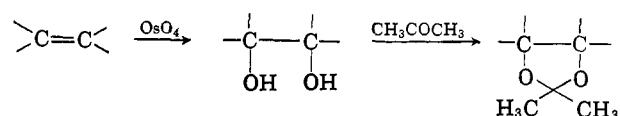
procedure[8] is well suited for mass spectrometry, since the reactions are quantitative, yielding an isomerically pure product, and are easily carried out on a milligram scale, thus avoiding the generally recognized disadvantages of OsO_4 (high cost and toxicity). Moreover, the use of OsO_4, a *cis*-specific oxidant, results in products (*cis* → *erythro*, *trans* → *threo*) which retain the configurational identity of the olefin. We have found that OsO_4 may easily be labeled with O^{18}, providing a simple route to O^{18}-labeled diols. The resulting O^{18}-isopropylidenes are quite useful in corroborating proposed fragmentation paths. The choice of acetone[10] as a reagent for condensation with the diols was made primarily for two reasons. First, isopropylidene derivatives are well suited for gas chromatographic separation of geometrical isomers,[8] providing a highly useful comparative technique, either alone or in

(1) R. Ryhage and E. Stenhagen in "Mass Spectrometry of Organic Ions," F. W. McLafferty, Ed., Academic Press Inc., New York, N. Y., 1963, Chapter 9.

(2) See for instance: C. Asselineau, J. Asselineau, R. Ryhage, S. Ställberg-Stenhagen, and E. Stenhagen, *Acta Chem. Scand.*, 13, 822 (1959); R. Ryhage, S. Ställberg-Stenhagen, and E. Stenhagen, *Arkiv Kemi*, 18, 179 (1961); G. Odham, *ibid.*, 22, 417 (1964).

(3) B. Hallgren, R. Ryhage, and E. Stenhagen, *Acta Chem. Scand.*, 13, 845 (1959).

(4) See, for instance, N. Dinh-Nguyen and R. Ryhage, *J. Res. Inst. Catalysis Hokkaido Univ.*, 8, 73 (1960).

(5) N. Dinh-Nguyen, R. Ryhage, and S. Ställberg-Stenhagen, *Arkiv Kemi*, 15, 433 (1960).

(6) (a) N. Dinh-Nguyen, R. Ryhage, S. Ställberg-Stenhagen, and E. Stenhagen, *ibid.*, 18, 393 (1961); (b) G. W. Kenner and E. Stenhagen, *Acta Chem. Scand.*, 18, 551 (1964).

(7) H. Audier, S. Bory, M. Fetizon, P. Longevialle, and R. Toubiana, *Bull. soc. chim. France*, 3034 (1964).

(8) K. Tanaka and E. C. Horning, to be published.

(9) Ethyl palmitoleate and the methyl esters of myristoleic, palmitoleic, palmitelaidic, oleic, elaidic, petroselinic, cis- and trans-vaccenic, cis-5-eicosenoic, cis-11-eicosenoic, erucic, and nervonic acids.

(10) Condensation with formaldehyde and acetaldehyde also yields products which exhibit useful mass spectra (unpublished experiments of R. E. Wolff and G. Wolff).

direct combination with a mass spectrometer. Second, the ready availability of acetone-d_6 provides a convenient means of deuterium labeling for the elucidation of fragmentation mechanisms, including unambiguous identification of the position of the 1,3-dioxolane ring in the chain, as will be discussed.
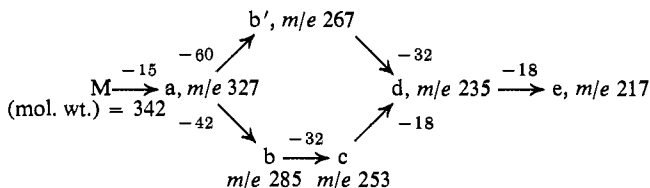
Discussion of Mass Spectra

Mass spectra of isopropylidenes derived from unsaturated fatty esters show that these molecules all exhibit the same principal modes of fragmentation, differing only in the small but diagnostically important cleavages α to the dioxolane ring. The mass spectrum of methyl *erythro*-O-isopropylidene-9,10-dihydroxyhexadecanoate (I, Figure 1) derived from methyl palmitoleate, serves as a typical representative of the compounds studied. In addition, spectra of the hexadeuterio- (II, Figure 2) and di-O^{18}-isopropylidene (III, indicated by asterisks, Figure 1) analogs of I have aided in the confirmation and elucidation of the modes of formation of fragment ions.
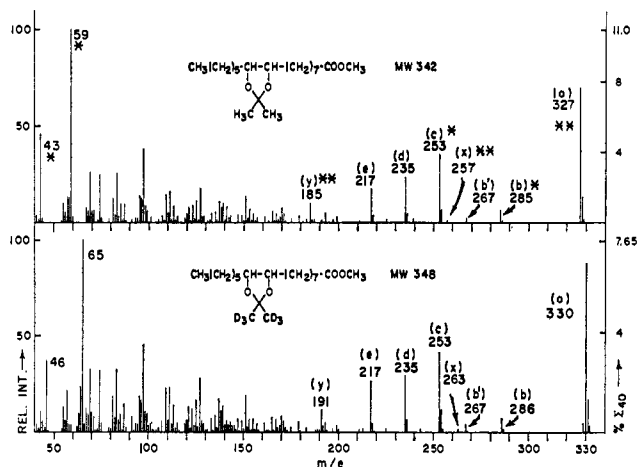

Figure 1. Mass spectrum of the O-isopropylidene derived from methyl palmitoleate. Location of 1,3 dioxolane ring oxygens in this spectrum is indicated by the appropriate number of asterisks, taken from the mass spectrum of the di-O^{18}-isopropylidene derived from methyl palmitoleate.

Figure 2. Mass spectrum of the O-isopropylidene-d_6 derived from methyl palmitoleate.

Table I. Metastable Peaks from the Mass Spectrum of the O-Isopropylidene Derived from Methyl Palmitoleate

Calcd.	Found	Transition indicated, m/e
248.4	248.8	327 → 285
224.6	224.8	285 → 253
218.3^a	218.3	253 → 235
200.4	200.4	235 → 217
218.0^a	218.3	327 → 267
206.8	206.8	267 → 235

^{a} The two metastable peaks occurring near m/e 218 are resolved by the corresponding transitions in the case of the O-isopropylidene derived from ethyl palmitoleate: m/e 218.3 (218.3 calcd.) and 231.8 (231.6 calcd.), respectively.

The molecular weight is clearly indicated by loss of a methyl group from the 2,2-dimethyl-1,3-dioxolane ring, giving rise to an intense peak at M − 15 (a, m/e 327). This peak thus shifts as expected to m/e 330 in the spectrum of II (Figure 2), due to the presence of one trideuteriomethyl group. This tertiary carbonium ion is extremely well stabilized by two adjacent ether oxygens, and thus constitutes a localized charge center from which the principal modes of decomposition are initiated. The presence of metastable peaks (Table I) indicates that decomposition occurs from ion a (M − 15) by two paths.

Elimination of the elements of acetic acid from a to yield ion b′ is confirmed by the loss of the remaining trideuteriomethyl group, as well as both labeled oxygens from the dioxolane ring, as indicated in Figures 2 and 1, respectively. Mechanistic involvement of a hydrogen α to the dioxolane ring would be par-

a, m/e 327

b′, m/e 267
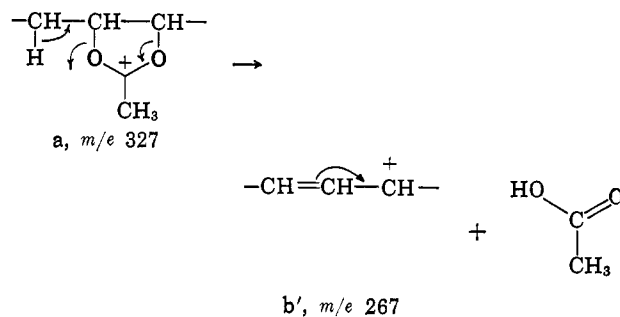
ticularly attractive, yielding the allylically stabilized ion, b′. Further loss of methanol *via* a cyclic elimination process[11] results in ion d, m/e 235. Analogous elimination of ethanol occurs from the corresponding ethyl ester.

The second path of decomposition originating with ion a first involves loss of 42 mass units, containing only one of the dioxolane ring oxygens (b, Figure 1). Ion b shifts one mass unit to m/e 286 in Figure 2, indicating elimination of ketene with rearrangement of one hydrogen from the methyl group attached to the ring. This may be formulated as a concerted mechanism resulting in a protonated epoxide ion.

a, m/e 327   b, m/e 285
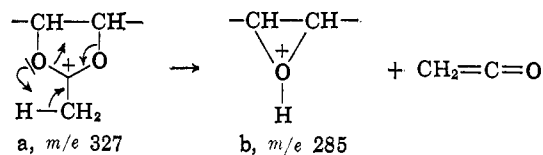
The next step in the decomposition, loss of 32 mass units from b to c, m/e 253, at first appeared to be analogous to numerous other cases in which fragment ions from long-chain methyl esters eliminate methanol.[12] However, comparison of the mass spectrum of I

(11) Simple 1,2-elimination of methanol is ruled out by the mass spectrum of I partially labeled with deuterium at C-2, in which the label is completely retained in ion d.

(12) Elimination of methanol from fragment ions is a common process in the mass spectra of branched, keto, hydroxy, and methoxy methyl esters.[1]
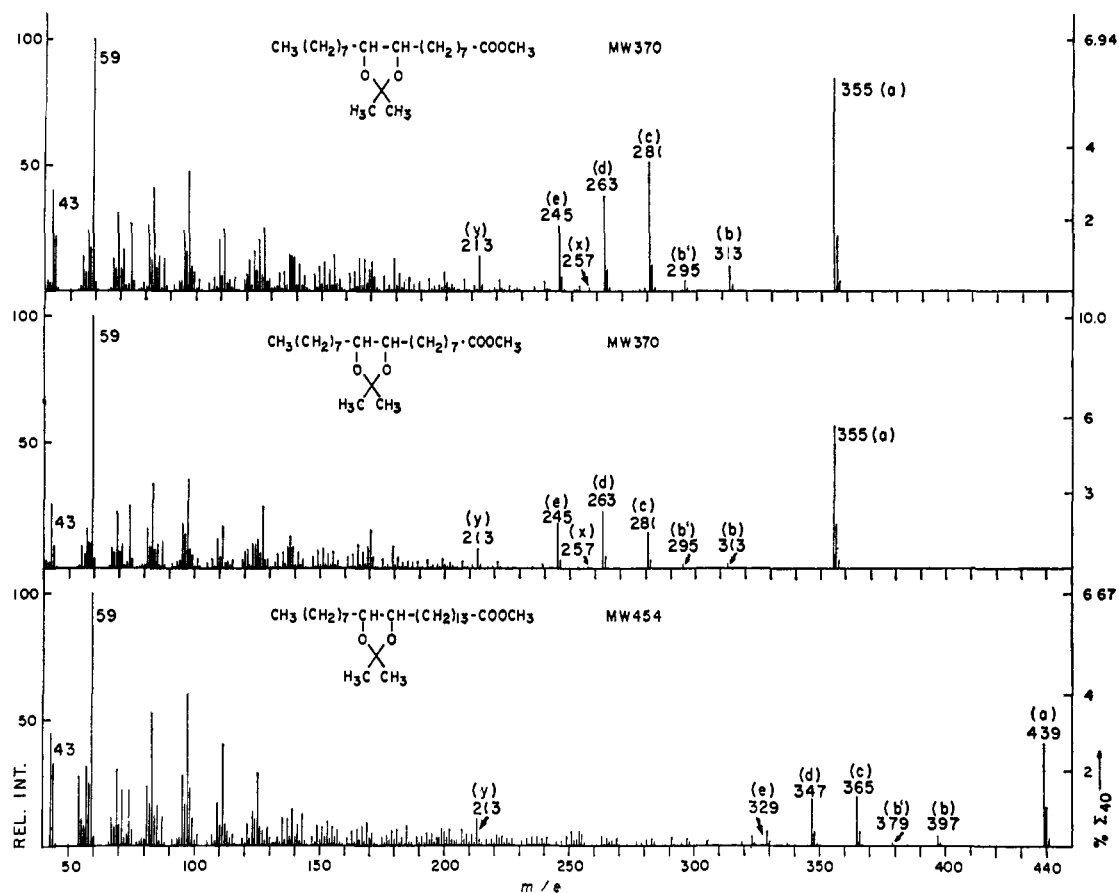
Figure 3. Mass spectrum of the O-isopropylidene derived from methyl oleate.
Figure 4. Mass spectrum of the O-isopropylidene derived from methyl elaidate.
Figure 5. Mass spectrum of the O-isopropylidene derived from methyl nervonate.

(Figure 1) with its hexadeuterio analog II (Figure 2), shows that the rearranged hydrogen (deuterium in Figure 2) present in ion b discussed above has been lost to the extent of 91% in the transition b → c. The rather surprising conclusion has therefore been reached that elimination of methanol in the transition b → c involves the highly specific (>90% in every case) abstraction of a previously rearranged hydrogen, *even though its proximity to the methoxyl group varies, up to a chain separation of at least 15 carbon atoms.*[13] Evidence for this process may be summarized as (i) the presence of metastable peaks (see Table I) indicating the single-step processes a $\xrightarrow{-42}$ b $\xrightarrow{-32}$ c from the nondeuterated compound (see Table I), and b $\xrightarrow{-33}$ c[14] in the case of the hexadeuterio analog; (ii) mass spectra of the deuterium (Figure 2) and oxygen-18 (Figure 1) labeled analogs; (iii) the analogous elimination of ethanol in the spectrum of the O-isopropylidene derived from ethyl palmitoleate. Due to the general steric requirements[15] that the two groups

(*i.e.*, methoxyl and hydrogen) must approach each other within at least several angstroms, it seems probable that a statistically large number of ions must exist in a coiled or helical form prior to transition b → c. Such a conclusion may not be unrelated to the fact that normal fatty esters have been shown to eliminate two- and three-carbon fragments from within the hydrocarbon chain.[1]

Ion c therefore contains two oxygen atoms: one from the carbonyl group in the original ester linkage, and one from the 1,3-dioxolane ring, as borne out by the presence of one O^{18} in Figure 1 (*m/e* 253). The latter oxygen is then lost in the elimination of water which results in ion d, containing no O^{18} (Figure 1). Since *m/e* 235 (d, Figure 1) also arises *via* loss of methanol from ion b' as discussed earlier, there are two species of ion d. Although both have the same empirical composition, it should be assumed that their actual structures may be somewhat different, since the mechanisms of transitions b' → d and c → d cannot be determined from the evidence presently available. Further expulsion of water yields ion e (*m/e* 217, Figure 1), a hydrocarbon fragment.

The exceptional stabilization of ion a, which results in every case in an intense peak, plus the virtual absence of any observed molecular ions, indicates that the great majority of fragment ions come from ion a

(13) The O-isopropylidenes derived from methyl nervonate (*cis*-15-tetracosenoate; Figure 5) and its d_5 analog provide the greatest distance between the 1,3-dioxolane ring and the methoxyl group in the series of compounds studied.
(14) Calcd., *m/e* 223.8; found, *m/e* 223.9.
(15) Djerassi and co-workers have demonstrated the importance of interatomic distances between participating groups in mass spectrometric rearrangements through their studies of γ-hydrogen rearrangements in 11- and 15-keto steroids.[16a,b]
(16) (a) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, Calif., 1964, Chapter 20; (b) C.

Djerassi, G. von Mutzenbecher, J. Fajkos, D. H. Williams, and H. Budzikiewicz, *J. Am. Chem. Soc.*, 87, 817 (1965).

rather than M. However, one expects, and finds, a relatively small number of ions due to simple bond cleavage α to the 1,3-dioxolane ring. The resulting secondary carbonium ions (e.g., m/e 185, 257 in Figure 1) are stabilized by one of the adjacent ether oxygens. These ions, though of relatively low intensity, are of considerable structural significance, since they mark the position of the double bond before oxidation. The ion bearing the carbomethoxy group (ion x), Figures 1–4) is always of lower abundance than the one containing the hydrocarbon chain (ion y). This effect becomes more pronounced as the chain length increases, so that in extreme cases (e.g., the O-isopropylidene derived from methyl nervonate, Figure 5) only ion y may be observed without resorting to higher operating pressures in the mass spectrometer. Identification of ions x and y is facilitated in several ways. Of course, for mono-O-isopropylidenes, x and y may only assume certain mass values.[17] Furthermore, since the principal modes of fragmentation (i.e., ions a through e) are always the same, small differences arising from positional isomerism are more easily detected. Finally, the routine use of acetone-d_6 in addition to ordinary acetone in the condensation reactions provides an easily observed shift of six mass units in x and y (e.g., Figures 1 and 2) due to the presence of two trideuteriomethyl groups on the 1,3-dioxolane ring.

In the lower mass range two of the most prominent peaks in every case are m/e 43 and 59, which shift to m/e 46 and 65 in the mass spectra of the hexadeuterio derivatives, and contain one O^{18} in the case of the O^{18}-labeled compounds. These ions are also formed in the fragmentation of O-isopropylidene derivatives of pentoses and hexoses,[18] and undoubtedly represent[18] the acetyl ion, CH_3CO^+, and protonated acetone, $CH_3C(=O^+H)CH_3$, formed from the 1,3-dioxolane ring. The presence of the latter ion is analogous to the well-known formation of H_3O^+, NH_4^+, H_3S^+, etc., in the mass spectra of alcohols, amines, and sulfides, respectively.

As might be expected, the *erythro* and *threo* isomers give rise to mass spectra which show intensity, but not mass, differences. In those pairs studied[19] the intensity of ion c is at least twice as great in the spectra of *erythro* isomers derived from *cis* fatty esters, than in the case of *threo* derivatives. These intensity differences are evident by comparison of mass spectra of the O-isopropylidenes derived from a representative pair of esters such as methyl oleate (Figure 3) and methyl elaidate (Figure 4), which indicate that *cis* and *trans*

isomers may in general be differentiated by the mass spectra of their O-isopropylidene derivatives. However, since the relative intensity of ion c varies somewhat with chain length and position of the ring in the chain,[20] determination of the configuration of an unknown unsaturated ester may be difficult unless a closely related compound is available for comparison.

Experimental Section

Mass Spectra. Spectra were determined with an Atlas-Werke CH-4 mass spectrometer, equipped with a gas chromatographic inlet system[21] (1% SE-30, 140–190°). The transfer system (including He separators) was maintained at 175° and the ion source 250°. Ionizing potential was 20 e.v.,[22] and ionizing current was 60 μa. Spectra from 1–20-μg. samples were recorded in 1–4 sec. on the apex of the gas chromatographic peak, as indicated by the gas chromatogram continuously produced by the total ionization monotor.

Preparation of OsO^{18}_4. OsO_4 (2 mg.) was dissolved in dioxane (approx. 100 μl.), to which was added H_2O (100 μl.) containing 11.2% excess O^{18}, and an approximately equal amount of dioxane. The mixture was added directly to the reaction mixture after standing 10 min. Incorporation was generally 10–11% per oxygen, as determined from the peaks due to ion a (M − CH_3) in the mass spectra of the resulting O-isopropylidene derivatives.

O-Isopropylidene Derivatives. These compounds were obtained essentially by the procedure of Horning.[8] OsO_4 (2 mg.) and the unsaturated ester (1 mg.) were added to a pyridine–dioxane mixture (1:8) and allowed to stand for 1 hr. at room temperature. The mixture again stood for 1.5 hr. after addition of a suspension of Na_2SO_3 (6 ml.) made by addition of 16% Na_2SO_3 in H_2O (8.5 ml.) to methanol (2.5 ml.). After centrifugation, the supernatant was dried under vacuum, taken up in ether, and re-evaporated. The resulting diol was condensed with acetone (1 ml. or less) or acetone-d_6 (0.2–0.3 ml.) in the presence of anhydrous copper sulfate (50 mg.) upon heating for 2 hr. at 50°. The reaction mixtures were injected directly on the gas chromatographic column connected to the mass spectrometer.

(17) Ion x = m/e 159 + n(14) and ion y = m/e 101 + m(14), where n and m are integers. The fixed relation between x and y may be expressed as M(mol. wt.) = (x) + (y) − 100.

(18) D. C. DeJongh and K. Biemann, *J. Am. Chem. Soc.*, **86**, 67 (1964).

(19) The methyl esters of palmitoleic and palmitelaidic, oleic, and elaidic, and *cis*- and *trans*-vaccenic acids.

(20) For instance, the relative intensities of ion c from the O-isopropylidene derivatives of: methyl erucate, 28%; methyl myristoleate, 65%.

(21) R. Ryhage, *Anal. Chem.*, **36**, 759 (1964).

(22) Use of higher energies, e.g., 70 e.v., made no appreciable difference in the relative intensities of the peaks in the spectrum.