METHODS

Mass Spectra of Fatty Acid Derivatives, of Isopropylidenes of Novel Glyceryl Ethers of Cod Muscle and of Phenolic Acetates Obtained with the Finnigan Mat Ion Trap Detector[1]

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The applicability of the Finnigan MAT Ion Trap Detector (ITD) mass spectrometer for structure determination in some selected fatty acids and their derivatives has been investigated. Isopropylidene derivatives of novel glyceryl ethers isolated from cod flesh and of phenolic acetates are included to indicate the potential for diverse structures and to clarify the protonation of ions. The ITD is a simple and unsophisticated gas liquid chromatograph-mass spectrometer, but the spectra obtained are in most respects comparable to those from more conventional electron impact mass spectrometers. However, due to the comparatively high background pressure ($\sim 10^{-3}$ torr) in the ionization chamber of the ITD, there is a tendency for both neutral and ionized molecules to acquire protons from other molecules or fragments through collision. In many cases, the molecular ion was observed as the protonated molecular ion (M+1), as in chemical ionization mass spectrometry. These interactions can be minimized if the sample load is decreased. Phenolic acetates exhibit not only protonation of the molecular ion, but also protonation of stable fragmented neutral molecules or ions.
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Gas liquid chromatography-mass spectrometry (GC-MS) is an established technique in the analysis of complex mixtures, and is critical to many applications of analytical chemistry because of its combination of sensitivity and a wide range of applicability. Several more sophisticated models of GC-MS instruments have been available to a certain proportion of lipid chemists for approximately two decades, but GC-MS has not been commonly accessible on a direct user basis because of the high cost and complexity of operation. During the last decade the miniaturization of personal computers into data handling microprocessors has proceeded with unexpected rapidity; the union of this development with GC-MS has led to the Finnigan Ion Trap Detector, commonly called an ITD. This is a novel form of mass spectrometer (1,2) available at about one-quarter the price of conventional GC-MS equipment and designed to capitalize on the IBM-PC for control, data storage and data processing. It provides direct comparison with a library of 38,000 spectra on hard disc. This report illustrates some applications appealing to lipid chemists and clarifies the current capability of this relatively inexpensive apparatus.

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EXPERIMENTAL PROCEDURES

Methyl 16-methylheptadecanoate was obtained from Analabs Inc. (North Haven, Connecticut) and pure *cis*-9-octadecenoic acid from Applied Science Lab. Inc. (State College, Pennsylvania). Phenols were purchased from Aldrich Chemical Co. Inc. (Milwaukee, Wisconsin).

Preparation of fatty acid derivatives. The vicinal diol of methyl *cis*-9-octadecenoate was prepared essentially according to the procedure of McCloskey and McClelland (3). The 0-isopropylidene derivative of the diol was obtained by condensing the diol (5 mg) with acetone (1 ml) in the presence of anhydrous copper sulfate (50 mg) upon heating for 2 hr at 50 C. The trimethylsilylether derivative of the diol was prepared by treating the diol (10 mg) with hexamethyldisilazane (0.3 ml) and trimethylchlorosilane (0.1 ml) in the presence of pyridine (1 ml). An N-octadec-9-enoylpyrrolidide of *cis*-9 octadecenoic acid was prepared by heating the methyl ester (0.5 mg) with freshly distilled pyrrolidine (1 ml) and acetic acid (0.1 ml) for 30 min at 100 C (4).

Preparation of phenolic acetates. Phenolic acetates were made by treating the phenols (10 mg) with a mixture of acetic anhydride (1.5 ml), 3 N NaOH (0.5 ml) and crushed ice (5 g) for 5 min (5). The phenolic acetates were extracted with diethyl ether after acidifying with 3 N HCl.

Extraction of cod flesh lipids and preparation of isopropylidene-glyceryl ethers. Lipids from filets (without skin) of cod caught in January 1986 from the Middle Bank off the east coast of Nova Scotia were extracted by the method of Bligh and Dyer (6). The total lipid was separated into polar and nonpolar fractions according to the procedure of Galanos and Kapoulas (7). The nonpolar lipid fraction was subjected to preparative thin layer chromatography (TLC) on "Prekotes" Adsorbosil-5 silica gel TLC plates (20 × 20 cm; Applied Science Laboratories, College Park, Pennsylvania). The development was in hexane/diethyl ether/acetic acid (85:15:1, v/v/v). The bands were visualized by spraying a 0.01% solution of 2′7′-dichlorofluorescein in ethanol and viewing under UV light. The diacylglyceryl ether (DAGE) band was scraped off the TLC plate and extracted from the silica gel using a mixture of $CHCl_3/CH_3OH/H_2O$ (5:5:1, v/v/v), according to the procedure of Fine and Sprecher (8).

The isolated DAGE was saponified with KOH/C_2H_5OH according to the AOCS official method Ca 6b-53. The unsaponifiable matter was extracted with diethyl ether and subjected to preparative TLC as described earlier. The 1-0-alkyl glycerol fraction was isolated and converted to the isopropylidene derivative according to Mueller et al. (9).

GC-MS analyses. GC-MS data was obtained by using a model 700 Finnigan MAT Ion Trap Detector system

controlled by an IBM Personal Computer XT (PC XT). The ITD was connected to a Perkin Elmer 990 gas chromatograph. The wall-coated flexible fused silica GC column was fed through a heated transfer line directly to the ITD gas inlet. Chromatography of the above polar derivatives of octadecenoic acid and of phenolic acetates was executed on a flexible fused silica capillary column (10 m × 0.21 mm) coated with OV-101 methyl silicone. The column temperatures were respectively 130 C and 225 C for the phenolic acetates and the fatty acid derivatives, at a helium carrier gas pressure of 13 kPa. Underivatized fatty acid methyl esters were analyzed on a Supelcowax-10 (bonded Carbowax-20M) fused silica capillary column (30 m × 0.21 mm) operated at 190 C/68 kPa and connected to the ITD in the same fashion. The isopropylidene derivatives of glyceryl ethers were analyzed on a DB-Wax fused silica capillary column (30 m × 0.25 mm; a bonded polyethylene glycol column from J&W Scientific Inc., Rancho Cordova, California) operated at 210 C/68 kPa.

RESULTS AND DISCUSSION

A notable feature of the unit is that the PC XT controls and monitors the vacuum system, system temperatures and scanning of the ITD and also handles all data acquisition and processing.

The principle of the apparatus is described elsewhere (1). The effluent from any gas liquid chromatography (GLC) column, packed or open-tubular, can be accessed via a heated transfer line, but we prefer to pass the exit end of the flexible fused silica column directly into the inlet port of the ITD. The latter operates at high vacuum and the limited capacity of the turbomolecular pump requires that carrier gas flow also be limited. This places minor constraints on GLC operating conditions, but the high thermal stability of bonded liquid phases in flexible fused silica columns (10) permits higher temperatures (up to 280 C) to be used in lieu of high carrier gas flow. In the same way as the phenomenal sensitivity of the Chromarod-Iatroscan TLC-FID system, compared to plate TLC and reagent sprays, requires users to rethink their sample sizes (11), the ITD also functions best with the lowest possible sample entering the system. If the system is overloaded there is a greater tendency for both neutral or ionized molecules to acquire protons from other molecules or fragments, leading to protonation of the parent molecule and other fragments. Figure 1 illustrates this for methyl 16-methylheptadecanoate (iso-18). The comparison shows that in regular electron impact mass

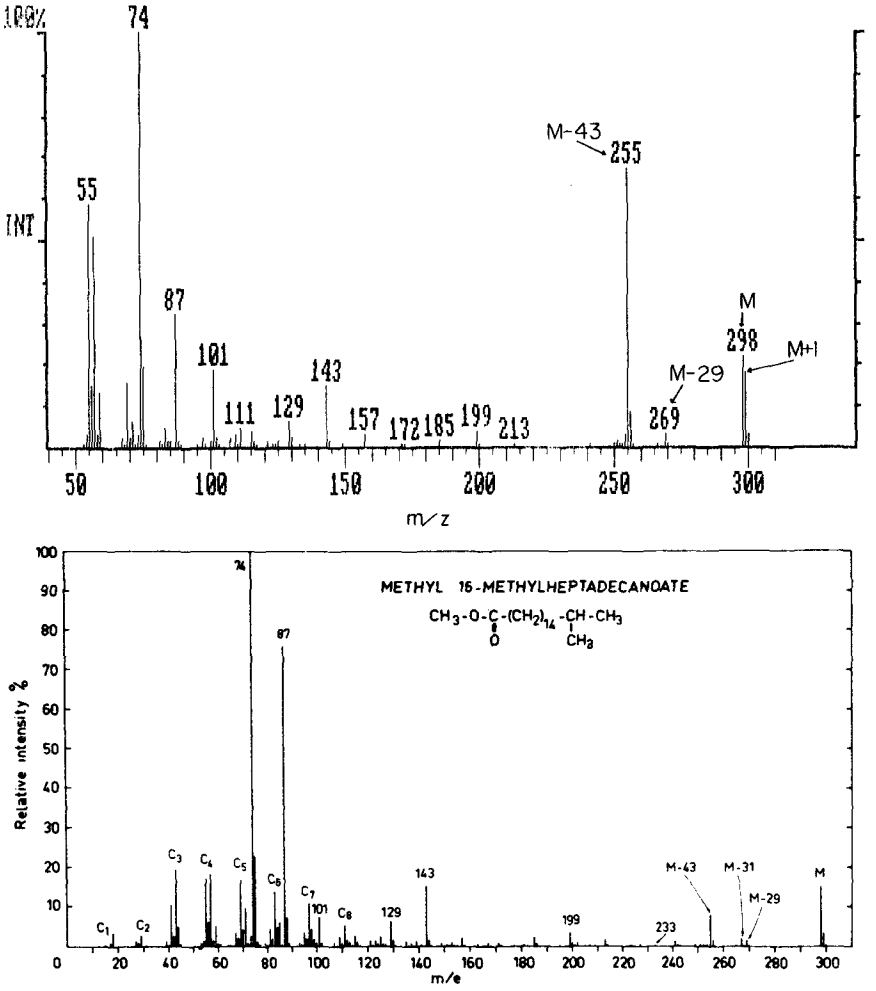

FIG. 1. Mass spectrum of methyl 16-methylheptadecanoate from ITD GC-MS (top) and from a conventional electron impact MS (bottom reproduced from Ref. 8).

spectrometry the ratio of intensities of M^+ to $(M+1)^+$ is 21 (12), which is close to the natural isotope ratio for C_{18}, whereas in the ITD spectrum the molecular ion is accompanied by an almost equal proportion of $(M+1)^+$. However, the ratio of M-43 (m/z 255) to the adjacent M-42 is nearly the same, and most other fragments are similar in proportion in both spectra.

Recently Tulloch and Hogge (13) observed that intensities of $(M+1)^+$ in mass spectra of fatty acid methyl esters exceeded theoretical values on a Finnigan model 4000 GC-MS. Large $(M+1)^+$ ions have been observed commonly with shorter chain methyl esters such as methyl decanoate. They showed with deuterium labeling that a hydrogen radical migrates internally, most favorably from the C-4 position of the fatty acid chain, to the ionized carbonyl oxygen of M^+, and the resultant ion on collision with a neutral molecule transfers the hydrogen as a proton, giving $(M+1)^+$ (Fig. 2). Probably the same mechanism is operating in the ITD to produce the relative abundance of $(M+1)^+$ ions. This proton transfer is more pronounced in the ITD than in the majority of fatty acid spectra obtained by more conventional GC-MS, but is not by any means unique to the ITD. In the ITD the background carrier gas pressure (without the sample) inside the ion chamber is about 10^{-3} torr (14). This is much higher than in the conventional magnetic sector mass spectrometer, which operates around 10^{-6} torr. If the sample pressure inside the ionization chamber exceeds 10^{-2} torr, secondary collisions of the ions or molecules with each other become important (15,16). A high ion residence time (the ions spend time in the ion source and in flight to the detector) could also lead to interaction of ions and neutral molecules. The ion residence time in the ITD is of the order of 10^{-3} seconds (17) compared to 10^{-5}–10^{-6} seconds for many conventional mass spectrometers (15). Thus, any abnormal high intensity or abundance of $(M+1)^+$ in ITD is obviously due to the secondary collisions brought about by the relatively high ion residence time and high sample pressure in the ionization chamber.

The ITD is quite useful in determining the position of double bonds in unsaturated fatty acids. This was demonstrated with the pyrrolidide of *cis*-9-octadecenoic acid and of the isopropylidene and trimethylsilyl ether (TMS) derivatives of the methyl esters of the vicinyl dihydroxy oxidation product. For example, the ITD mass spectrum of methyl erythro-O-isopropylidene-9,10-dihydroxyoctadecanoate (Fig. 3) was almost identical to that published by McCloskey and McClelland (3). The molecular ion and the $(M+1)^+$ ion were absent, but the molecular weight was clearly demonstrated by a loss of a methyl group from the 2,2-dimethyl-1,3-dioxalane ring, giving rise to an intense peak at M-15 (a, m/e 355). Other characteristic fragments appeared at m/z 313 (b), 295 (b′), 281 (c), 263 (d), 256 (x), 246 (e) and 214 (y). The peaks at e and y were one unit higher and the peak at x was one unit lower than those reported in the literature (3). This is probably the result of secondary collisions of fragmented ions. The ions x and y are diagnostic of the original position of the double bond in monoethylenic fatty acids, which are formed by simple cleavage of the C-C bond alpha to the 1,3-dioxalane ring. The ITD-mass spectrum of the TMS derivative of the vicinal diol prepared from methyl *cis*-9-octadecenoate did not exhibit the M^+ or $(M+1)^+$ (Fig. 4), a result similar to that from a conventional mass spectrometer (18). The two ions at m/z 215 and 259, which represent the cleavage between the two carbon atoms which originally constituted the double bond, were

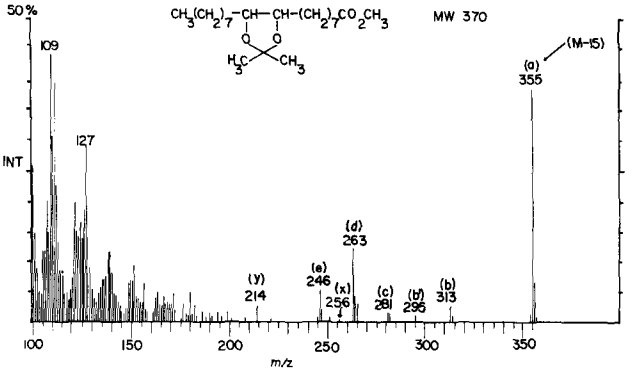

FIG. 3. ITD mass spectrum of methyl erythro-O-isopropylidene-9,10-dihydroxyoctadecanoate.

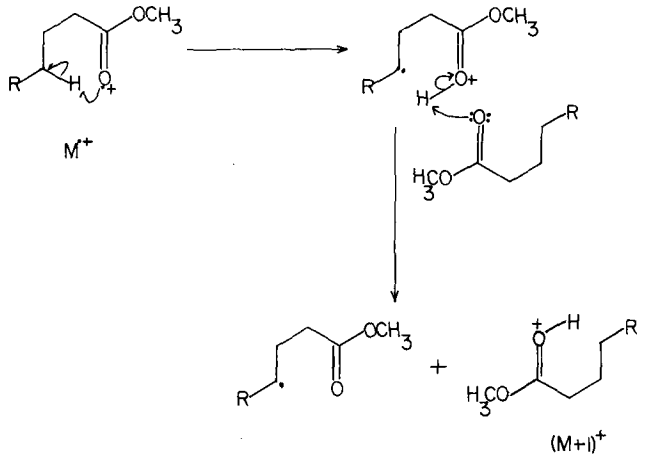

FIG. 2. Postulated mechanism for the formation of $(M+1)^+$ in the ITD mass spectrum of fatty acid methyl esters.

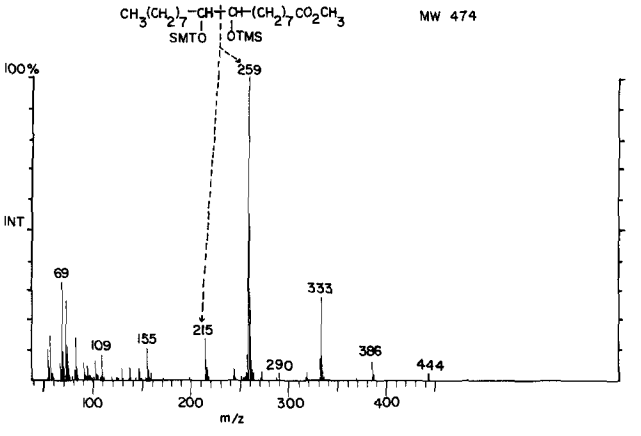

FIG. 4. ITD mass spectrum of the TMS derivative of 9,10-dihydroxyoctadecanoate.

particularly prominent, with m/z 259 being the base peak. Pyrrolidide derivatives of unsaturated fatty acids have distinctive mass spectra with important ions that can be used to locate the position of the double bonds (4,19). Figure 5 (top) illustrates the ITD mass spectrum of the pyrrolidide derivative of *cis*-9-octadecenoic acid. The ITD mass spectrum was almost identical to that obtained on a quadrupole GC-MS (4,19; for an example, see Fig. 5, bottom). The peak at m/z 336 in the ITD spectrum is due to the protonated parent molecule and confirms the molecular weight of 335 for the pyrrolidide derivative. The base peak was the McLafferty rearrangement ion at m/z 113 (not shown in Fig. 5) which is typical of N-acylpyrrolidides (19). Prominent and characteristic fragments appeared at m/z 126, 140, 154, 168, 182, 196, 208, 210, 222 and 236. This series spaced at 14 atomic mass units (amu) is generally observed except in the vicinity of the double bond where the interval is 12 amu, occurring between m/z 196 and 208. According to the rules proposed by Andersson and Holman (4), this confirmed that the double bond resided between carbons 9 and 10 in the N-octadec-9-enoylpyrrolidide.

Protonation is observed not only with the parent molecule, but also with fragmented ions and daughter molecules. During our work with phenols of the suberin of tomato locule protoplasts (5), we observed that this protonation of fragmented ions or molecules is quite pronounced in the ITD spectra of acetylated phenols. The ITD mass spectrum of vanillin acetate is shown in Figure 6. At the time this spectrum was obtained the importance of minimizing sample size was not clearly understood, hence the importance of M+1 (m/z 195). The base peak was at m/z 43, while ions representing acetyl (m/z 151) and ketene (m/z 152) eliminations were about 40 and 50% of the base peak. A similar behavior was also observed with the ITD mass spectrum of p-acetoxybenzaldehyde. A previously published spectrum of vanillin acetate obtained with a conventional mass spectrometer showed that the base peak was at m-43 (m/z 151) due to the elimination of an acetyl radical (20). The peak at m/z 43, due to acetyl ions, was about 85% of the base peak (m/z 151), and the peak at m/z 152, which is due to the elimination of ketene, was around 15% of the base peak (20). The greater intensity of the m/z 43 peak of phenolic acetates in the ITD spectra could be rationalized by considering that the m/z 43 peak is not only due to the acetyl ion but also to a protonated ketene. As depicted in Figure 7, ketene (which is eliminated from the parent molecule) could acquire a proton, through collision, from a fragmented ion such as structure H. The predominance of m/z 43 peak in the mass spectra of phenolic acetates has also been observed by other workers in the GC-MS field (21). The ITD spectrum of vanillin acetate exhibits a prominent peak at m/z 153 (Fig. 6). However, in the reports for magnetic sector MS (20) and in GC-MS (21), the intensity of this ion was much less than that of m/z 151 and m/z 152. The predominance of m/z 153 in the ITD spectrum is obviously due to secondary collisions; it could have formed by the abstraction of a hydrogen radical by the odd-electron ion (B) from another odd-electron ion such as the molecular ion (E). The corresponding ion in the ITD spectrum of p-acetoxybenzaldehyde appeared at m/z 123. It had the same intensity as that of m/z 121.

We have recently examined Atlantic cod flesh lipids by TLC and Iatroscan-TLC/FID (11) and found that cod flesh lipids contain trace levels (<0.05% by wt) of DAGE. There have been no previous reports concerning the existence of DAGE in cod flesh. DAGE are the major lipid components of the livers in some elasmobranch fish such as sharks (22,23). The occurrence of DAGE in marine fish flesh is not commonly mentioned except in some deep-

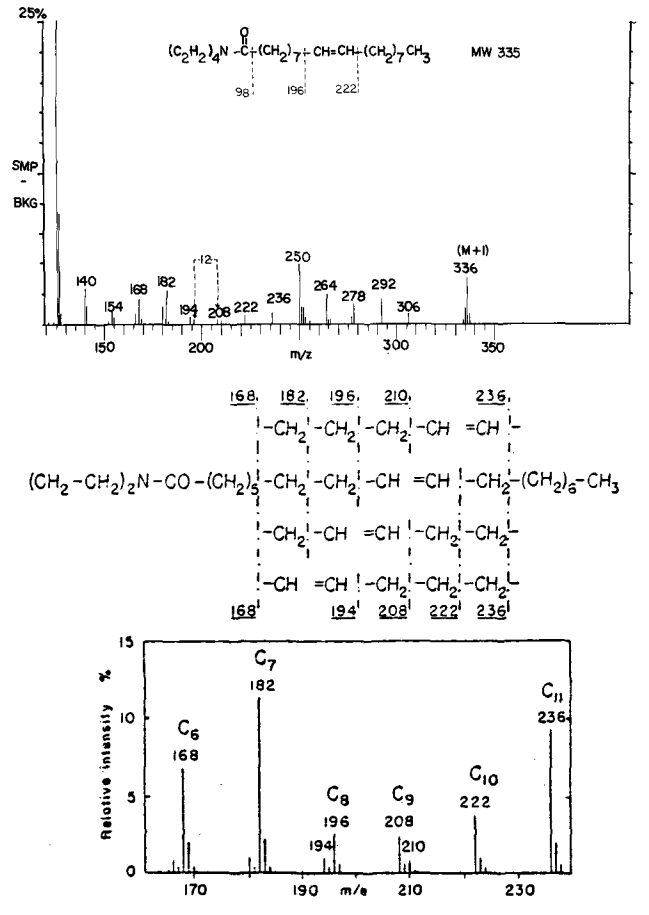

FIG. 5. Mass spectrum of N-octadec-9-enoylpyrrolidide from ITD GC-MS (*top*) and from a conventional GC-MS (*bottom*, reproduced from Ref. 19).

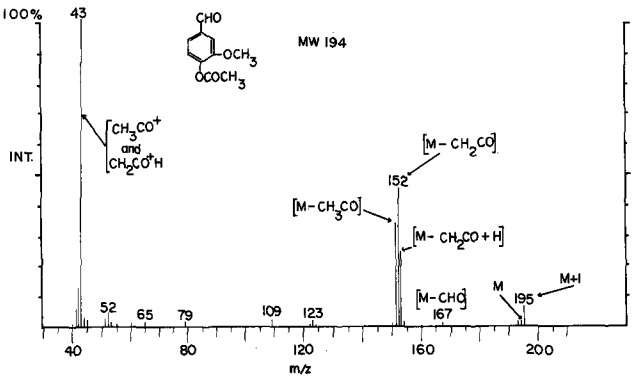

FIG. 6. ITD mass spectrum of vanillin acetate.

sea teleost fish (24,25). The ITD was used to verify, after deacylation, the original existence of DAGE of cod flesh lipids as well as to characterize the individual 1-0-alkyl moieties of DAGE. Figure 8 shows the reconstituted ion chromatogram patterns from the ITD of the isopropylidene derivative of glyceryl ethers, before and after hydrogenation, analyzed on a DB-Wax flexible fused silica capillary column. From the ITD mass spectra, 14:0, 16:0 and 16:1 were identified as the major alkyl chains of the cod flesh glyceryl ethers. ITD mass spectra also showed moderate or minor amounts of 14:1, iso-15:0, anteiso-15:0, iso-15:1, 15:0, 15:1, iso-16:0, iso-16:1, anteiso-17:0, 17:0, 18:0, 18:1 and 18:2 alkyl chains. The mass spectra and the comparison of the peak areas before and after hydrogenation confirmed the identity of all the components. To demonstrate the versatility of the ITD, the mass spectra of a major (1-0-alkyl 16:0, peak no. 10), moderate (1-0-alkyl iso-15:0, peak no. 3) and a very minor (1-0-alkyl 18:2, peak no. 20) component are illustrated in Figure 9. The fragmentation pattern for the isopropylidene glyceryl ethers obtained from the ITD were very characteristic and were not different from those obtained from any conventional GC-MS (9,26). The ITD spectra yielded characteristic ions of M-15 (due to the loss of a CH_3 group from the isopropylidene ring of the parent ion) and m/z 101 (the base peak), both indicative of a glyceryl ether structure (9,26,27). All the major components exhibited the molecular ion and also the protonated molecular ion (e.g., m/z

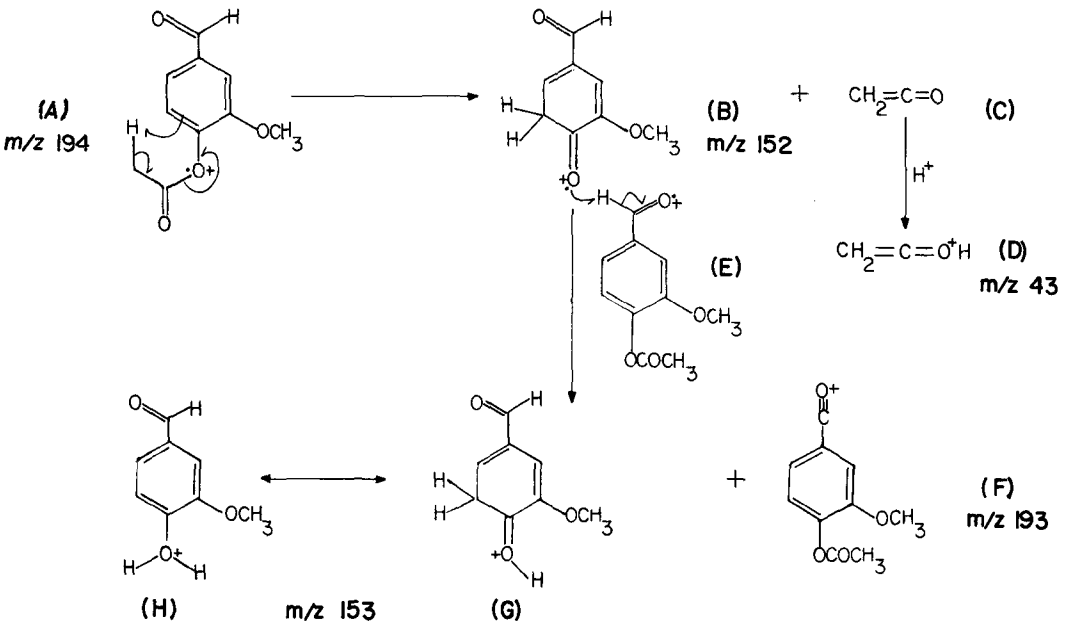

FIG. 7. Proposals for mechanism of formation of m/z 43, 152, 153 and 193 ions in the mass spectrum of vanillin acetate.

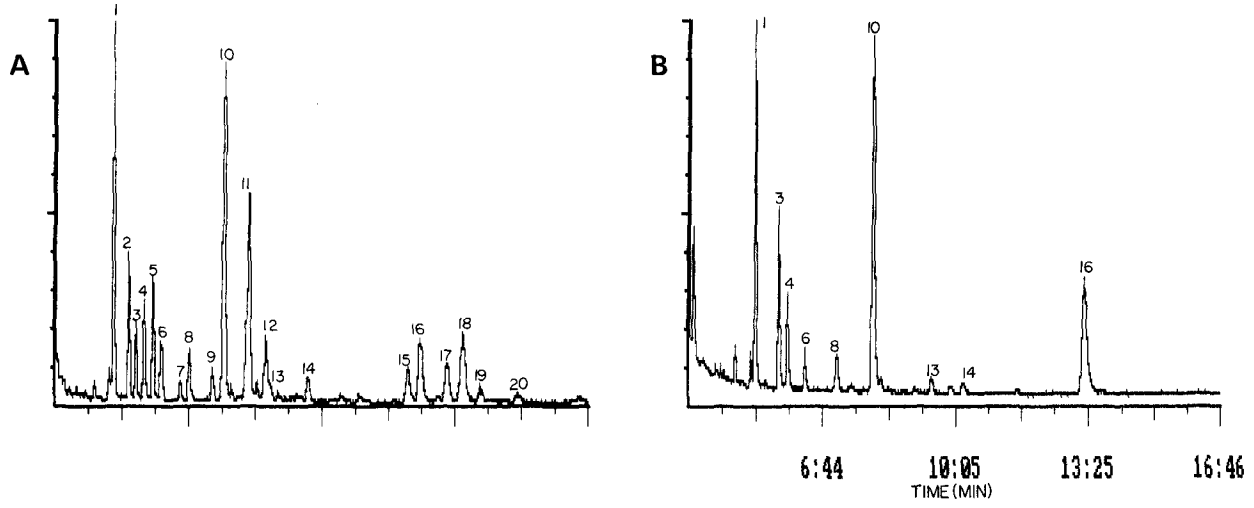

FIG. 8. Reconstituted ion chromatograph from the ITD-GC analysis of isopropylidene derivatives of deacylated glyceryl ethers from cod flesh lipids. A, unhydrogenated; B, hydrogenated. (*Note:* there is slight change in the retention time between A and B.) Peak identification: peak No. (1-0-alkyl group) 1 (14:0), 2 (14:1), 3 (iso-15:0), 4 (anteiso-15:0), 5 (iso-15:1), 6 (15:0), 7 (15:1), 8 (iso-16:0), 9 (iso-16:1), 10 (16:0), 11 (16:1), 12 (16:1), 13 (anteiso-17:0), 14 (17:0), 15 (unidentified impurity), 16 (18:0), 17 (18:1), 19 (18:1) and 20 (18:2).

357 for 1-0-alkyl 16:0 in Fig. 9). These two ions were not detected for moderate size or minor components. The complete absence or very low intensity of the molecular ion of the isopropylidene derivative of glyceryl ethers has also been noted by several other workers, even with conventional GC-MS (25-27). The presence of the M-CH_3 ions at 327 and 365 identifies the 1-0-alkyl moieties of the two peaks at 3 and 20 as iso-15 and 18:2, respectively. Hydrogenation results (Fig. 8, bottom) confirmed the identity of these components. The 1-0-alkyl 18:2 peak disappeared on hydrogenation and is converted to 1-0-alkyl 18:0. The position as well as the mass spectrum of

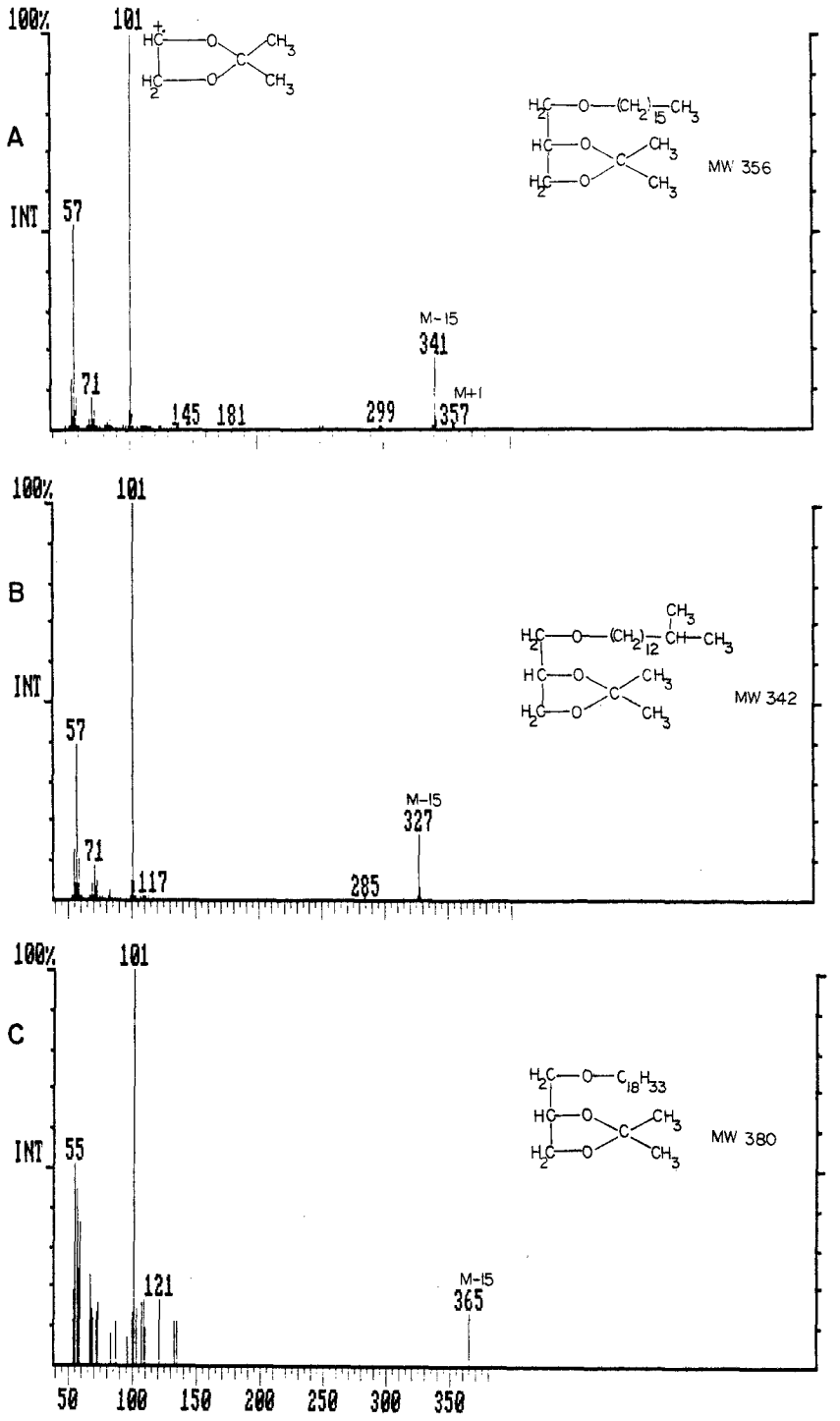

FIG. 9. Mass spectra of isopropylidene derivatives of deacylated glyceryl ethers of cod flesh lipids. A, 1-0-alkyl-16:0 compound (peak 10); B, 1-0-alkyl-iso-15:0 compound (peak 3); C, 1-0-alkyl-18:2 compound (peak 20).

peak 3 after hydrogenation remained unchanged, verifying the identity of peak 3 as 1-0-alkyl iso-15:0. As can be seen in Figure 8, the size of peak 3 increased after hydrogenation due to the contribution from 1-0-alkyl iso-15:1.

In spite of the favorable cleavage at the methyl branch, the ITD spectra do not differentiate between iso, anteiso and straight chain structures of a given chain length for the isopropylidene derivative of glyceryl ethers, as all these isomers produced identical fragmentation patterns. This is to be expected as the mass spectra are dominated by $(M-15)^+$, m/z 101 and ions of low masses. Nevertheless, the iso, anteiso and straight chain structures could easily be differentiated by their retention characteristics on both polar and nonpolar GLC columns (28). The unexpected identification of an 18:2 glyceryl ether in cod flesh illustrates that the ITD is quite sensitive and useful in characterizing trace level components present in a mixture.

The few results presented here demonstrate that the Finnigan ITD is an excellent substitute for more expensive and sophisticated GC-MS units, especially for those laboratories operating on a restricted budget. The sample size introduced into the ionization chamber should be kept as low as possible to minimize the secondary collisions of particles. Nevertheless, there is a possibility that these interactions could be utilized to advantage, especially by the protonation of the parent molecule to obtain the molecular weight, as in chemical ionization MS. Due to these collisions of particles, the ITD mass spectra should be expected to be slightly different from that of a classical mass spectrum. Our experience shows >95% correlation between the ITD spectra and that obtained with a conventional spectrometer for many lipid components. For highly unsaturated aliphatic compounds as well as aromatics, such as the phenolic acetates demonstrated above, the correlation will be expected to be much less, as the parent molecule could easily abstract a proton through collision. Very stable neutral fragments and ions have a greater tendency to acquire protons through collision, as the concentration of stable fragments is relatively higher than of unstable fragments. Ketene, obtained from phenolic acetates, is an example of such a stable neutral fragment. ITD spectra of compounds producing stable fragments will differ from the classical pattern to a moderate extent. Therefore, it should be emphasized here that the tendency for ionized molecules or fragments to become protonated varies with the type of compounds and with concentration. This may introduce uncertainty in structure assignment to fragments and in the final identification of the parent molecules, but if the fragmentation pattern of a particular class of compounds in the ITD is thoroughly understood, an unknown component of this particular class should be identifiable with little or no difficulty. However, identification of a completely unknown compound by ITD spectra alone will pose some difficulties. Nevertheless, the ITD is extraordinarily useful for confirmation of molecular structures when supporting data is available through other physical and chemical means. Another major problem with the ITD is the variation of the intensities of the ions, especially M^+ and $(M+1)^+$, with the concentration of the sample introduced into the ITD. This variability of ion concentration excludes the ITD from work with isotope-labeled metabolites and with stable isotope-labeled internal standards. Further, due to this variability of intensity of ions, the ITD is less suitable for quantitative work with either fragment ions or molecular ions.

The ITD has been in service for approximately one year in our laboratory and has been used for study of a variety of lipid classes. Further development of novel apparatus usually takes up to five years to mature and become widely known, and we expect this now familiar pattern of application and modification to develop quickly for the ITD.

ACKNOWLEDGMENT

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